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On Using VIKOR for Ranking Personnel Problem

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Abstract: Personnel selection problem implies more than one dimension to be optimized. Many conflicting criteria should be considered when comparing alternatives to choose among or rank them. In This article, a Multi-Criteria Decision Making (MCDM) problem is presented and a real-life international company personnel selection problem of a new manner is illustrated. The technique used in solution named *Vlse Kriterijumska Optimizacija I Kompromisno Resenje* in Serbian (VIKOR) is applied for ranking the alternatives.

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Keywords: Multi-Criteria Decision Making; Personnel; VIKOR.

1. Introduction

Personnel selection problem is a well known Multi Criteria Decision Making (MCDM) problem which involves many conflicting attributes. Personnel training process is very crucial in developing organizations. It implies more than one dimension to be optimized. Many conflicting criteria should be considered when comparing alternatives to choose among or rank them. The merit of MCDM techniques is that they consider both qualitative parameters as well as the quantitative ones, MCDM includes many solution techniques such as Simple Additive Weighting (SAW), Weighting Product(WP) [3], and Analytic Hierarchy Process (AHP) [7]. The personnel selection problem, from the multi-criteria perspective, has attracted the interest of many scholars as in [5,6].

In this paper a new personnel training selection problem existed in a multi-national company is presented. The technique named *Vlse Kriterijumska Optimizacija I Kompromisno Resenje* in Serbian (VIKOR), a branch of MCDM methods, is applied to rank the candidates for an international course of one year duration provided by the company to its employees. The rest of the paper is structured as following; in section 2 the VIKOR method is illustrated, section 3 is made for case study, finally section 4 is for conclusion.

2. VIKOR

A MCDM problem can be concisely expressed in a matrix format, in which columns indicate criteria (attributes) considered in a given problem; and in which rows list the competing alternatives. Specifically, a MCDM problem with m alternatives (A_1, A_2, \dots, A_m) that are evaluated by n criteria (C_1, C_2, \dots, C_n) can be viewed as a geometric system with m points in n -dimensional space. An element x_{ij} of the matrix indicates the performance

rating of the i^{th} alternative A_i , with respect to the j^{th} criterion C_j , as shown in Eq. (1):

$$D = \begin{matrix} & C_1 & C_2 & C_3 & \cdots & C_n \\ \begin{matrix} A_1 \\ A_2 \\ A_3 \\ \vdots \\ A_m \end{matrix} & \begin{bmatrix} x_{11} & x_{12} & x_{13} & \cdots & x_{1n} \\ x_{21} & x_{22} & x_{23} & \cdots & x_{2n} \\ x_{31} & x_{32} & x_{33} & \cdots & x_{3n} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ x_{m1} & x_{m2} & x_{m3} & \cdots & x_{mn} \end{bmatrix} \end{matrix} \quad (1)$$

The VIKOR method was introduced as an applicable technique to implement within MCDM [4]. It focuses on ranking and selecting from a set of alternatives in the presence of conflicting criteria. The compromise solution, whose foundation was established by Yu [10] and Zeleny [11] is a feasible solution, which is the closest to the ideal, and here "compromise" means an agreement established by mutual concessions.

The VIKOR method determines the compromise ranking list and the compromise solution by introducing the multi-criteria ranking index based on the particular measure of "closeness" to the "ideal" solution. The multi-criteria measure for compromise ranking is developed from the L_p -metric used as an aggregating function in a compromise programming method. The levels of regret in VIKOR can be defined as:

$$L_{p,i} = \left\{ \sum_{j=1}^n [w_j (x_j^* - x_{ij}) / (x_j^* - x_j^-)]^p \right\}^{1/p}, \quad 1 \leq p \leq \infty, \quad (2)$$

where $i = 1, 2, \dots, m$. $L_{1,i}$ is defined as the maximum group utility, and $L_{\infty,i}$ is defined as the minimum individual regret of the opponent.

The procedure of VIKOR for ranking alternatives can be described as the following steps [2]:

Step 1: Determine that best x_j^* and the worst x_j^- values of all criterion functions, where $j = 1, 2, \dots, n$. If the j th criterion represents a benefit then $x_j^* = \max_i f_{ij}, f_j^- = \min_i f_{ij}$.

Step 2: Compute the S_i (the maximum group utility) and R_i (the minimum individual regret of the opponent) values, $i = 1, 2, \dots, m$ by the relations:

$$S_i = L_{1,i} = \sum_{j=1}^n w_j (x_j^* - x_{ij}) / (x_j^* - x_j^-), \quad (3)$$

$$R_i = L_{\infty,i} = \max_j [\sum_{j=1}^n w_j (x_j^* - x_{ij}) / (x_j^* - x_j^-)], \quad (4)$$

where w_j is the weight of the j th criterion which expresses the relative importance of criteria.

Step 3: Compute the value $Q_i, i = 1, 2, \dots, m$, by the relation

$$Q_i = v(S_i - S^*) / (S^- - S^*) + (1-v)(R_i - R^*) / (R^- - R^*), \quad (5)$$

where $S^* = \min_i S_i, S^- = \max_i S_i, R^* = \min_i R_i,$

$R^- = \max_i R_i,$ and v is introduced weight of the strategy of S_i and R_i .

Step 4: Rank the alternatives, sorting by the $S, R,$ and Q values in decreasing order. The results are three ranking lists.

Step 5: Propose as a compromise solution the alternative (A') which is ranked the best by the minimum Q if the following two conditions are satisfied:

C1. "Acceptable advantage":

$Q(A'') - Q(A') \geq DQ$, where A'' is the alternative with second position in the ranking list by $Q, DQ = 1/(m - 1)$ and m is the number of alternatives.

C2. "Acceptable stability in decision making":

Alternative A' must also be the best ranked by S or/and R . This compromise solution is stable within a decision making process, which could be: "voting by majority rule" (when $v > 0.5$ is needed), or "by consensus" ($v \approx 0.5$), or "with vote" ($v < 0.5$). Here, v is the weight of the decision making strategy "the majority of criteria" (or "the maximum group utility"). $v = 0.5$ is used in this paper. If one of the conditions is not satisfied, then a set of compromise solutions is proposed [2].

Recently, VIKOR has been widely applied for dealing with MCDM problems of various fields, such as environmental policy [8], data envelopment analysis [9], and personnel training selection [1].

3. Case Study

A multi-national company that works in Tele-Communications is willing to select one employee from its personnel to join a two-year course provided by one of its suppliers in Europe. The course is budgeted by 100,000 Euros for one person; the supplier company will pay the fees, and the whole charges of the selected employee suggested by the multi-national company in order to train and teach the rest of the company during the orientation phase after the supplier company installs and provides its software packages. The company restricted the selection to middle management in the technical support department found in the whole company branches and offices. After many procedures and tests done, four candidates are eligible to have the opportunity of the course, the multinational company Human Resources department specifies five criteria to compare the four candidates and put them through many tests for them in order to select only one. The process of ranking the four candidates in order to select optimally one is a typical MCDM problem.

The Human Resources department set two exams to the six candidates; first the fluency in the foreign language test, and second is computer skills test including basic programming concepts. Both tests are combined to be one grade out of 100 points. The human resources department set the first criterion C_1 to be the age of the candidate, the younger is preferable. C_2 is set to be the experience years in the field; C_3 is the number of years passed by the candidate inside the company. C_4 is the average point attained by the candidate on the performance assessment annual report during the last 5 years; and finally C_5 is the grade obtained by each candidate in the two exams set by Human Resources department. Table 1 shows the five criteria, their weights, and their computation units. The Human Resources department presented the data included in the decision matrix found in Table 2 showing the four candidates, and their performance ratings with respect to all criteria. All candidates are indexed by the term (CAND) for simplicity.

Table 1. Criteria and their computation units

Criterion Index	Criterion Description	Computation Units	Weights
C ₁	Age	No. of Years	0.30
C ₂	Work Experience	No. of Years	0.15
C ₃	Company Experience	No. of Years	0.20
C ₄	Annual Assessment Report	Average of 5 years	0.10
C ₅	Human Resources Tests	Grade (1-100)	0.25

Table 2. Decision matrix

	C ₁	C ₂	C ₃	C ₄	C ₅
CAND1	48	23	10	70	78
CAND2	42	15	12	80	70
CAND3	36	16	16	62	95
CAND4	45	10	20	77	68

By applying the procedure of VIKOR, we can calculate the S , R and Q values as shown in Table 3 to derive the preference ranking of the candidates. Management should choose the third candidate because he has the minimum S , R , and Q values; also, the two conditions mentioned earlier in section 2 are satisfied.

Table 3. Ranking lists and scores

	S	R	Q	Rank
CAND1	0.5556	0.3000	1	4
CAND2	0.4023	0.1600	0.39007	2
CAND3	0.2608	0.1000	0	1
CAND4	0.3917	0.2250	0.534521	3

4. Conclusion

A VIKOR method is presented to solve a real-life personnel training problem existed in multinational company. A MCDM problem of a new manner is introduced. The VIKOR method is employed to rank the candidates. It might be combined to other techniques in further research. The MCDM problem should be reformulated and solved if any parameter or alternative is added or deleted because of its sensitivity to any changes.

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Assessment of the Knowledge of Midwives Regarding Prevention of Low Apgar Score

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Abstract: Health professionals and midwives in particular, are responsible for the management of a pregnant woman during antenatal clinic, labour, puerperium and including neonatal care. They have to acquire knowledge of obstetric practice. Lack of knowledge may lead to mismanagement of labour, poor delivery technique which leads to babies born with low Apgar score of 7 or less at 5 minutes. The objective of the study was to assess the knowledge of midwives regarding the prevention of low Apgar scores among neonates. The study was designed as a quantitative and descriptive research. A representative sample of 100 midwives working in the maternity units of three district hospitals was selected. A self-administered questionnaire with closed questions was used to collect data. A purposive sampling method was used to select participants. The findings revealed that midwives perceived themselves to be having knowledge related to midwifery practice; however they were lacking knowledge of some skills related to midwifery care such as gestational period for engagement of the fetal head in primigravida. Protocols on the management of conditions contributing to low Apgar scores among neonates should be developed. The protocols should be in line with the Guideline for Maternity Care in South Africa. All midwives need to be trained in the implementation of these protocols.

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Keywords: Midwives; knowledge; prevention; low Apgar score; neonates

1. Introduction and Background

Health professionals and midwives in particular, play an important role in rendering maternal and childcare services, including neonatology. Midwives are responsible for the management of a pregnant woman during antenatal clinic, labour, puerperium and including neonatal care. They have to acquire knowledge of obstetric practice because in the management process they are exposed to various challenges such as conditions affecting pregnancy, difficult labour and resuscitation of hypoxic neonates (MacDonald & Van Der Walt, 2003).

It is the responsibility of the midwife to ensure that a woman gives birth to a healthy newborn baby with an Apgar score of 10/10 at one minute after birth. The Apgar score is a method that was introduced by an American anaesthetist called Virginia Apgar in 1953. The scoring system was intended to evaluate and record the physical condition of the baby in numerical terms at one minute after birth and if necessary it may be repeated at five minutes (Myles, Fraser & Cooper, 2004).

According to the World Health Organization (2003), midwives are responsible for clinical practice in midwifery discipline where they provide supervision, care and advice to the woman before conception, during pregnancy, labour and puerperium. Midwives must give health education during antenatal

clinic on topics such as the dangers of alcohol intake during pregnancy which may lead to a newborn born with a low Apgar score due to fetal alcohol syndrome (Viljoen, 1999). Midwives must have the ability to estimate fetal weight through abdominal inspection and palpation to identify macrosomic infants. Macrosomic were found to be the significant risk factors of low Apgar score (Essel & Opai-Tett, 1995).

Squire and Frances (2004) indicate that intrapartum asphyxia and chronic asphyxia were found to be contributory factors to low Apgar scores. Monitoring of the fetal condition by a midwife during labour is essential to ensure that delivery of the baby takes place before the occurrence of lack of oxygen supply to the fetal brain. experienced midwives with knowledge detects signs of fetal distress, identifies and grades meconium-stained liquor and seeks medical assistance for immediate management in order to prevent a low Apgar score (Myles *et al.*, 2004).

The knowledge and experience of midwives in the manipulation and interpretation of a cardiotocograph is very important in the identification of fetal distress which may result in a low Apgar score (Sellers, 2001a; Zapta-Vazquez, Rodríguez-Carvajal, Sierra-Basta, Alonzo-Vázquez and Echeverrilluz-Equiluz, 2003). Bunchman, Pattison and Nyathikazi (2002) reports from the study that 25% of fetal distress was due to incorrect interpretation of cardiotocograph

by midwives, 185 were due to inadequate fetal monitoring by midwives and in 7% there was no response to poor progress in labour by midwives. The role of a midwife is to evaluate carefully the progress of labour through the monitoring of maternal and fetal condition. The primary outcome is initiation of labour and delivery within 24 hours (Moodley, Venkatachalam & Songca, 2003).

Midwives keep clear and accurate records of the progress of pregnancy; labour and puerperium period. Apply the basic skills and techniques such as internal examination and pelvic assessment, cutting and suturing for an episiotomy in the second stage of labour (Searle, 1987a).

The South African Nursing Council regulation which governs the practice of a midwife (R2488) states that in case of antepartum haemorrhage, a midwife shall not carry out internal examination to avoid aggravating the condition and refer the woman to the medical practitioner for emergency management (SANC, 1990). Maternity Guidelines of South Africa states that the partogram must be promoted as the only legitimate record of labour progress to the extent that failure to use a partogram would be seen as negligence or indefensible in a medico-legal context (Department of Health, 2007).

The incidences of high or low statistics of babies born with low Apgar scores depend upon the knowledge of midwives in the management process prior to conception, during pregnancy, labour and neonatal care (Sellers, 2001a). Babies who fail to respond to resuscitation at birth have low Apgar scores of 6 or less at one minute and are sometimes referred to as being "flat" or "depressed" (Squire & Frances, 2004). An Apgar score of 8/10 usually indicates that the neonate is in good condition and that few or no problems may be expected (Nolte, 1998).

2. The Problem Statement

The practice of obstetric nursing requires a midwife practitioner who is competent to practice independently in providing antenatal services during pregnancy, progress of labour and conducts delivery on her own for a normal healthy baby. Vhembe district statistics from three particular hospitals, that is Hospital A, Hospital B and Hospital C revealed that out of 1 218 deliveries in a month, 43 neonates were born with low Apgar scores. Contributory factors might be many and varied (MacDonald *et al.*, 2003). The researcher is concerned about constant statistic of low Apgar scores among neonates which is not coming down and focused on the knowledge of midwives regarding prevention of low Apgar scores among neonates.

3. Purpose of the Research

The purpose of the study was to assess the knowledge of midwives with regard to the prevention of low Apgar scores among neonates.

4. Objectives of the Study

To describe the knowledge of midwives regarding the prevention of low Apgar scores among neonates.

5. Materials and Methods

This study used a quantitative and descriptive research design (Polit & Hungler, 1995). A sample of 100 midwives working in maternity units at three district hospitals was selected. A self-administered questionnaire was used to collect data. A non-probability purposive sampling method was used to select participants.

The research study was conducted in a clinical setting at Government hospitals in the Vhembe district of Limpopo Province. The district has seven district hospitals and one regional hospital which serve as a referral hospital to which the six district hospitals refer patients for specialised services. The seventh hospital is for maximum security psychiatric patients. Three hospitals were chosen as sites for the study to be conducted. The choice appeared to be relevant because they were located less than 40 kilometres from one another.

The questionnaire was used as an instrument for collecting self-reported data from the midwives. A self-administered questionnaire with closed-ended questions was designed. Structured questions were formulated to assess and describe the competencies of midwives with regard to the prevention of low Apgar scores among neonates. Structured procedures and a formal instrument were used to collect numerical information under controlled conditions. Midwives in this study provided relevant data in relation to the study (Dempsey & Dempsey, 1992; Mouton, 1996). Questionnaires were distributed to all midwives practicing in the maternity units of three different hospitals and they were allowed to complete them in the presence of the researcher and bring them back.

The population in this study was all midwives practising in maternity units of three selected hospitals of the Vhembe district. Only midwives who were allocated and practicing in maternity units were included in the study. Midwives were required to have at least one year's experience of practising in maternity, and six months in the labour ward (Brink, 2003; Brink & Wood 1998). A sample size of 100 midwives was sufficient to achieve saturation of the theoretical categories.

For the purpose of this study a non-probability purposive sampling approach was used to select the hospital sample and participants. The researcher used

her own knowledge about the population and its elements to handpick cases to be included in the study. All available midwives practicing in the maternity departments who were judged to be typical of the population in question or particularly knowledgeable about the issue of the prevention of low Apgar scores among neonates from three hospitals were chosen (Brockopp & Hastings-Tolsma, 1995; Burns & Grove, 2003). This meant that not all midwives working at the three selected hospital had a chance to be part of the study.

Data obtained from the completed questionnaires were subjected to analysis by the Statistical Packages of social sciences (SPSS) programme to establish frequencies and percentages. The purpose of the data analysis was to impose some order on a large body of knowledge so that a general conclusion could be communicated in a research report. Statistical procedures enabled the researcher to reduce summaries, organize, evaluate, interpret and communicate numerical information (Polit & Hungler, 1999).

Mouton (1996) maintains that since scientific research is a type of human conduct, it follows that the research has to correspond with the generally accepted norms and values. Ethics is doing what is right and good during research and all ethical principles to be applied to the research process were observed. The researcher protected the rights of the midwives and those of the institutions in which the study was conducted. According to (Nieswiadomy, 1993; Seaman, 1998), midwives have several rights such as the right not to be harmed, the right to maintain self-respect, dignity and the right to privacy, informed voluntary consent, confidentiality and anonymity, and the right to refuse to participate or to withdraw from participation without any fear of discrimination.

Pilot study was conducted in order to double check the instrument before commencing the major study to ensure that it worked properly (Polit & Beck, 2004). A purposive sample of 10 midwives was drawn from three different hospitals and was informed about the purpose and outcome of the study. This is done to prevent frustrations and irritability if instrument is out of order as it may result into losing respondents, time, patience and motivation (Hicks, 1996; Mouton & Marais, 1991). The respondents involved in the pilot study were not included in the major study (Brink & Wood, 1998). The researcher was able to test the use of the questionnaire and assess whether the questions were understood (Streubert & Carpenter, 1995). This further determined the reliability of the questionnaire (Abdellah & Levine, 1986).

In ensuring reliability in this study, the same tool or instrument was used at three different hospitals and yielded the same results. This is supported by De Vos

and Fouche (1998) and Crookes and Davies (2004) who refer it as “the extent to which independent administration of the same instrument yields the same results under comparable conditions.” the same instrument was used several times in different situations the outcome or results were the same. Reliability and validity are related to each other (Polit & Hungler, 1999).

According to Myles *et al.* (2004) and Mashanzi (2000), midwives has been trained to assess and make a decision to a save life by the South African Interim Nursing Council (SANC). Fullerton and Ingle (2003) state that Knowledge is the pre-requisite for proper management of the woman during antenatal care and labour.

6. Results

Respondents were asked to rate their knowledge on the scale below. “Strongly agree” and “agree” were combined to denote “agree”; “disagree” and “strongly disagree” were combined to denote “disagree” and missing responses by “mis”.

Table 1. “Knowledge of Midwives in Prevention of Low Apgar Score”

Knowledge of the Midwives	Agree	Disagree	Mis	Total
1. During palpation the palmar surfaces of the fingers determine the soft consistency of the fetus	64.3	29.5	6.3	100.0
2. Walking the fingertips of both hands is an excellent method of locating fetal position	68.4	26.3	5.3	100.0
3. Recurrent pregnancies lead to poor fetal growth	55.4	40.0	4.2	100.0
4. Thick and tight cervix facilitates good progress of labour	11.6	88.4	-	100.0
5. The hands grasp the fetal mass in the centre to assess the fetal weight and size	49.4	44.3	6.3	100.0
6. Meconium-stained liquor is an indication of amniotitis	32.7	64.1	3.2	100.0
7. In primigravida the head should have engaged at 38-39 weeks of pregnancy	63.2	35.7	2.1	100.0
8. Posterior position of the fetus leads to prolonged labour	77.9	22.1	-	100.0
9. Resuscitation of a newborn requires extra skills and experience	86.3	13.7	-	100.0
10. Presence of moulding 3+ is a good indication of descent of the head during labour	19.0	81.0	-	100.0
11. Continuous strong	84.2	15.8	-	100.0

contractions may lead to rupture of the uterus				
12. In multiparous women duration of second stage of labour should not exceed 30 minutes	69.5	30.5	-	100.0
13. Fetal heart rate is monitored ¼- hourly during the active phase of labour	60.0	40.0	-	
14. Pelvic assessment is done at 34- weeks of pregnancy in primigravida	69.5	30.6	-	100.0
15. Full urinary bladder facilitates descent of the fetal head during labour	16.8	81.1	2.1	100.0
16. Diabetes mellitus during pregnancy may cause obese babies leading to difficult delivery	90.5	8.4	1.1	100.0
17. A rise in blood pressure of 160/100 during the second trimester is considered pathological and needs advice for rest	65.2	33.7	1.1	100.0
18. Maternal condition is recorded on the partogram	84.2	13.7	2.1	100.0
19. PIH causes decrease in uterine blood flow and placental dysfunction if not treated properly	73.7	22.1	4.2	100.0
20. Descent of the fetal head takes place in deflexed head	33.6	58.9	7.4	100.0
21. Antepartum haemorrhage, prolonged labour and premature labour are conditions that compromise the fetus	71.6	27.4	1.1	100.0
22. Vacuum extraction is the best in the case of delayed second stage of labour	44.2	54.7	1.1	100.0
23. Bishop score of “6” is a good indication of induction at 42 weeks pregnancy	52.6	42.1	5.3	100.0

7. Discussion

Results revealed that midwives have knowledge related to most midwifery skills that would prevent low Apgar score among the neonates. But not necessarily to all skills outlined in the questionnaire. All midwives are expected to have acquired knowledge to achieve competency level of 100% in midwifery practice. The birth of the baby is more than the start of new life (Drake, 2010). However, they were lacking knowledge in performing some of the midwifery skills which are considered to be critical for the best possible outcome of the neonates as follows:

- During palpation the palmar surfaces of the fingers determine the soft consistency of the fetus (64.3%)

- The hands grasp the fetal mass at the centre to assess fetal weight and size (49.4%)
- Recurrent pregnancy leads to poor fetal growth (40.0%)
- In primigravida the head should have engaged at 38-39 weeks of pregnancy (63.2%)
- Meconium-stained liquor is an indication of amnionitis (64.1%)
- Descent of the fetal head takes place in a deflexed head (58.9%)
- A Bishop score of 6 is a good indication of induction at 42 weeks of pregnancy (52.6%)
- Fetal heart rate is monitored ¼-hourly during the active phase of labour (60.0%)

Identified Lack of Knowledge as Perceived by Midwives

The Australian Nursing and Midwifery Council (2009) indicate that midwives acquire theoretical knowledge during their period of training which is applied in practical situations when performing midwifery clinical skills. According to the findings, midwives perceived themselves to be lacking knowledge in performing some skills related to midwifery care. A discussion of key areas in relation to lack of knowledge when performing midwifery skills follows.

During palpation the palmar surfaces of the fingers determine the soft consistency of the fetus and the hands grasp the fetal mass in the centre to assess the fetal weight and size.

The findings reveal that 64% and 49% of the midwives are lacking knowledge related to that: “during palpation the palmar surfaces of the fingers determine the soft consistency of the fetus and the hands grasp the fetal mass in the centre to assess the fetal weight respectively”. Lack of knowledge in performing the above skills predisposes poor location of fetal position, inability to estimate fetal weight and size, and possible undetected big baby (macrosomia). If labour is progressed and the baby allowed to be delivered normally, baby will be born with a low Apgar score due to difficult delivery of a big baby (Chiarella *et al.*, 2008). Mocanu *et al.* (2000) reports similarly from the study conducted at the American College of Obstetrics and Gynaecologists in America involving 175 000 deliveries with the aim of evaluating the impact of macrosomic babies and neonatal outcome. The results indicated that 2 345 caesarean sections which were done were due to macrosomic babies (exceeding 4500g) in order to prevent low Apgar scores among the neonates. Caesarean section was recommended for all suspected fetal weight exceeding 4500g. Contrary to the stud Askham and Barbour

(1996); Barbour (1990) reports that women preferred to tell midwives their problems rather than the doctor during antenatal clinic where abdominal palpation takes place. They consider midwives to have more knowledge about childbearing than men (doctor or accoucher). Midwives had considerable midwifery knowledge and skills which needed to be put into practice in midwifery units.

Robinson (1990) also reported similar findings, that midwives had considerable knowledge and experience of providing midwifery care and women felt more comfortable when they were cared for by midwives. Hodnett, Gates, Hofmeyr and Sakala (2007); The Royal College of Obstetricians and Gynaecologists (2001) reported that there is relationship between the fetal size and shoulder dystocia. Similar findings were reported by Sokol and Blackwell (2003) and Langer, Berkus, Huff and Samueloff (1991), that a big baby is associated with a difficult delivery and a low Apgar score. All midwives should be able to perform abdominal palpation to estimate fetal weight and identify macrosomia.

Recurrent pregnancy leads to poor fetal growth

The findings reveal that 55% of the midwives are lacking knowledge related to that "Recurrent pregnancies lead to poor fetal growth." According to Sellers (2001b), recurrent pregnancy is a predisposing factor for a big baby leading to difficult delivery and low Apgar score if allowed to deliver vaginally. Lack of knowledge prevents midwives from anticipating the outcome of recurrent pregnancies, which may result in babies born with low Apgar scores. The findings might conclude that midwives are lacking in knowledge related to the impact of recurrent pregnancies on the unborn baby.

In primigravida the head should have engaged at 38-39 weeks of gestation

The findings of the study reveals that 63% of the midwives are lacking knowledge related to that: "In primigravida the head should have engaged at 38-39 weeks of pregnancy." According to Sellers (2001a), the fetal head should have engaged into the pelvic brim two weeks before commencement of labour. Failure of engagement is a sign of inadequate pelvis or the presence of some abnormality in the lower pole of the uterus. That will lead to prolonged labour causing fetal distress and a baby born with a low Apgar score. Midwives should apply theoretical knowledge during pelvic palpation and assessment to identify engagement of the fetal head in primigravida. The findings of this study concluded that midwives lacked the knowledge related to engagement of the fetal head in primigravida during pregnancy. Failure of the fetal head to engage at 38-39 weeks of pregnancy requires prompt attention

and referral to the hospital for hospital delivery to prevent a low Apgar score.

Meconium-stained liquor is an indication of amnionitis

The findings of this study reveal that 64% of the midwives are lacking knowledge of differentiating meconium-stained liquor and amnionitis. Myles & Strassner (2005) report that meconium-stained liquor is detected in three grades and it is an indication of fetal distress which causes a low Apgar score. Amnionitis usually occurs if membranes have been ruptured for more than 24 hours. It is a sign of infection. It may have an offensive smell and is very dangerous to the baby. Midwives should report the woman to the doctor for prophylactic treatment including antibiotics to be administered to prevent a low Apgar score.

Davis and Henderson-Smart (2001) report that a dexamethasone injection is given to infants who are exposed to amnionitis to extubate the lungs after delivery. Zapata-Vazquez et al. (2003) report similar from the study conducted at Carlos Urziaz Jiménez hospital in Merida, Mexico involving 387 neonates with the aim of evaluating the impact of amnionitis on newborn babies. The outcome of the study indicated that 83 neonates had low Apgar scores of less than 7 at five minutes. Among the 83 neonates with low Apgar scores, 26 were due to amnionitis which was not detected and treated properly before delivery due to lack of knowledge of midwives. It was also associated with prolonged rupture of membranes for more than 24 hours or the woman had undergone multiple vaginal examinations during labour by midwives.

Similarly, lack of knowledge of the midwives had resultant into 46% of neonates with low Apgar scores and 6% died within 24 hours of delivery from 102 neonates who had amnionitis at Francisco maternity hospital in France. It was also indicated that amnionitis was associated with placental insufficiency with subsequent fetal hypoxia, fetal distress and low Apgar score if not treated properly (Wiswell, 2001). Ghidini and Spong (2001) and Davis and Henderson-Smart (2001) share similar views, that amnionitis is an infection of the amniotic fluid and prophylactic treatment should be given prior to delivery to prevent a low Apgar score. Lack of knowledge by midwives leads to poor management of women with amnionitis and causes low Apgar scores among the neonates. Richardson, Tarnow-Mordi and Escobar (1998); Ward and Sinn (2003) shared similar views that prolonged rupture of membranes is associated with amnionitis. If midwives lack the knowledge concerning it, it may result in babies born with low Apgar scores.

The perinatal care survey in South Africa in 2001 analysed the causes of perinatal deaths from 78 343 births. The outcome showed that 3 045 neonates died

and the most common primary causes were spontaneous premature labour associated with amnionitis (Pattison, 2001).

Descent of fetal head takes place in a deflexed head

The findings of this study reveal that 58% of the midwives are lacking knowledge about the situations or conditions which facilitated descent of the fetal head. Descent of the fetal head takes place in situations where the fetus is in a complete attitude (flexion) while still in the uterus. In deflexed head, there is no flexion of the head. During labour, it will lead to poor progress which may result in deep transverse arrest. Caesarean section may be performed or rotation and flexion of the head with application of a forceps delivery resulting in poor neonatal outcome. The baby may be born with a low Apgar score (Myles *et al.*, 2004).

A Bishop score of 6 is a good indication of induction at 42 weeks of pregnancy

The findings of this study reveal that 52% of the midwives are lacking knowledge regarding the favourable features of a Bishop score for induction of labour. According to Bishop (1994); Buchanan, Macer and Yonekura (2005), induction of labour is commenced following full assessment of the woman by the doctor. Midwives should have knowledge of the fact that the favourable induction feature is a score of 6-13. Levis (2007) reports that a score of 5 or less is unfavourable for induction of labour. A score of 6 and above indicate that the cervix is ripe. Induction of labour can therefore be initiated and may have a high probability of being successful.

Midwives must have knowledge of the Bishop Score so that they can be able to advocate for their patients. According to the SANC (R2598, 1984; R2488, 1990), a midwife acts as an advocate for the patient. She speaks on behalf of the woman in labour in order to protect her from possible abuse, neglect or harm. Augustine and Orhue (2005) report the findings of the study conducted at the University Hospital, Benin City in Nigeria involving 90 primigravida with a low Bishop Score of less than 6. The outcome indicates that methods used for induction failed due to unripe cervix. Ezimokhai and Nwabine (1998) report similar findings that a Bishop score of less than 6 had poor induction outcome as the cervix was not yet ripe and ready for dilatation.

Fetal heart rate is monitored ¼-hourly during the active phase of labour

The findings of this study revealed that 60% of the midwives are lacking knowledge related to monitoring fetal heart ¼ hourly during active phase of labour. This is one of the critical skills which 100% knowledge is expected from all midwives responsible for

management of labour. This may help with the detection of any sign of early or late deceleration and indicate urgent action to be taken to prevent a low Apgar score. Various methods can be used for monitoring the fetal heart during labour. Bunchman *et al.*, (2002) report findings from study conducted in metropolitan and rural hospitals in South Africa involving 102 perinatal deaths due to asphyxia neonatorum. Out of these babies, in 80 cases fetal monitoring was done ¼-hourly, 55% with cardiotocograph, 32% using the fetal scope and 13% using the hand-held Doppler. Early and late decelerations of the fetal heart were detected which resulted in poor neonatal outcome. However, findings not reported in numerical terms.

According to Maternity Guidelines in South Africa (Godi, Mhlanga, Saloojee, Steinberg and Tlebere, 2007), all findings of maternal and fetal condition, including progress of labour, are recorded on the partogram by midwives who are monitoring and progressing labour. Failure to use a partogram during labour or incorrect recording with misinterpretation of the findings constitutes substandard care. Poor progress with complications such as fetal distress will result in babies born with low Apgar scores. The WHO (1994) also reported similar views that the partogram was an important tool for monitoring labour and identifying women in need of obstetric intervention.

Orji (2008) conducted a study of 463 women with normal labour with the aim of evaluating the progress of labour using the modified WHO partogram. Labour was monitored and plotted on the partogram by midwives. The results indicated that 102 women who had crossed the action line had delayed first stage of labour which further resulted in poor neonatal outcome. Low Apgar scores were reported in 36 babies at one-minute and five-minute intervals, 25 babies had asphyxia neonatorum and there were 5 stillbirths (Orji, 2008). Bosse, Massawe and Jahn (2002) reported similar findings from their studies, that the partogram was analysed to evaluate neonatal outcome. The results yielded low Apgar scores of less than 7 at five minutes.

8. Conclusion

The study included 100 midwives who agreed to participate in the study and a self-administered questionnaire was used to collect data. Data were analysed by using a computer programme for statistical analysis, Statistic Package for Social Sciences (SPSS). The knowledge of midwives was assessed by addressing the objectives and the purpose of the study, which were achieved. The findings of the study reveal aspects where midwives have acquired the knowledge needed in managing pregnancy and labour as well as the areas where they are lacking knowledge. Maternity care forms an integral component of primary health

care, and is one of the priority reproductive issues that require urgent attention. For this reason all midwives need to be equipped with knowledge to provide quality midwifery care as they are dealing with two lives, that of the mother and the baby, and to curb maternal and neonatal complications.

Recommendations Related to Improving the Knowledge of Midwives on Aspects of the Prevention of Low Apgar Scores

Standardised clinical guidelines should be included in the structured learning curriculum for undergraduate nurses.

Protocols on the management of conditions contributing to low Apgar scores among neonates should be developed. The protocols should be in line with the Guideline for Maternity Care in South Africa. All midwives need to be trained in the implementation of these protocols.

Midwives should meet monthly to discuss problems experienced and update each other on the latest developments in midwifery care.

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A Study on Seed Hydro-Priming Effects on Morphological Traits, and Qualitative and Quantitative Yield in Soybeans under Farm Conditions (Iran)

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Abstract: To study the Seed Hydro-Priming effects on soybeans morphologic, and qualitative and quantitative traits, a research was carried out in Ardebil Islamic Azad University research farm, in 2008. This research was conducted in factorial based on complete block randomized design. One of the Seed Hydro-Priming factors was 8, 12, 16 and 20 hours which were soaked in tap water and dried to 30percent moisture. A seed sample was also considered as an observation sample (without pretreatment). The second cultivar factor was Williams and LV₍₁₇₎. Results indicated that there is a significant difference at 1percent level between hydro-priming durations on plant height, grain yield, oil content, weight of sub-stems, number of sub-stem and germination percentage. In most traits other than sub-stems weight and number of sub-stems, 8-hour Hydro-Priming provided the best yield. Moreover, cultivar interaction effects on plant height trait in Hydro-Priming were significant at 1percent. The results to the average comparison table indicated that Williams cultivar had the most height with 8-hour Hydro-Priming. Also, there was a significant difference among cultivars on number of sub-stem, weight of sub-stems and plant height at 1percent and in most traits LV₍₁₇₎ had a better yields comparing to the Williams cultivar. Considering the results, see Hydro-Priming due to the short growth period and to increase the yield and better green in farm seems to be of significance. Also, 8-hour Seed Hydro-Priming is suggested for soybeans.

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Keywords: Seed Water Pre-Treatment, oil and protein percentage

Introduction

Deploying seedling is a critical step in the plant production process. Seeds uniformity and germination percentage in direct cultivation could have a great impact on production quality and performance. In recent years, a lot of efforts have been made to improve the germination condition, seed and seedling growth strength in special environments. (Ellis, 1989; Srinivasan et al, 1999; Drewet et al, 1997) Priming is among the main methods in increasing seeds germination strength. Priming includes various seed improving methods in which seeds are controllably discharged. (Faruq et al, 2006b) The main objective in seed priming is partial water discharge so that seeds pass the germination in the first stage (physical water absorption) and second stage (biochemical processes initiation and sugars hydrolysis) and halt in the

germination third stage (sugar consumption by the embryo and radicle growth). (Bradford, 1995) Seed priming is of various types according to the priming solution. The main and common point for all solutions is related to the optimal concentration and proper preservation period. Also, the seed should not be immersed in the prime solution during priming. Putting the seed with 50% of the height for a better exchange of oxygen is recommended. (Van Vactor, 2000; Hardegree et al., 2002) One of the main priming methods is to prime by water. Priming by water is very simple and cheap, and the water absorption amount is controllable through the calculating the time which the seeds are in contact with water. (Jusi and Sharifzade, 2006; Ashraf and Fulad, 2005; Faruq et al, 2006b)

Using increasing seed strength treatments could lead into rapid germination, consistent emergence and

plant strong deployment. (Afzal et al, 2002; Ashraf and Fulad, 2005; Faruq et al, 2006a) Priming by water affects DNA and RNA synthesis, alpha-amylase activities and better embryo growth. By improving the germination rate, growth consistency, seedling vigor and deployment, plant growth improves. (Basra et al., 2005; Ruan et al., 2002; Harris et al., 1999) It is reported that hydro-priming improves the cottonseed germination under tension and non-tension conditions. (Casenave and Toselli, 2007) Raaj and Mehra (2002) have also reported growth improvement and seedling deployment in canola under tension condition. Kaya et al (2006) have reported more germination and seedling growth in hydro-primed sunflower seeds under drought and salinity tensions. Additionally, Ghassemi- Golezani et al (2008) reached more seed yield in pea seeds under 16-hour hydro-priming treatment. Ghassemi-Golezani et al (2008) showed that hydro-priming results in seedlings growth rate, percentage, yield and yield components. They also reported that hydro-priming has a better effect on lentil seedling growth rate and percentage comparing to osmo-priming. Berg et al (1989) reported increase in production in subsequent to soybean seeds pretreatment. During their research on wheat seeds, Bosra et al (2005) came to this conclusion that, aqueous pretreatment for 24hours has a high effect on germination rate. Aqueous pretreatment in sunflower seedling weight is more tangible comparing to osmotic treatment in osmotic tension condition. (Demir Kaya, 2006) Pill (1986) reported that, parsley pre-germinating decreases the growth duration and increases shoot dry weight. Since there is not enough comprehensive data on hydro-priming application and effects on soybean cultivars primary growth and yield, the following research tries to study hydro-priming treatments effects on morphologic traits and qualitative and quantitative yield under farm condition.

Materials and Methods

The soybean seeds (Williams and LV₍₁₇₎) which were provided by Moqan Agricultural Research Center were divided into five equal portions and a sample with 10percent moisture was collected in a plastic bag in refrigerator in 3to 5°C as the control sample. The other four samples were soaked in an incubator with 17.3 °C in distilled water for 8, 12, 16 and 20hours. Pretreated seeds were scattered on a table in laboratory environment between 20to 22°C to reach 30percent moisture. To determine the seeds moisture, 2 5-gram replicates of each treatment were separately beaten within porcelain to turn into granule. Beaten samples were weighed again and put

in an oven with the temperature of 130°C for an hour. Consequently, samples were brought out of the oven and weighed. Seeds moisture percent were calculated through the following equation:

$$\text{Equation 1: } MC = \frac{\text{Sample Wet Weight} - \text{Sample Dry Weight}}{\text{Sample Wet Weight}} \times 100$$

This research was conducted in in Ardebil Islamic Azad University research farm, in 2008. The region climate was semi-arid and cold with an altitude of 1350m above sea-level. The research was carried out as a factorial based on complete block randomized design in 3replicates. Each test unit included five implant lines with five meters length. On row from sides and half a meter from the row beginning and end were omitted as margin and sampling was done on the three medial rows. Soybean seeds were planted with 20seeds in a square meter on May 25, 2008. Immediately after observing the first seedlings, counting the grown seedlings in each test unit began and continued daily for 10days. The growing percentage was determined, considering implanting density and total grown seedlings, by the period ending. During crop ripening, 10plants were randomly harvested from each test unit and plant height, sub-stem weight and number of sub-stems was measured. Final harvest for each test unit was done when seed moisture reached to 17percent. In this stage, plants in 1 square meter in each plot were harvested. Subsequently, seeds were separated from pods and seed yield per unit area were separately weighed and recorded for each treat and replication. Also, oil and protein percentage in laboratory were calculated by Soxhlet apparatus. Data variance analysis was done as factorial in complete block randomized design for all studied traits. All statistical analyses and average comparisons were carried out by SPSS software. The diagrams were drawn by Microsoft Excel.

Results and Discussion

Plant Height: According to the data analysis and Table 1, there is a significant effect between plant height and replication, cultivar, treatment and cultivar with treatment interaction at 1percent level. According to the average comparison table, Williams cultivar has a higher plants comparing to LV₍₁₇₎ and among the treatments, 8-hour aqueous pretreatment has the most high. Control treatment and then other treatments are prioritized, respectively. Cultivar with treatment interaction has a significant relation with the plant height. According to the variance analysis and trait average

comparison between cultivars' traits, there is a significant difference between the cultivars based on germination percentage. Since Williams cultivar has the most germination during 8-hour pretreatment, hence, it has the most plant height. Caur et al (2002) reported that, re-exposed to the water, pretreated seeds grow faster and germinate more comparing to the control seeds and finally result in plant better deployment under drought tension. They also showed that pea seeds pretreatment by 4% mannitol, increases the plant height to 17percent within 130days after planting, comparing to the control seeds seedlings.

Germination Percentage: According to the average comparison table and variance analysis (Figure 5 and Table 1) there is a significant difference between cultivars and treatments in this trait at 1percent level. Among the cultivars, Williams cultivar and among the treatments, aqueous 8-hour treatment had the highest germination percent. This trait shows that 8-hour aqueous pretreatment is the best seed aqueous pretreatment for germination, growing speed and yield in Ardebil condition. Also, the 20-hour aqueous pretreatment had the lowest effect in the farm. During their research on wheat seeds, Bosra et al (2005) came to this conclusion that, aqueous pretreatment for 24hours has a high effect on germination rate. Also, Casiro et al (2004) came to this conclusion that aqueous pretreatment is the most effective method for improving onion seeds germination. The aqueous and matric pretreatment had a higher effect on wheat germination rate and percentage comparing to osmotic pretreatment with NaCl. (Bosra et al, 2005)Ghassemi- Golezani et al (2008) showed that hydro-priming results in increase in growth rate and percentage along with the increase in yield and yield components. They also reported that hydro-priming had a better effect on lentil seedling growth percentage and rate. Biyoli and Black (1978) and Khan (1992) have reported that the growth duration in farm could be decreased to 50% by priming. Seed priming could help the seedling extraction before soil forms crust and result in damage.

Number of Sub-Stems: There was no significant relation found between replication effects and treatment interaction on cultivar on number of sub-stems. However, considering the variance analysis table (Table1) cultivar and treatment effects on this trait were significant. Among the cultivars, LV₍₁₇₎ cultivar had the most number of sub-stems and among the treatments, the 20-hour seed aqueous pretreatment had the most number of sub-stems. It should be mentioned that control treatments had no

significant difference in 8 and 12-hour treatments. The reason to this result could be the plant strength in control treatments of 8 and 12-hour aqueous pretreatment and low plant density in area unit in 20-hour pretreatment. Kaur et al (2002) have reported increase in amylase enzymes and sucrose synthase in shoot and root of treated seedlings. They claimed that priming leads to the increase in amylase enzymes activity and converting the savings substances into transitional substances and as a result increase in plant growth.

Weight of Sub-Stems: There was no significant relation found between replication effects and treatment interaction on cultivar on weight of sub-stems (Table 1). Since this trait is dependent on weight and number of stems, they have more correlation so their results are similar to number of sub-stems. Data mean comparison shows that among the cultivars, LV₍₁₇₎ has the highest weight of sub-stems and among the treatments, the highest weight is related to 16 and 20-hour aqueous pretreatment. Hydro-priming affects DNA and RNA synthesis, ATP availability, alpha-amylase activity and embryo's better growth. Hence, germination better rate, growth consistency, seeding vigor and deployment leads to better plant growth. (Basra et al., 2005; Ruan et al., 2002; Harris et al., 1999)

Grain Yield: According to the variance analysis table (Table 1) there is a significant difference between economic yield in replications, cultivars and treatments. The interaction between cultivar and treatment is not significant in this trait. Average comparison results show that among the cultivars, LV₍₁₇₎ has the highest economic yield and among the treatments, 8-hour aqueous pretreatment has the highest yield. It should be mentioned that there was no significant difference found between 8-hour and 12-hour treatments. 20-hour aqueous treatment had the lowest economic yield. Pod number, plant height and grain dry weight are among the factors which could affect the yield. In aforementioned traits also, 8-hour aqueous pretreatment had the highest yield. Rashed et al (2006) reported that barley seed pretreatment could increase the yield up to 53percent. Increase in seed yield has been observed in corn and rice seeds due to the seed pretreatment. Faruq et al (2006) and Harris et al (1999) believe that the rice seed yield due to the pretreatment is a result of growth percentage improvement and yield execution such as seed weight. Ghassemi- Golezani et al (2008) showed that hydro-priming could lead into seedling growth rate and percentage and also yield and yield components. Kahlon et al (1992) Hussain et al (2006) reported higher seed yield in

hydro-primed seeds of sunflower and wheat, respectively. Moreover, Ghassemi- Golezani et al (2008) obtained a higher pea seed yield in 16-hour hydro-priming.

Harris et al (1999) reported that hydro-priming results in corn, pea and Upland rice better seedling deployment and vigor which increases the growth, flowering maturity and yield.

Oil and Protein Percentage: The data variance analysis related to the oil percentage (Table 1) indicates that all effects except the treatments effects are not significant. Data mean comparison show that among treatments, 8-hour aqueous pretreatment, control treatment and 12-hours aqueous pretreatment had the highest oil percentage and 20-hour aqueous pretreatment had the lowest oil percentage. According to the variance table (Table 1) the protein percentage in replication, cultivar, treatment and cultivar treatment interaction was not significant. This shows that treatments are not effective in protein yield. Ashrafi and Razmjou (2009) in a study on safflower claimed that 6 hours of hydro-priming could improve the hydro-primed seeds physiologic and biochemical characteristics and this leads to increase in oil and protein in seeds. Hydro-priming results in better growth a plant system protection against tension and increase in oil and protein amount. Seeds priming affects DNA and RNA synthesis and also improves the embryo's growth. (McDonald, 2000) Results from this research show conformity with previous studies in oil percentage

while the protein percentage was not in accordance with previous studies.

Results: Priming in improving seed germination and seedling deployment is accepted in arid and semi-arid regions due to its positive effects. Considering Iran's location which is situated in arid and semi-arid region and two crises of moisture and temperature which are considered to be of significant factors in seed germination and seedling deployment stage, especially rain fed conditions, rapid deployment could be of a great help in better water resources use. In such situation, using seed priming technique to reach a scientific result from laboratory to farm is of importance. One of the main existing concerns is conducting laboratory research in this field without evaluating their results in greenhouse and farm conditions. Hence, if there is a possibility to use this technique well, we could benefit from each condition of water cultivation in more rapid deployment with lower irrigation and success in delayed plants in rain fed condition with temperature and moisture fluctuations. According to the results in this research, due to the growth short period and using seed aqueous pretreatment in increasing the yield and improvement in growth, the activities before seed aqueous pretreatment seem to be of essence and the 8-hour aqueous pretreatment is recommended for soybeans.

Table 1. Analysis of variance for the evaluated traits at different Hydro-Priming levels in Williams and LV₁₇ cultivars in 2008

Source Variations	of df	Mean Square						
		Plant height	Number of Sub-Stems	Weight of Sub-Stems	Oil percentage	Protein percentage	Grain yield	Germination percentage
Replication	2	148.146**	1.213 ^{ns}	0.245 ^{ns}	0.196 ^{ns}	2.359 ^{ns}	49409.085**	36.273 ^{ns}
Cultivar	1	329.425**	5.208**	7.792**	0.768 ^{ns}	0.481 ^{ns}	17079.941**	188.351**
Hydro-Priming	4	161.822**	3.737**	2.917**	3.644**	6.686 ^{ns}	19956.800**	431.148**
C * H-p	4	341.735**	0.047 ^{ns}	0.161 ^{ns}	0.197 ^{ns}	3.354 ^{ns}	1456.316 ^{ns}	2.230 ^{ns}
Error	18	0.447	0.368	0.429	0.357	76.022	2975.028	18.723
CV (%)		0.93	13.84	10.11	3.99	4.70	16.66	10.69

* and ** Significantly at $p < 0.05$ and < 0.01 , respectively

Table 2. Comparison of Means of traits at different Hydro-Priming levels in Williams and LV₁₇ cultivars

Hydro-Priming levels	Characters				
	Number of Sub-Stems	Weight of Sub-Stems (gr)	Grain yield(gr/m ²)	Oil percentage	Germination percentage
Without pretreatment	3.86 BC	5.88 C	326.7 ABC	15.48 AB	41.85 B
8 hours	3.56 C	5.88 C	409.6 A	15.83 A	54.01 A
12 hours	4.15 BC	6.24 BC	348.3 AB	15.08 AB	37.72 BC
16 hours	4.83 AB	7.10 AB	297.3 BC	14.53 BC	37.56 BC
20 hours	5.52 A	7.33 A	255.3 C	13.87 C	31.19 C

Differences between averages of each column which have common characters are not significant at probability level of 5%.

Table 3. Comparison of Means of traits at Characters

Cultivars	Characters		
	Number of Sub-Stems	Grain yield(gr/m ²)	Germination percentage
Williams	3.97 B	303.56 B	42.97 A
LV ₁₇	4.80 A	351.28 A	37.96 B

Table 4. Comparison of Means of cultivar interaction effects with trait

Hydro-Priming levels	Cultivars	Character
		Plant height(cm)
Without pretreatment	Williams	77.20 B
	LV ₁₇	70.38 DE
8 hours	Williams	86.81 A
	LV ₁₇	72.33 CD
12 hours	Williams	73.06 C
	LV ₁₇	66.86 F
16 hours	Williams	70.47 DE
	LV ₁₇	68.60 EF
20 hours	Williams	67.76 F
	LV ₁₇	64.00 G

Differences between averages of each column which have common characters are not significant at probability level of 5%.

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Study on the architecture of Iran by new perspective to future

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ABSTRACT: Today, the necessity of energy saving is one of categories to be noticed more than before. Accurate controlling the amount of energy consumed in building and providing the required designing criteria to save energy is quite vital. Traditional architecture as applied in Iran is of a great value for its extensive capabilities to provide solutions for effective use of energy. So, by taking advantage of collective wisdom of the architects of precedent generations as well as historical experience, an Iranian architect has achieved to this capability and contemporary artists could be inspired by this achievement to design new and modern buildings. Since, Zavareh is one of ancient cities of Iran and comprehensive studies have not been made on this city, then desirable recognition of climatic aspect of this city may be valuable to know the Iranian local /domestic architecture. In the present paper, the residential architecture of this city is going to be studied to provide reasonable solutions for designing. Then, by application of these solutions, designing regulations, optimized materials and architectural regulations consistent with the climatic conditions of this city will be established and finally an effective measure to achieve a lasting architecture is taken. This paper will mainly focus on studying typology of residential constructions of it and checking its climatic features against sustainable architectural factors and those elements specific to sustainable and climatic architecture of it. The methodology applied in this research is based on a descriptive and analytical approach and the documents compiled are documentary resulting from many field studies. To do this, many of the constructions built in this city have been visited. The theoretical framework of this research demonstrates that the conditions of traditional architecture in each region have been affected by climatic conditions.

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Key words: architecture, climate, sustainability, vernacular

INTRODUCTION

The tranquility of those people who resided in different four regions of Iran has been always disrupted due to humidity or aridity and high temperature or high coolness and the traditional architect could overcome these difficulties and could establish innovative methods taking advantages of available facilities and employing the natural forces and the energy existing in the nature. Today, these have been forgotten.

One of the most important features of desert regions is comparing the residential buildings with the region the city is located therein. Since, this city is treated as a city with desert climatic features, then compliance of residential units with environmental conditions is quite inevitable. In the present paper, to examine the climatic-based architectural qualifications of housing development in it, 9 houses as indicators located in this city were selected and the climatic-based architectural qualifications of the foregoing city as a desert-based city the houses built therein are consistent with climatic conditions were examined. We are going to provide the solutions of architectural designing with this city to

establish the appropriate designing regulations and criteria. These actions may have a significantly effect on heating- cooling energy saving. It is worthy to note that the world is developing and the natural resources are diminishing. Given the resources found in it, five types of houses are identified. The general characteristics of housing varieties of this city were reviewed.

It is as an ancient city is 119 Km from northeastern of Isfahan province and it is located in a hot and arid region. Given the arid nature and undesirable soil texture, extensive salt lands and broad sandy places, the vegetation density of this region is very poor and it is mostly of desert step type. From ecological point of view, this region is undesirable for residency. From water network point of view, its region is treated as a poor region. Given the climatic condition and natural location, there are not permanent surface water network in this region (Consulting Engineers, 2006). The water supplied by Ghanat. In the past, the water required for city was supplied by glacier and some cisterns.

CLIMATE STUDY

Given the data and information on temperature, humidity and precipitation as registered for this city, we found that different features of desert weather such as low precipitation and fluctuation of temperature during in one day and low humidity are the most important environmental limitation of this city. (Municipality of Zavareh, 2009) and this environmental limitation may play a significant role to form the environment. The environmental conditions can be summarized as follows: this city has a cold winter and warm and dry summer and the average temperature reaches to 17.7°C (Consulting Engineers, 2006). The minimum temperature is -9°C and the maximum temperature reaches to 44°C in July-August. The temperature fluctuates within the range of 25°C during in one day in summers. Given these data, the weather dryness is demonstrated. This dryness will cause the water to be evaporated in this region and may have a significant effect on crops, as well. The blowing of warm wind may intensify the dryness (Ghaffari, 2000).

The most degree of relative humidity is registered in December with 6.3% and the least degree of relative humidity is registered in June with 25.8% (Consulting Engineers, 2006). The annual precipitation is 103 mm on average.

Low precipitation may result in barren lands and these lands are covered by the sands carried by the dry winds from the desert. The occurrence of frost during more than three months is likely and it may be intensified by the blowing of cold and ruthless winds.

The blowing of dry and burning winds usually accompanied with dust will intensify the dry and harsh heat of the summer. Under such conditions, the blowing of some winds will relieve the heat of the desert weather (Ghaffari, 2000).

Wind is one of important weather factors and elements in this region which play an effective role in positioning the urban elements and proper direction of housing and other urban spaces. In general, by taking advantage of shadow, achievement of tranquility conditions and desirable spaces cannot be guaranteed. However, air flowing. Taking advantage of desirable winds and avoiding undesirable winds in this region may be the most influential factors. In general, no scientific research was made on the winds blowing in it. Given the climatic studies of here, we have to reach some applied solutions for human comfort in this city by taking advantage of climatic-architectural facilities including providing effective ventilation, protecting the walls and windows against sunlight and undesirable winds, preventing heating of internal air during summer days and minimizing it in the night.

THE STRUCTURE AND TEXTURE OF THE CITY

In addition to climatic factors, some probable risks such as earthquake and water shortage have given rise to innovation and evolution of constructional forms such as dome, arch, windbreak and the cover of some parts of roof as hollow and using mud-bricks. Paying attention to aesthetics issues in application and composition of these forms and controlling the filled and vacant spaces are one of the features of buildings in valuable texture of the city.

The texture of this city has a checkered grid with an organic order and the courses are consistent with this grid. The existence of alley and roofed and indirect passages and narrow alleys with high walls provide the minimum sunlight and the maximum shadow for the people. Since, the grid is placed against the windward, the blowing of undesirable winds are prevented. In consequent of this type of urban planning, the hierarchy of observing the bounds and social fields are established.

The skeleton of it has two main axes which in its intersection, the city's gravity center with concentration of main architectural elements have been conceptualized and then the city can be divided into different places.

Historically, it is treated as an ancient city and most of its old-textured houses have architectural and cultural values and they are still used for living. The houses have a single unit and the yard and the spaces surrounding it have an ordered pedestal with geometrical shapes. Most of these houses have a separate and splendid yard. According to studies made on these houses, they are classified into three categories in terms of spatial arrangement: single- central yard, two-central yard and three-central yard and sometimes one of the open spaces have been designed as in the form of garden.

The most of houses are built in one level, and they have a basement and sometimes a windbreaker connected to the basements. The people spent their times in the deep and cold basements during the summer. Some of these houses have a two-level basement. The most of houses had a compact and inner plan and the external surfaces were minimized to lower heat exchange. The existence of dome has caused a part of roof to be protected by direct sunlight. During studying the houses, it was found that the most of houses with fountain and Hasht Behesht have two unilateral windbreakers. These facilities caused the air to be flowed and ventilated naturally within the internal space of the house.

While contacting with moisturizing elements such as fountain, flowerbed, trees and the wall of basement, the air flow could compensate the moisture shortage and

provide the comfort conditions for the residents during warm and arduous days of summer (Ghahramani, 1996).

In houses with Hasht Behesht, in addition two windbreakers, two general elements with rectangle-shaped section of 20*70 are extended by the roof.

The red tape of entering the house from the alley and the arrangement and organization of spaces: door, front yard some of them were common with two or three adjacent house, entrance hall, yard, veranda and the closed space, separate the building from the outer life stream. The entrance hall of the houses is usually a semi-opened space which is connected to the yard through the dark and tortuous corridors.

The corridors play the role of connection of entrance hall with yard(s) and or connection of other elements of the house with each other. In houses with fountain for instance, the lobby and lateral spaces are connected by the corridor. This phenomenon will increase the purification of these spaces and the designing of niches has visually improved the quality of this space.

Veranda is a semi-opened space and it faces the yard. It is a place for sitting, sleeping and a part connect the different spaces of the house with the yard and it is higher than the yard and it is located in the outer section of the residential buildings in the southwestern side of the building. By providing the shadow, the veranda prevents entering sunlight to inner space of the house. In wintertime, the veranda causes the sunlight to be penetrated as inclined. There is a veranda in southeastern side of the yard and it is used as a canopy for adjacent house. In accordance with the results of studies, the ratio of filled and vacant spaces is 3 to 2. The results of studies are given in figure 1.

Yard is an integral part of the houses built in this city. Having geometrical shape (square or square-rectangle), they have many functions. The yard has a central function in all of these houses and acts such as the heart of the house. Different spaces of the houses such as room, veranda, corridor, terrace, portico and platform with a defined arrangement are located around the yard. All spaces are indirectly or gradually related to the yard. Four fronts of the yard, even those parts which form the walls are defined.

In terms of spatial feeling, the yard will act such as unroofed room which the most daily tasks of the housewives are performed there. In general, the yard makes possible the free connection with the nature. Since they have not any view, they are used easily. In each of sides of the yard, the height of roof of fountain, storeroom, veranda, room with tree doors and room with five doors are different from each other which is very influential to direct recognition of the spaces.

Using the topography and vegetation density is the simplest form to provide the natural shadow. During the

climatic examination of Iranian traditional buildings, Ghobadian states that a large oak tree and or the grass with an area of 500 sq. m may have a significant role in cooling the air during a sunny day in summertime. It is equal to operation of a cooling system during 20 hours for ten rooms. As the existence of water resources in the region may moderate the temperature during a one day, it is able to diminish the temperature fluctuation as a small climate inside the building (Ghobadian, 2004) Water basin and the flowerbed are integral parts of the yards.

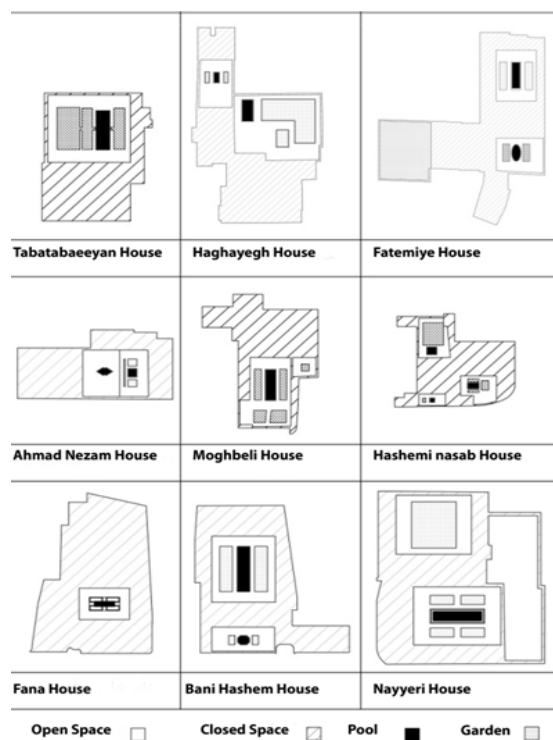


Figure 1. Location of garden and pool in samples houses

RESULTS

Given the points mentioned in this paper, it is time to develop the designing regulations and criteria for this city. To use the pattern of neighboring and local garden, a green space to be able to provide the desirable shadow with its mass trees will have the most effective role during hot hours a day. To create a green space to be able to provide the humidity and tranquility in its environment is one of the objects of such spaces (Consulting engineer, 2006). While absorbing the sun rays, the green surfaces prevents reflection the rays again and unwanted heating (Haghpanah, 2009). To plant the trees in the eastern and western sides to protect against sunlight (Ghobadian, 2004). Due to evaporation of water, the plants have a significant role in cooling the environment (Hamzehlou, 2006).

One of the characteristics of the plants is to direct and diminish the violence of the wind. By planting the tress, burning, cold and dusty winds are prevented. Furthermore, sound transmission is prevented, the air is filtrated, the temperature is moderated and the biological desirable conditions are met (Farokhyar, 2008).

- To cultivate the plants such as the bushes of tamarisk for stabilization of running sands and prevention of dust.
- To plant the trees with long roots for prevention of soil movement in desert lands with running sands.
- To avoid the blowing of the wind by planting trees. The trees shall play the role of windbreaker in wintertime (Saied sadr, 2001).
- To plant the long trees in lines by observing the distances by the vicinity of the building to direct the air flow (Farokhyar, 2008). In addition to increasing the relative humidity, the trees make the shadow during the summer (Hamzehlou, 2006). Specifically, by planting the autumn trees in the southern side of the building, we can use their shadows as well as sunlight during wintertime (Ghobadian, 2004).
- To cover the external margin of the building using the bushes and ever-green plants to diminish waste of energy.
- To provide a proper distance between the external walls and the bushes and the leaves and branches of adjacent trees to make possible the heat reflection from these walls (Consulting engineer, 2006).
- In addition to the cases as mentioned above, the plants can be used to communicate with architectural context as discussed below:
- To use the tree for a desirable landscape and use of the tree to prevent the view.
- To use green space with vegetation diversity compatible with the climatic conditions of the city may be employed as an architectural element for designing the urban spaces. These are classified into autumn and ever-green tress in terms of length and different types of them are used on the basis of the dimension and function of space. In this way, a combination of urban context with green space appropriate with its function is provided.
- Due to the dryness of the environment, we shall do our best to increase the air humidity. To do this, the following points shall be observed:
- Providing the water basin in the context may increase the humidity (hamzehlou, 2006).
- Sprinkling the yard and the plants in afternoon will evaporate the water and may be effective in cooling the weather and diminishing the air dryness.
- It is recommended that the waterscapes are placed in the places that their humidity is directed toward the building (Farokhyar, 2008).
- Due to rapid evaporation, the accumulation of water shall be avoided in the places exposed to the sun and or the water shall be directed inside the building and or a proper space shall be provided between the water and the building (Saied sadr, 2001).
- To cause the air to be flowed in the distance between the compressed limits of vegetation and building limits for taking advantage of its humidity.
- For taking advantage of the coolness and heat of the ground, the buildings shall to be constructed on the ground floor (Farokhyar, 2008).
- To make use of the current of cold air and take advantage of natural air conditioning, the following points shall be observed:
- By proper designing the area, scheme, construction form and ceiling and application the solutions such as ups and downs or land features and or walls or adjacent building as wind protector or wind direction, the desirable wind blowing in the region may be lead to the desired direction or the undesirable wind may be avoided (Yazdi, 2009).
- Using duct for air ventilation and air exit and providing free spaces to create and move the cold air (Ghobadian, 2004).
- Using the direction of wind blowing to make coolness and direction toward the building.
- To avoid the wind as tunnel- shaped forms.
- To take advantage of proper wind blowing and to place the water basin in its direction to reach a desirable breeze (Saied sadr, 2001).
- Openings shall be place in pressurized and suction place and the height of the bottom of window from the ground of the room shall be 0.5 to 1.5 m.
- To increase the height of a part of central space for chimney and to direct the warm air upward and outside the building.
- To use one-level buildings and low height in the fronts exposed to severe and harsh winds during wintertime.
- To provide external spaces for taking advantage of desirable breeze (Keshtkaran, 2009).

- To provide airproofs or doubled entrance or entrance yard and to heighten the entrance surface against external finished ground.
- To construct the building within the land on the foothills behind the wind and to cover the faces exposed to the wind using soil and to get light from internal yards.
- To use integrated and isolated doors in the facades exposed to the wind and to seal all doors, windows and openings.
- To minimize the number of entrance doors and to place the main entrance door in direction of windward and to fully protect the main entrance door by the tree (Watson, 2003).
- To predict the compact plans and to develop the settings with compact texture.
- To install air vent with automatic apertures and to install the aperture or cap on the chimney.
- To take advantage of air flow for air ventilation in the spaces (Moradi, 2005).
- To place the openings horizontally for controlling the wind blowing and current of cold air toward the inside of the space (Farokhyar, 2008).
- To provide mesh-like guards opposite the facades against the wind and to construct the thick walls for the buildings exposed to wind.
- To install the aperture or moving grid of thermal insulation at the back of the windows or to use internal staircases.
- To consider the direction of wintertime winds and to prevent constructing the single buildings in the field and unprotected areas.
- To provide steep roofs and to establish the steep slopes in wind direction (Consulting engineer, 2006).
- To erect the building in direction of radiation of minimum sun energy in warm conditions.
- To use the common walls in constructional complex and to form a compact texture.
- To provide a bounded parking in the western side of the building.
- To open the main spaces toward internal yards or open spaces located in the shadow.
- To provide the spaces under the roof to be ventilated and doubled roof or using thermal insulation on the roofs.
- To avoid providing the window for the roof, except for the summertime which in this case it shall be fully covered by the shadow.
- To avoid providing window for eastern and specifically for the western facades, otherwise to limit the number and area of such windows and to provide a vertical canopy for them.
- To take advantage of roof projection and roofed veranda or balcony to create the full shadow on the external surface of the glass-made window, detachable and walls exposed to the sun.
- To take advantage of the proper canopies (external canopy, as possible) for glassed surface and detachable and to provide the gap in connection point of the canopy above the window and related façade.
- To use wooden aperture or insulated moving grid at the back of the windows (Consulting Engineers; 2006).
- To use the soil as thermal insulation due to its trivial variation against temperature variations by constructing the building inside the land, to embank at the back of the walls and to cover the external margins of the building using bushes and ever-green flowers to create a desirable and ideal environment and condition when the natural temperature of the environment is not favorable (Varmaghani, 2009).
- To avoid constructing the building on negative gradients and indented parts.
- To consider the southern wall to provide the heating. This point forms the basis of those buildings referred to solar buildings (Farrokhhyar, 2008).
- To erect the interconnected buildings in the middle parts of southward slopes.
- To erect the building toward the direction of maximized sunlight under cold conditions.
- To provide heat generating spaces such as kitchen in the center of building plan and to provide insignificant spaces such as storeroom as thermal insulation in the walls or cold parts of the building (Rashidi, 2009).
- To develop underground construction with central yard in such a way that its roof is one meter lower than the ground level. In this way, light, sun's heat and fresh air will be transferred inside the building.
- To take advantage of stone-made foundation beneath the rooms to save the additional heat and to restore the saved heat in the night (Varmaghani, 2009).
- To use the proper thermal insulations in the external walls specifically in the roof.
- To avoid installing the large windows on the facades of the building.
- To take advantage of doubled-wall windows and even three-wall ones and to provide the thermal insulated sheets inside of the detachable parts.

- To take advantage of all types of curtains, moving insulated grid for preventing heat loss of the building through the detachable parts.
- To organize the plan in such a way that sunlight to be shined on the internal spaces.
- To leave southern frontier of the building at least by 30 degree from each side.
- To allocate the southward space for living and to allocate insignificant spaces in southeastern and northwestern parts of the building.
- The depth and the position of the window on the façade shall in such a way that enough sun shining inside the internal spaces is met.
- To Take advantage of general windows installed on the southern facades or the windows and ceiling skylight (to prevent heat loss through these windows, required measures are to be taken) (Consulting Engineers, 2006).
- To provide proper canopies for the windows assisting sun shining during winter and preventing sun shining during the summer.
- To provide the reflective surfaces in the grounds in the vicinity of the sunshade windows.

Conclusion

Zavareh is often sunny during the year and a significant amount of energy may be saved in the environment. Then, through making the scientific studied on using this energy, it can be utilized ideally. One of the common methods to use this energy is direct sun radiations together with direct radiation. In this method, the sun's light shines on the building and after passing through the window's glass, the internal spaces will become warm. For indirect sun radiations, these radiations will shine on the absorbent mass placed between the sun and inside the house. When this mass becomes warm, the absorbed energy will transfer to the rooms. A body or intermediate space such greenhouse, sunshade space provided under the roof and or thermal wall may play the role of this mass. The greenhouse is better to be used in the southern side of the building. To prevent heating the greenhouse, the construction material with high thermal capacity and dark- colored surfaces shall be applied.

Undoubtedly, the material used in the buildings shall be of heavy and compact material. The thermal insulation with bright colors and smooth surfaces shall be used on the roof surface and external walls exposed to the sun. To minimize the heat, it is recommended that proper materials to be used for the area of the building.

The old city has maintained its relation with the nature and a reasonable and logical relationship between the human and its surrounding environment is always seen thanks to the order and harmony. While architecture has

been forced to manipulate the nature to construct the building, it has not destroyed the nature and has been able to establish a good balance in this relation.

While absorbing the sun rays, the green surfaces prevents reflection the rays again and unwanted heating. To plant the trees in the eastern and western sides to protect against sunlight. Due to evaporation of water, the plants have a significant role in cooling the environment.

In each region, proper selection to erect the building is the first and the most important measures to be taken for designing appropriate with that region. In this case, we can take advantage of climatic factors such sunlight and wind during winter and summer seasons to protect the building against the sunlight and to decrease the heat loss. Furthermore, natural condition, need to private spaces, control and decrease sound are some other factors that may influence the erection process of the building.

To allocate the least side to southeastern and northwestern sides and to place the proximities in these sides and to avoid selecting the eastward or westward slopes for constructing the building.

Due to increasing the air and environment temperature in the afternoon, the eastward turning of the building will cause the western side to be exposed to sun shining very short.

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Demonstration of Size-Based Separation of Molecules by Gel Chromatography: An Exercise for Biology Beginners

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Abstract: An introductory laboratory exercise has been designed for biology beginners to visualize and analyze the chromatographic separation of a mixture of blue dextran, α -chymotrypsinogen (protein) and potassium ferricyanide on a Sephadex G-75 column (60×1.0 cm). Separation of the two coloured components *i.e.* blue dextran (blue colour), α -chymotrypsinogen (colourless) and potassium ferricyanide (yellow colour) of a green-coloured mixture can be visually seen in the form of blue- and yellow-coloured bands distant by a colourless zone. The elution volumes of different components in the mixture were found similar to the elution volumes of these components, when loaded individually onto the same column. Such demonstration of separation of different components in a mixture on a gel chromatographic column is an interesting exercise for biology beginners (undergraduate students) to learn separation technique on the basis of size.

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Keywords: Biology beginners; gel chromatography; laboratory exercise; molecular separation

1. Introduction

Protein separation techniques have been important since the 1940s in order to understand structure-function relationship of proteins. Now these techniques have become the integral part of biochemistry and biotechnology curricula as protein is the translational product in molecular biology experiments. Even biological researches have reached to an advanced level involving some of the biochemical techniques. Therefore, practical exercises on biochemical techniques have moved from specialized graduate courses to undergraduate biochemistry and a few biology programs for the entry level students. In order to move with the growing scientific pace, it is important to keep one abreast of these biochemical techniques. To include a laboratory separation technique in the undergraduate biology program, it is required that the laboratory exercise should be relatively inexpensive, require minimum instrumentation (equipment) and simple to handle.

Gel chromatography, also known as gel filtration, molecular sieve chromatography, gel permeation chromatography or size exclusion chromatography is one of several biochemical techniques used to isolate and purify a protein from a given mixture. The principle of separation is based on the difference in the size (molecular weight) of different biomolecules. The stationary phase used in gel chromatography is porous gel particles/beads which allow access to different sized molecules to different extents. Molecules, which are able to

completely access these gel particles are retained in the stationary phase and elute later from the chromatographic column. On the other hand, molecules bigger than the pore size of the gel particles do not enter the gel beads and elute earlier. Therefore, separation of molecules differing in size takes place on the chromatographic column in such a way that those bigger molecules elute faster followed by smaller molecules. In other words, the elution volume of a molecule on a gel chromatographic column is inversely proportional to the size of the molecule.

Although a number of exercises on gel chromatography are available in the literature (Wallach, 1982; Versee, 1985; Dixon, 1985; Malhotra and Kumar, 1989; Rowe, 1993; Davis and Brunauer, 2008), they might be either difficult to follow or practice independently by biology beginners. The following laboratory exercise may be a good alternative for inclusion in the undergraduate biology curriculum in order to give a proper understanding of the gel chromatographic technique to these students. The approach is intended to be general but emphasis has been given to visualize the separation of coloured molecules in the given mixture. The students can prepare their own gel chromatographic column, learn column packing and equilibration, load the sample, collect and monitor the fractions, plot the elution profile and analyze their results (active learning about separation of molecules on the basis of size). They can work in pairs but each of them should be able to perform the experiment at

least once. This exercise can be completed in three days with three hours laboratory sessions. The whole exercise is comprised of three parts: (i) packing and equilibration of the gel chromatographic column, (ii) sample loading and elution of different components of the given mixture and (iii) elution of individual components from the same column and analysis of results. Although this laboratory exercise is not new and has been used since the 1960s, use of coloured mixture and visual separation of two coloured and one colourless component in the mixture will surely add to students' learning about size-based molecular separation through this technique. For students without any biochemistry/ biotechnology background, it would be necessary to use a visual mode for demonstrating the principle of a biochemical technique to make it more understandable.

2. Materials and Methods

Chemicals

Sephadex G-75, α -chymotrypsinogen, blue dextran and potassium ferricyanide were procured from Sigma-Aldrich Inc., USA. All other chemicals used in this study were of analytical grade.

Absorbance measurements

The concentrations of blue dextran, protein and potassium ferricyanide were determined by measuring the absorbance at 540, 280 and 420 nm, respectively, using Thermospectronic Genesys 10 UV spectrophotometer.

Preparation of a gel chromatographic column

Sephadex G-75 powder form (5 g) was allowed to swell in 200 ml of water at 90°C for 3 h as recommended by the manufacturer. Fines were removed by repeated decantation before packing of the gel into the column. A glass burette was mounted onto a table in a vertical position with the help of an iron stand with two clamps. The radius (r) of the glass burette was determined at three different places along the height of the burette by collecting a known volume of water of 2 cm height (h) in the burette. The volume (V) of the collected water was taken as equal to the volume of a cylinder and the radius was obtained by substituting the values of V , π (3.14) and h (2 cm) into the formula, $V = \pi r^2 h$. The lower end of the burette received a disc of glass wool previously boiled in water and its surface was covered by a few glass beads. The burette was filled with buffer [0.02M sodium phosphate buffer, pH 7.0 containing 0.15M NaCl (PBS)] up to one fourth of its height and the gel slurry was poured slowly into the column with the help of a glass rod in a single operation. The gel was left for 1 h to settle under gravity and the outlet was opened slowly with a flow rate of 5 ml/h. The flow rate was increased gradually after the gel settled

down. Three bed volumes of the buffer (PBS) were passed through the column at a flow rate of 40 ml/h to equilibrate and stabilize the gel bed. The column is supposed to be stable if there is no change in the gel length during operation.

Sample application and elution

Before application of the sample, most of the buffer above the gel surface was removed and the outlet was closed. The sample containing mixture of blue dextran (3 mg), α -chymotrypsinogen (6 mg) and potassium ferricyanide (2 mg) or individual components in 1 ml of PBS were layered gently on top of the gel bed with the help of a micropipette and allowed to drain into the bed by slowly opening the outlet. Once the sample had passed into the gel, 1 ml of PBS was applied in the same way at least two times and finally connected to a reservoir containing PBS. The elution was performed with a constant flow rate (30 ml/h) and fractions of 2 ml size were collected in tubes. Absorbance was recorded at 540 nm for blue dextran (blue-coloured solution), 280 nm for protein (colourless solution) and 420 nm for potassium ferricyanide (yellow-coloured solution). Absorbance values were plotted against the elution volume to get the elution profile(s) of the given sample(s). The volume required to elute the component at its maximum elution (peak position) was taken as the elution volume of the component.

3. Results and Discussion

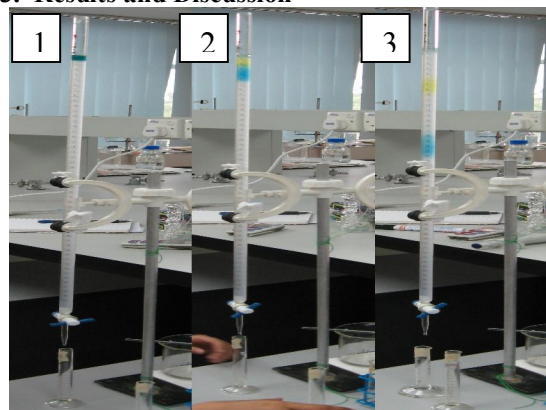


Figure 1. Separation of various components of a given coloured mixture on a Sephadex G-75 column (60 × 1.0 cm). The green-coloured mixture (3 mg blue dextran + 6 mg α -chymotrypsinogen + 2 mg potassium ferricyanide) in 1 ml of 0.02M sodium phosphate buffer, pH 7.0 containing 0.15M NaCl was applied onto the column and the elution was performed at a flow rate of 30 ml/h. Lane 1 shows photograph taken soon after loading the green-coloured sample. Lanes 2 and 3 show photographs taken 5 min and 20 min, respectively after application of the sample.

Figure 1 shows visual chromatographic separation of different components of the mixture (green in colour) containing blue dextran (blue in colour), α -chymotrypsinogen (colourless) and potassium ferricyanide (yellow in colour) on a Sephadex G-75 column. The first lane shows the sample (green-coloured mixture), when loaded onto the column. Lane 2 shows separation of different components of the mixture into distinct blue- and yellow-coloured bands. The blue-coloured band represented blue dextran while potassium ferricyanide band was of yellow colour. It is important to note that the colourless, α -chymotrypsinogen band was yet to be seen as separation was incomplete. Being bigger in size with very high molecular weight (2×10^6), blue dextran was completely excluded by Sephadex G-75 gel particles and moved faster through interstitial spaces available in the column. On the other hand, potassium ferricyanide, being a low molecular weight (329) compound had both the inner spaces and interstitial spaces of the gel column available to it and therefore, took longer time to pass through the gel column. Lane 3 shows a very clear visual demonstration of the separation of two coloured components of the mixture, as they were seen to be well separated and far apart from each other on the column. The middle colourless zone between the blue- and the yellow-coloured bands was the protein, α -chymotrypsinogen with a molecular weight of 25000. Elution of these components from the column in different fractions also reflected their separation from each other as fractions collected very early were of blue colour representing elution of blue dextran, followed by colourless fractions of the protein, α -chymotrypsinogen and finally yellow-coloured fractions of potassium ferricyanide. Therefore, visual monitoring of coloured bands on a column helps students to understand the principle of gel chromatography involving separation of the molecules based on their size.

Figure 2 shows elution profiles (absorbance versus elution volume) of three components of the mixture, when monitored at 540, 280 and 420 nm for blue-coloured, colourless and yellow-coloured fractions, respectively. Peak A represents elution profile of blue dextran, monitored at 540 nm with an elution volume of 22 ml. Elution volume of the blue dextran represented void volume (V_o) of the column, as it was completely excluded by all the gel particles, resulting its elution with the interstitial volume (void volume) of the column. The protein, α -chymotrypsinogen eluted right after the blue dextran peak with an elution volume of 30 ml, when monitored at 280 nm (Peak B). This is

understandable as its molecular weight (25, 000) lies between blue dextran and potassium ferricyanide. Therefore, some of the inner spaces and all interstitial spaces of the gel column would have been available to it. The last peak (Peak C) eluted from the column showed elution of potassium ferricyanide, when monitored for absorbance at 420 nm. Its elution volume (50 ml) was equal to the sum of the void volume (V_o) and the inner volume (V_i) of the column, as both the interstitial spaces and inner spaces of the gel column were available to it. Therefore, subtracting the void volume (22 ml) from the elution volume of potassium ferricyanide (50 ml) yielded the value of the inner volume (28 ml) of the column. Thus, all the three components of the mixture, differing in their molecular weights, were successfully separated by this column.

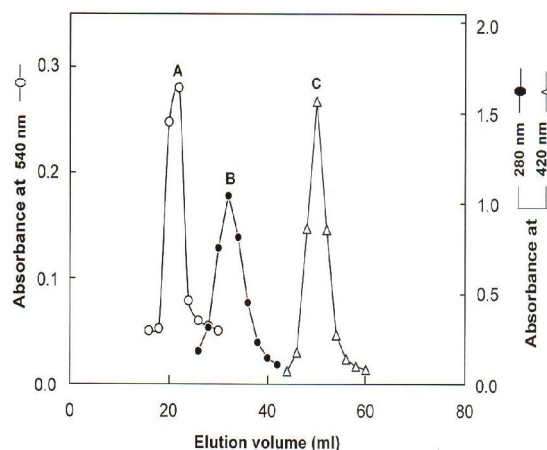


Figure 2. Elution profile of the mixture containing blue dextran, α -chymotrypsinogen and potassium ferricyanide on Sephadex G-75 column (60 \times 1.0 cm). The column was monitored for blue dextran (fraction number 8-15) at 540 nm (A), α -chymotrypsinogen (fraction number 13-21) at 280 nm (B) and potassium ferricyanide (fraction number 22-30) at 420 nm (C).

To further check the elution behaviour of these components present in the mixture on Sephadex G-75 column (60 \times 1.0 cm), these components were passed through the same column individually. The elution profiles of blue dextran, α -chymotrypsinogen and potassium ferricyanide are shown in Figure 3 A, B and C respectively. As can be seen from the figure, all these components eluted from the column in the form of a single symmetrical peak. Furthermore, their elution volumes (22 ml for blue dextran, 30 ml for α -chymotrypsinogen and 50 ml for potassium ferricyanide) were also found to be similar to those obtained from the mixture (Figure 2).

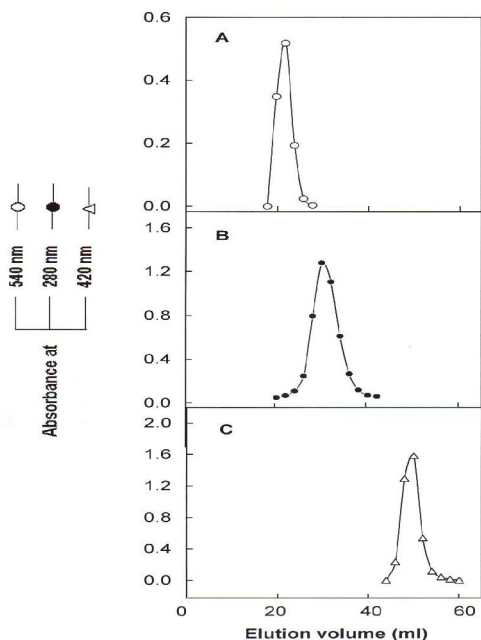


Figure 3. Elution profiles of blue dextran (A), α -chymotrypsinogen (B) and potassium ferricyanide (C) on Sephadex G-75 column (60 \times 1.0 cm). Sample size and other conditions were the same as described in 'Materials and Methods' section.

In order to evaluate the impact of this exercise in the context of active learning, a questionnaire (Table 1) stating several queries about the exercise can be made and distributed among biology students. It was done with our biochemistry II year students and found that >95% students answered all queries in affirmative. This was indicative of getting the clear understanding and verification of the theoretical principle of a biochemical technique through this exercise.

In conclusion, the exercise described here is a simple visual method for the introduction of gel chromatographic technique for biology students at undergraduate level. The technique is useful in being simple, economical, independent of the use of any advanced instrumentation and interesting to visualize the separation of molecules. Similar kind of exercises can be developed to teach other chromatographic techniques to these students using coloured mixtures.

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Table 1: Questionnaire about gel chromatographic technique

S. No.	Question
1.	Were you eager to see the inclusion of this separation technique in your practical curriculum?
2.	Were you excited to see the separation of different sized components on a column with your naked eyes?
3.	Did you find it easy to repeat the experiment yourself without the help of anybody?
4.	Did you find the exercise good enough to see the verification of theoretical knowledge about gel chromatography?
5.	Did you feel more educative about the principle of gel chromatographic technique compared to your theoretical knowledge?
6.	Can you teach your younger colleagues about gel chromatographic technique with greater confidence?
7.	Would you like to include similar type of exercises in your practical curriculum, if you are appointed as a teacher in any university?
8.	Do you think that this exercise has increased your interest and motivated you in this field of science?
9.	Out of several exercises in your practical curriculum, would you like to place this exercise in the preferential pool?

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Assessment of Households' Access to Electricity and Modern Cooking Fuels in Rural and Urban Nigeria: Insights from DHS Data

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Abstract: Nigerian domestic energy crises are significantly paradoxical given the high spectrum of energy resources that the country is naturally endowed with. This study analysed the factors influencing access to electricity and use of modern cooking fuel in Nigeria. The data were the 2008 Demographic and Health Survey (DHS) comprising 34070 respondents. The data were analysed with descriptive statistics and Seemingly Unrelated Bivariate Probit (SUBP) regression. The results show that 45.57 percent of all the households had access to electricity with 82.25 percent in urban and 28.72 percent in rural areas. Also, 0.82 percent and 0.13 percent of urban and rural respondents respectively primarily used electricity for cooking, while 44.82 percent and 9.87 used kerosene. However, 83.99 percent and 42.53 percent of urban and rural households respectively used wood for cooking. The results of the SUBP regression show that access to electricity and modern cooking energy sources significantly increased ($p < 0.01$) among urban dwellers, educated household heads but declined with resident in northern Nigeria. It was concluded that Nigerian government needs to properly design some institutional mechanisms and approaches for increasing access to modern energy to reduce indoor pollution and other associated health hazards.

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Keywords: modern energy, electricity, kerosene, fuel wood, Nigeria

Introduction

Historical review of electricity generation in Nigeria dates back to 1896 when electricity totaling about 60KW was produced in Lagos (Niger Power Review, 1985). Thereafter, the Public Works Department was commissioned by the government in 1946 to undertake responsibilities for electricity supply in Lagos State (Okoro and Chikuni, 2007). However, in 1950, Electricity Corporation of Nigeria (ECN) was established by law as a central body for distributing electricity in the country, although other bodies like Native Authorities and the Nigerian Electricity Supply Company (NESCO) obtained license to produce electricity in some other parts of the country. Simultaneously, another body that was called Niger Dams Authority (NDA) was also legislatively permitted to produce electricity which was sold to ECN (Manafa, 1995). However, in order to ensure efficiency through proper production coordination and distribution, ECN and NDA were in 1972 merged into the National Electric Power Authority (NEPA).

Over the past few decades, there had been some concerted efforts by the government to meet increasing electricity demand in the country as a result of rapid urbanization, industrialization and population increases. However, despite supposed huge budgetary allocations, majority of Nigerians still do not have access to electricity, and supply is very erratic for households with connectivity (Okoro and Madueme,

2004). No doubt, NEPA grew to become an household name due to intermittent power cuts and was angrily retagged "Never Expect Power Always" (Adenikinju, 2005). In 2000, efforts to privatize NEPA led to adoption of a holistic power sector restructuring reform which transformed it into seven companies that were meant to generate power (GenCos), one for transmission (TransysCo), and eleven for distribution (DisCos) (Oyeneye, 2004). These arrangements, which came into effect in January 2004 were finalized by changing the name of NEPA to Power Holding Company of Nigeria (PHCN). The reforms were also meant to dissolve the monopolistic power of NEPA by attracting some independent power producers (IPPs).

However, despite a change of name, there have not been any significant improvements in service delivery by PHCN. Therefore, with hope of regular access to electricity of several Nigerians dashed, PHCN had been retagged as "Problem Has Changed Name". Therefore, it will be an understatement to assert that energy problem in Nigeria had over the past few decades grown from bad to worse. The crises, like cancerous cells had rapidly spread in magnitude of unimaginable dimensions to all sectors of the economy. No doubt, an important premise for desiring regular supply of clean energy is its direct linkage with households' welfare. This had been widely brought to fore by multiple indicators of welfare, being synchronized into the framework for understanding the

multidimensional nature of poverty. Moreover, desirability of clean energy is justified because it minimizes domestic air pollution that often constitutes some adverse health effects (Emmanuel and Samuel, 2012; Adenikinju, 2005)

The dimension of energy poverty in Nigeria is not warranted given enormous energy resources the country is naturally endowed with. Impact of erratic access to clean energy is largely aggravated by growing household poverty despite recent economic reforms and widely applauded growths. Specifically, recent evidences suggest that relative poverty increased from 54.4 percent in 2004 to 69.0 percent in 2010 (National Bureau of Statistics, 2010). No doubt, growing dimension of poverty severity had over the years transmitted into energy poverty. This resulted from households' adjustments of expenditure patterns in a manner that ensures preference for basic needs. It is also surprising that huge budgetary allocations to address the country's growing energy crises by previous governments were mere pretext to embezzle and mismanage public funds.

Over the years, government's abject failure to address dilapidating state of old power generating infrastructure, perfected corrupt practices among government workers, targeted destruction and theft of key transformers have been responsible for the country's wailings over the energy woes. Shaad and Wilson (2009) noted that given Nigeria's enormous energy resources (oil and gas reserves, abundant sunlight and significant hydropower potential), inadequate access to energy should not be witnessed

It should be further emphasized that there is wide gap between access by urban and rural households to clean energy supplies. About 73% of Nigerian population lack access to electricity although this may increase to about 90 percent for rural areas if properly disaggregated. Poor rural electricity supply attests to the window dressing nature of many rural electrification projects and lack of strong political will to offer permanent solution to the problem. It should be noted that energy needs for cooking represent the bulk of energy demand in Nigeria, although about 67 percent of the population uses dirty energy sources in form of fuel wood or charcoal. This should raise a lot of environmental concerns because of its inefficiency contributions to indoor air pollution. Similarly, households also use kerosene for cooking although sometimes adulterated with petrol or diesel and expensive (Shaad and Wilson, 2009). To make up for electricity supply shortages, markets for petrol and diesel generators are flourishing although this sometimes makes up at 400 percent of grip price (Osunsanya, 2008; Shaad and Wilson, 2009).

Unfortunately, however, economic development is directly linked to access to clean

energy (Dorf, 1978; Adegbulugbe, 2006). Given the tragic situations that many Nigerian households have found themselves in relation to access to clean energy, it is unlikely that efforts by the government to achieve the Millennium Development Goals (MDGs) can yield any positive outcome. This is because of the consensus among energy policy experts that achievement of most of these goals is diametrically linked to access to clean energy. Specifically, access to electricity is essential for efficient service delivery in health, education and sanitation sectors, and for ensuring reduction in indoor pollution (Shaad and Wilson, 2009).

This study can be motivated from the fact that understanding the factors influencing choice of energy at the household level is important for policy formulation. Specifically, the pattern of energy utilization is potentially able to enhance our understanding of the nature of environmental pollution resulting from domestic cooking and lighting activities. Similarly, ability to determine the socio-economic characteristics of households that engage in usage of one form of energy can inform policy through assessments of demographic dynamics within the society and provision of adequate incentives for rapid economic development.

There is a strong correlation between access to electricity and socio-economic development of a country. Some empirical studies on domestic energy demand had also focused on sources of energy and factors responsible for choices made by the households. Some authors such as Onyekuru and Eboh (2011) and Shittu *et al.* (2004) have found positive relationship between income and improved energy demand in some studies on Nigeria. Shittu *et al.* (2004) also found household heads' age as an important factor that influenced demand for biomass fuel in Ogun state. Babanyara and Saleh (2010) found that fuel wood rural-urban migration, poverty and hikes in price of kerosene were critical factors influencing demand for fuel wood in urban Nigeria. This study seeks to determine the factors explaining access to electricity and improved cooking fuel in Nigeria using the Demographic and Health Survey data of 2008. In the remaining parts of the paper, materials and methods, results and discussions and conclusions have been presented.

Materials and Methods

Sources of data

The study used the Demographic and Health Survey (DHS) data that were collected in 2008. In the sample selection process, the 2006 Population and Housing Census sampling frame was used. In this sampling frame, the primary sampling unit (PSU) that was referred to as a cluster for the 2008 NDHS was defined on the basis of Enumeration Areas (EA) from

the 2006 EA census frame. Samples were selected using stratified two-stage cluster design consisting of 888 clusters with 286 in urban areas and 602 in rural areas. A representative sample of 36,298 households was selected, with a minimum target of 950 completed respondents per state. In each state, the number of households was distributed proportionately among its urban and rural areas. However, only 34070 households fully completed the survey thereby giving 98.3 percent response rate.

Estimated model

Different alternative methods exist for analyzing the data given that the dependent variables are bivariate (1 if using improved cooking energy sources and 0 otherwise or 1 if having access to electricity and 0 otherwise). It is possible to consider Probit or Logit method but due to endogeneity nature of cooking fuel variable in explaining access to electricity, our estimated parameters would be inefficient. Therefore, Seemingly Unrelated Bivariate Probit (SUBP) is the best approach for modeling the data in such a way that parameter efficiency can be ensured. Therefore, estimation of the equations simultaneously is required as discussed by Maddala

(1983). The structural recursive form of the model can be stated as:

$$Q_{i1} = \gamma + Q_{i2} + \delta_i \sum_{i=1}^n X_i + z_i \quad \text{i.}$$

$$Q_{i2} = \alpha + \beta_i \sum_{i=1}^n X_i + v_i \quad \text{ii.}$$

Q_{i1} and Q_{i2} are latent bivariate variables of using improved cooking fuel and having access to electricity, respectively. Also, $\alpha, \beta, \gamma, \delta$ are the estimated parameters and X_i are the socio-economic variables of the households. Included explanatory variables are Ownership of generating set (yes = 1, 0 otherwise), household size, urban residence, north zones, sex, age, years of education. The error terms of the model are dependent and distributed as a bivariate normal such that: $E(v_i) = E(z_i) = 0$, $var(v_i) = var(z_i) = 1$ and $\rho = cov(v_i, z_i)$. The Wald test, which is reflected by statistical significance of ρ was used to determine whether the models would be best estimated jointly in a recursive manner or not.

Results and Discussions

Households' access to electricity and choice of primary cooking energy

Table 1: Access to Electricity in Urban and Rural Nigeria across Different Types of Cooking Energy Choices

Energy category	Urban			Rural			All		
	No	Yes	Total	No	Yes	Total	No	Yes	Total
Electricity	0	88	88	1	29	30	1	117	118
LPG	2	85	87	6	21	27	8	106	114
Natural gas	6	177	183	0	24	24	6	201	207
Biogas	4	36	40	4	16	20	8	52	60
Kerosene	328	4,479	4,807	664	1,640	2,304	992	6119	7111
Coal, lignite	4	70	74	16	26	42	20	96	116
Charcoal	50	438	488	163	218	381	213	656	869
Wood	1,418	3,143	4,561	15,058	4,550	19,608	16476	7693	24169
Straw / shrubs / grass	28	66	94	199	30	229	227	96	323
Agricultural crop	2	1	3	26	9	35	28	10	38
Animal dung	0	0	0	2	5	7	2	5	7
No food cooked in HH	52	228	280	470	136	606	522	364	886
Other	10	9	19	31	2	33	41	11	52
Total	1,904	8,820	10,724	16,632	6,706	23,346	18536	15526	34070

Table 1 shows the distribution of households based on access to electricity and the choice of primary cooking fuel. The sources of energy that were indicated by the households can be broadly classified into traditional (wood, charcoal, coal, straw, agricultural crop, animal dung and others like plastics) and modern (electricity, kerosene, gas including LPG, Natural gas and biogas) (Hemlata, 1990; Olatinwo and Adewumi, 2012). In the combined data, 45.57 percent of the households had access to electricity. However, 82.25 percent of urban households had access to electricity, while only 28.72 percent had access in rural areas. More specifically, about 0.75

percent of the households that are with access to electricity in the combined data primarily used electricity for cooking. Also, 0.1 percent of the households that had access to electricity in urban area were using electricity as the primary cooking energy. In rural areas, 0.43 percent of the households with access to electricity were using electricity as the primary cooking energy. However, of the urban households that had access to electricity, 50.78 percent and 35.63 percent were primarily using kerosene and wood as sources of cooking fuel respectively. Low usage of electricity as cooking fuel can be traced to erratic supply in both urban and rural areas.

Table 2: Distribution of households' cooking energy types in urban and rural Nigeria

Energy Group	Urban		Rural		All	
	Freq	%	Freq	%	Freq	%
Electricity*	88	0.82	30	0.13	118	0.35
LPG*	87	0.81	27	0.12	114	0.33
Natural gas*	183	1.71	24	0.10	207	0.61
Biogas*	40	0.37	20	0.09	60	0.18
Kerosene*	4,807	44.82	2,304	9.87	7,111	20.87
Coal, lignite	74	0.69	42	0.18	116	0.34
Charcoal	488	4.55	381	1.63	869	2.55
Wood	4,561	42.53	19,608	83.99	24,169	70.94
Straw / shrubs / grass	94	0.88	229	0.98	323	0.95
Agricultural crop	3	0.03	35	0.15	38	0.11
Animal dung	0	0.00	7	0.03	7	0.02
No food cooked in household	280	2.61	606	2.60	886	2.60
Other	19	0.18	33	0.14	52	0.15
Total	10,724	100.00	23,346	100.00	34,070	100.00

Table 2 shows the frequency and percentage distributions of urban and rural households across the different energy choices. Based on internationally accepted definition, electricity, LPG, natural gas, biogas and kerosene are the energy sources that can be classified as improved. These sources are characterized by high efficiency, low environmental pollution and reduced health hazards. It reveals that only 0.82 percent and 0.13 percent of urban and rural respondents respectively primarily used electricity for cooking. Non-usage of electricity for cooking by many households can be linked to complete lack of access to electricity and erratic supply to households that have connections (Adenikinju, 2005). Despite the huge capital that Nigerian government annually spend on power projects, there have not been any results to show for it. Also, wide gap between installation capacity and power needs of growing populations have resulted in load shedding. Non-responsiveness of PHCN officials often result in long delay in repair of faulty transformers and other problems. This may make an area to be deprived of access to electricity for very long period of time. In some instances, PHCN does not base electricity billing on amount used, but on expected income from an area. This often makes monthly charges not to reflect usage because of the monopolistic and oppressive role played by some PHCN officials (Okoro and Madueme, 2004; Iwayemi, 2008; Emmanuel and Samuel, 2012).

Similarly, liquefied gas was primarily used for cooking by 0.81 percent and 0.12 percent of urban and rural households respectively. Although Nigeria is endowed with a lot of gas reserves (Cole, 2004), domestic consumption is limited due to high price. It is often surprising that while Nigerian gases are being flared in the Niger Delta, supply for domestic usage is often erratic and price still high. Also, poverty

makes many households unable to invest in the gas cylinders and some have the impression that it is more expensive to use gas for cooking than using kerosene. Also, some households consider use of gas for cooking to be very risky due to higher tendency of fire accidents if mishandled. Shaad and Wilson (2009) submitted that if well managed, domestic shortages in energy demand in Nigeria can be minimized by using associated gas to meet local energy needs. This was also seen as a way to respond to new national legislation and international demands to halt gas flaring.

It should be noted however that while 44.82 percent of urban respondents primarily used kerosene for cooking, only 9.87 percent of the respondents from rural areas used it. In the combined data, 20.87 percent of the respondents were using kerosene for cooking. Shaad and Wilson (2009) submitted that when used for cooking, kerosene also releases some hazardous pollutants to the atmosphere and it is very expensive. It was noted that an average African household may spend between 10 - 15 percent of annual incomes on kerosene. In Nigeria, there have been several times with severe kerosene scarcity. During those times, kerosene was sold at prices that were far above the prices of other petroleum products and were mainly available in "black markets". Some greedy sellers were also in the practice of adulterating the product with petrol or diesel, leading to explosions that had destroyed several properties, claimed several lives and left many Nigerians permanently disabled.

Wood was primarily used for cooking by 83.99 percent of rural respondents, whereas 42.53 percent of urban respondents were using wood. In the combined data, 70.94 percent of the respondents were using wood for cooking. Use of wood for cooking has been largely traced to availability and low cost.

This energy source is responsible for significant indoor air pollution with significant health hazards. Many rural households spend quite a lot of time gathering fuel wood from the forest. This has some implications for deforestation. In some instances,

some households are addicted to using fuel wood to cook claiming that foods cooked therewith usually have better taste (Shaad and Wilson, 2009).

Factors explaining access to electricity and choice of cooking energy sources

Table 3: SUBP Results of the factors influencing access to electricity and choice of improved cooking energy

Variables	Parameter	Standard error	t-value	Parameter	Standard error	t-value
Cooking fuel	.6436176	.0695696	9.25	-	-	-
Generating set	-	-	-	.7318458	.0236063	31.00
Household size	.0154464	.0028229	5.47	-.1209401	.0041436	-29.19
Urban/rural	1.278897	.0237738	53.79	1.16109	.0190489	60.95
North zones	-.43726	.0217784	-20.08	-.8903611	.0212476	-41.90
Sex	-.0949333	.0214218	-4.43	-.0283239	.0239214	-1.18
Age	.0019078	.0005722	3.33	-.0130978	.0006641	-19.72
Year of education	.0765992	.0034589	22.15	.0913422	.004196	21.77
Constant	-.6869613	.0437225	-29.92	-.2834713	.0413051	-6.86
athrho	.1789575	.0396729	4.51			
rho	.1770712	.038429				

N = 34070
 Log likelihood = -28276.142
 Wald Chi Square = 16969.40***
 Likelihood ratio test Chi Square = 21.454***

Table 3 presents the results of SUBP regression. It is important to first discuss the significance of some diagnostic statistics. In the results as presented by STATA software, the parameter of rho seeks to confirm if the models are justified to be estimated simultaneously. This parameter is statistically significant as revealed by the computed Chi-Square value of 21.454 ($p < 0.01$). This confirms the endogeneity characteristic of the choice of improved cooking fuel variable. Similarly, the Wald Chi Square statistics is statistically significant ($p < 0.01$) and implies that the model produced a good fit for the data.

The parameter of improved cooking fuel is with positive sign and statistically significant ($p < 0.01$). This implies that those households that were using improved energy have higher probability of having access to electricity. This is expected because although not many households were using electricity as the primary energy source due to several reasons of which supply irregularity is paramount, use of improved energy sources is expected to be directly linked with high income status which automatically implies access to electricity.

The households that owned generating set have significantly higher probability of using improved cooking energy ($p < 0.01$). This is expected because those households that are able to afford the running and maintenance costs of generator should be able to afford improved cooking energy sources. The parameters of household size in the two models

imply that as household size increases, probabilities of having access to electricity and using improved cooking energy sources significantly increases and decreases ($p < 0.01$). Specifically, for the cooking fuel result, if the number of people within an household increases, their energy needs for cooking increases. Therefore, they may not be able to use stove or electricity to cook due to large volume of food that is involved. In rural areas, the cooking pots may be so bog such that it cannot be supported by a kerosene or coal stove. In this instance, use of fuel wood is inevitable.

The results also show that urban residents have significantly higher probabilities of having access to electricity and using improved cooking energy sources ($p < 0.01$). These results are expected because successive Nigerian governments have concentrated electricity supply efforts in the urban areas. Also, because poverty is concentrated in rural areas, this is also manifesting in energy poverty because the people are not able to afford use of improved energy sources. Specifically, majority of rural households convert their production time for fuel wood gathering. Furthermore, households in northern parts of the country also have significantly lower probabilities of having access to electricity and using improved cooking energy sources. These results are expected because poverty is concentrated in northern Nigeria. When the households are struggling to meet basic need of food, demand for improved energy sources will never be a priority.

Also, the parameter of gender in the electricity model is statistically significant ($p < 0.01$). This implies that households with male heads have significantly lower probability of having access to electricity. In the model for use of improved cooking energy, the parameter also has negative sign but statistically insignificant ($p > 0.10$). The results also show that as household head age increases, the probability of access to electricity increases significantly ($p < 0.01$). However, the probability of using improved cooking energy sources significantly decreased as age increased ($p < 0.10$). This can be explained from the fact that aged household heads may be inactive in the employment markets and thereby unable to afford the price of improved cooking energy. Also, they are likely to have large family size, requiring more cooking energy due to the large volume of food to be cooked at once. However, this finding is contrary to that of Olatinwo and Adewumi (2012) for a study on some rural households in Kwara state.

Also, the parameters of years of education in the two models are with positive sign and statistically significant ($p < 0.01$). This implies that as years of education increases, the probabilities of having access to electricity and using improved cooking energy sources increased. This is expected because education is expected to both impact access to electricity and use of improved cooking energy positively due to tendency of the educated to have high income, live in urban areas and live in houses where facilities for cooking with fuel wood are not easily provided.

Conclusion

Nigerian domestic energy crises are significantly paradoxical given the high spectrum of energy resources that the country is naturally endowed with. This study has shown that many households were not having access to modern energy sources and rural people were more deprived. This implies that reducing indoor pollution and exposure to cooking smoke as prerequisites for reducing some health hazards is guaranteed. The drive towards ensuring better access to cleaner and more efficient energy sources which is a global initiative for economic growth and development in Nigeria will therefore meet with serious setbacks. Nigerian government needs to properly design some institutional mechanisms and approaches for moving towards this goal. Such effort should also consider regional disparities in access to modern energy sources and ensure that each geopolitical zone addresses its energy needs from available resources without necessarily centralizing energy development activities and policies. Also, because biomass

constitutes the highest usage among households, government should design adequate programmes to ensure forest replanting across the country to averse the consequences of progressive deforestation. Development of more efficient biomass cooking stoves is important because it can save the volume of wood used for cooking and reduce the level of air pollution. The Nigerian government should also show more commitments towards solar energy utilization for domestic activities. This is going to reduce reliance of the people on other energy sources.

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TQM and Organization Performance: The Mediation and Moderation Fit

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Abstract: Total Quality Management (TQM) is a unified organizational setting to improve the quality at every function and level of organization. The objective of this study is to measure the effect of TQM practices on the performance of the telecom sector of Pakistan. Telecom sector is continuously striving to improve the quality of its services to achieve business objectives. A conceptual framework model to investigate the said relationship is developed and tested. The results are based on a survey instrument developed through an extensive literature review. To analyze the complex relationship between the variables, Structural Equation Modeling (SEM) methodology was employed. The data collected from 212 respondents was used to test the model by using AMOS 16. Analysis of the data supports a strong and positive association between the TQM practices and quality performance, innovation performance and organization performance (OP) respectively. This study found that innovation performance has partial mediating impact between TQM and OP, whereas, QP mediation impact was not established. Moreover, culture of support has a moderating role in the relationship between TQM practices and the OP.

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Key Words: Total Quality Management (TQM), Quality Performance (QP), Innovation Performance (IP), Organization Performance (OP), Culture of Support (CS).

1. Introduction

With the increasing trend of globalization and quality management/improvement practices, TQM has become a global phenomenon. Its emergence is one of the core developments in the field of operations management sciences and it has been widely adopted worldwide. Japanese companies are labeled as pioneers in TQM enactment, whereas Asia-pacific, European and American companies are known as followers. Particularly in the last two decades, TQM has received a great attention worldwide (Jung & Wang, 2006). Since the TQM philosophy is more frequently practiced in the manufacturing industry (Cassidy, 1996; Joiner, 2007; Prajogo & Sohal, 2003), and a little attention has been paid on the implementation of TQM and consequently its impact on the OP, particularly for the service industry (Breiter & Bloomquist, 1998; González, González, & Ríos, 1997; Lemak & Reed, 2000; Lindahl et al., 1995; Prajogo, 2005; Prendergast, Saleh, Lynch, & Murphy, 2001). This study focus to find out the relationship of TQM practices and the OP of the telecom firms of Pakistan. Telecom firms including the Cellular Mobile Operators (CMOs) are continuously putting their efforts to improve service quality through adoption of Quality Management Systems (QMS) like TQM and ISO standards.

2. Literature Review

TQM tools and procedures may vary but the fundamental philosophy and concepts are equally

germane to industries from manufacturing as well as service (Huq & Stolen, 1998). Similarly Prajogo (2005) also confirmed that TQM practices and QP are invariant between manufacturing and service industry, which infers that TQM is universal improvement initiative program and can be implemented in service industry. Further TQM and business performance were found positively correlated. (Salaheldin, 2009; Terziovski & Samson, 1999). There are several TQM practices and variables that have been underlined in the literature that can influence the OP. For instance, commitment of the management and leadership, focus on the customer, supplier relationship, design of quality, employee empowerment, benchmarking, statistical process control, employee involvement, empowerment and training (Ahire, Waller, & Golhar, 1996; Dale & Cooper, 1994). Karuppusami and Gandhinathan (2006) by using Pareto analysis technique on the literature review of Critical Success Factors (CSFs) on the implementation of TQM for the period 1989 to 2003 listed and arranged management leadership, process management, supplier management, service design, customer focus, employee relation, training, and quality of the data as the top eight CSFs of TQM.

Huang and Chen (2002) through a survey of Taiwan's firms revealed that TQM positively influence cost containment and performance. On the other hand, Terziovski and Samson (1998) found that the

integrated strategic quality orientation involving TQM and ISO 9000 quality standards are the most effective competitive strategy for sustainable performance. Similarly, Salaheldin (2009) revealed that operational as well as on the OP are significantly correlated with TQM. Moreover, Deming (1986), Joiner (2007) and Powell (1995) also confirmed the same results. On the other hand, there are also some findings about the weak rather irrelevant and negative relationship among TQM and performance (Powell, 1995; Yeung & Chan, 1998).

Some researchers, Demirbag, Tatoglu, tekinkus, and Zaim (2006) and Salaheldin (2009) have tested the impact of TQM separately on the financial and non-financial performance of organizations. Demirbag et al. (2006) found that the TQM practices indirectly effects financial performance. On the other hand Salaheldin (2009) using SEM illustrated that the CSFs of TQM (Strategic, Tactical and Operational) have a positive impact on financial as well as on the non-financial performance of Qatar based Small & Medium Enterprises (SMEs).

TQM facets can also be categorized into soft and hard TQM elements (Rahman & Bullock, 2005). The soft TQM elements include leadership, employee relation, employee involvement, focus on customer, strategic quality planning, process management, continual improvement, data and information analysis and knowledge and education. On the other hand, the hard elements include elements like quality tools and techniques, customer/supplier relation and product/process relations (Fotopoulos & Psomas, 2009; Jung & Wang, 2006).

Fotopoulos and Psomas (2009) found that quality improvement is primarily based on soft TQM elements and subsequently by the hard TQM elements. Further, in their research on the relationship of TQM factors and OP, they revealed that TQM practices like, top management role, employee participation, customer focus, quality management tools and techniques have a significant impact on the companies' performance (Fotopoulos & Psomas, 2010).

Leadership being a TQM element includes providing the vision and direction to the employees, improving the ability of information sharing and improving communication process, enhancing synergies value addition and bringing enlightenment (Zairi, 1994). Similarly, the senior management must understand the purpose and principles of TQM and should also consider the internal strategic management processes, training and development, participation of their staff, and their own role in implementing the TQM approaches in managing the OP (Taylor & Wright, 2003). Taking into account leadership as a soft TQM element, Zehir et al. (2012) in their research on management leadership provided that leadership is positively and significantly related to organizational

outcomes like innovativeness, quality performance and operational performance.

TQM focus on satisfying the customer needs. Goh and Ridgway (1994) argued that that to remain competitive organizations must satisfy their customer needs at reasonable cost. Sila and Ebrahimpour (2005) concluded that TQM impact business performance entailing customer focused results. Similarly Agus and Hassan (2011) revealed that TQM has a significant relationship with customer-related performance. Lorente, Dewhurst, and Dale (1999) found that TQM dimensions like customer focus, training, teamwork and empowerment can influence in bringing more innovativeness in business activities of organizations. Likewise, Prajogo and Sohal (2003) concluded that IP is significantly associated with TQM practices in non-manufacturing and manufacturing organizations Australia. More recently, Hung et al. (2011) in their research on high-tech industry of Taiwanese companies noted that TQM positively impact IP. However, the said relationship is mediated by organizational learning. When considering the mediation effect, Kim et al. (2012) suggested that quality management practices, being mediated by the process management, have a positive linkage with innovation.

Su, Li, Zhang, Liu, and Dang (2008) delineated that the relationship between quality management practices like TQM and OP is indirect; mediated through variables like QP and Research and Development (R&D) performance. In regards to the direct effect of TQM practices on quality Performance, Zehir et al. (2012) suggested that TQM is a quality oriented approach which has a direct effect on the quality performance of manufacturing, IT and service sector companies. Sharma and Gadeene (2001) argued that TQM is a holistic management philosophy and to have the full potential of the TQM on OP a holistic approach of TQM should be applied rather than on piecemeal basis. The importance of development of work environment and TQM driven cultural change is highlighted in the literature to enhance the performance outcomes of TQM implementation (Joiner, 2007; Montes, Jover, & Fernandez, 2003; Rad, 2008). High quality culture itself is considered as a significant TQM practice (Kaluarachchi, 2010). Likewise, the national cultural values have a significant influence on the organization's quality culture (Noronha, 2002). The sustainability of TQM can also result in a failure if human element of change in quality culture are ignored (Edwards & Sohal, 2003).

The extant literature is not fully matured and has research gap in the relationship of TQM practice and OPs in the service sector, especially telecom sector. This study is conducted to fill this knowledge gap. A theoretical model is developed to assess the

relationship among the TQM, QP, IP and OP for Pakistan's telecommunication sector.

2.1 Research Model

The theoretical model has been adapted/refined from the work of (Joiner, 2007; Prajogo & Sohal, 2003; Salaheldin, 2009; Su et al., 2008). Literature review on quality management implies that most of the TQM factors and the variables on which they impact involve more than one dimension and indicator; this suggests for the use of a latent variable model. A total of five latent variables are measured in the model on the basis of extensive support from the literature. This includes TQM, QP, IP, Culture of Support and OP. The variables are enlisted in Table 1 along with respective indicators.

Table 1: Research variables of the model along with their indicators.

Latent Variables	Indicators
Total Quality Management (TQM)	Employee Relations (ER), Leadership (LS), Customer Relations (CR), Product/Process Management (PPM)
Culture of Support (CS)	Co-worker Support (CS), Organizational Support (OS), National Culture Support (NCS)
Quality Performance (QP)	Service quality (SQ), Service Design (SD), Perceived Quality (PQ), Serviceability (SER)
Innovation Performance (IP)	Product Innovation (Pdl), Process Innovation (Pri), Innovation and Continuous Improvement (ICI)
Organizational Performance (OP)	Human Resources Results (HRR), Financial Performance (FM), Non-Financial Performance (NFM),

Figure 1 represents the research model and the hypotheses. The one-headed arrows therein show the hypothesized impact of one variable on another.

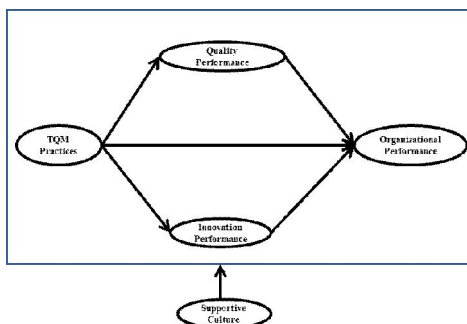


Figure 1: Research Model

2.2 Research Hypotheses

Reviewing the literature, it's quite evident that manufacturing and service industries business performances are impacted by TQM. TQM

significantly impacts the business performance of both. For instance, Powell (1995); Terziovski and Samson (1999) and Salaheldin (2009) revealed that the implementation of TQM has a significant positive impact on the OP (both financial and non-financial). Hence, the first hypothesis developed is;

H₁. TQM practices leads to a better OP.

Referring to the TQM literatures, studies have found that TQM has a positive and significant relationship with QP (Arumugam, Ooi, & Fong, 2008; Fotopoulos & Psomas, 2010). Likewise, Innovation in the business activities of an organization is positively and significantly influenced by TQM practices (Lorente et al., 1999; Pinho, 2008). Based on foregoing the second and third hypotheses are;

H₂. TQM practices leads to a better QP

H₃. TQM practices leads to a better IP.

Innovation relation with OP has been confirmed by (Huang & Liu, 2005; Lin & Chen, 2007; Pinho, 2008). Likewise, quality improvement has a positive impact on OP (Agus, 2005; Fotopoulos & Psomas, 2010). Su et al. (2008) found that the relationship of TQM practices and OP is indirect; mediated through variables like, QP and IP. The said relationships are investigated by testing the fourth, fifth, sixth and seventh hypothesis a;

H₄. QP leads to a better OP.

H₅. IP leads to a better OP.

H₆. QP mediates the relationship between TQM practices and OP.

H₇. IP mediates the relationship between TQM practices and OP.

The element of culture cannot be ignored while gauging the impact of TQM on OP. The culture of support moderates the relationship between TQM and OP (Joiner, 2007). This is tested in the last hypothesis:

H₈. CS moderates the relationship between TQM and OP.

3. Study Design and Methodology

3.1 Measurement Instrument

The instrument has been developed through literature review. Most of the items were adopted from different studies, such as (Curkovic, Vickery, & Droge, 2000; Demirbag et al., 2006; Joiner, 2007; NIST, 2002; Noronha, 2002; Prajogo & Sohal, 2003; Sila & Ebrahimpour, 2005) and augmented by the broad quality management literature. The content validity was established through interviews with the senior managers of quality assurance department (QAD) and project managers. The instrument was edited, items were added and deleted from the questionnaire. The questionnaire was then reviewed by the three academic scholars for comprehensibility and accuracy. To measure the items other than performance a 1-7 Likert scale was used (where 7 = strongly agree, 4 = about the

same and 1 = strongly disagree) and for performance items a 1-7 items scale was used (where 7 = above average, 4 = about the same and 1 = below average). A 7-point scale as compared to 5-point was used to achieve better consistency (Inman et al., 2011, p. 347). (See Appendix 1 for the details of all the items of the instrument and their corresponding literature). After the necessary amendments the questionnaire so formed was subjected for pilot testing.

Initially the reliability was checked from the data collected from a sample of 15 respondents (Three samples from each of the major telecom firm i.e. Telenor, Zong Ufone, Warid and Mobilink). Cronbach's alpha was calculated to analyze the reliability of the constructs. Alpha values from 0.70 or more are considered as good indicators of the reliability. The Cronbach's alpha values for all the constructs were from 0.76 to 0.94, therefore suggesting good reliability. A total of seventy nine items were the part of the questionnaire.

3.2 Sample

Pakistan telecom sector is the most growing service sector in the country having more than 10 billion US\$ Foreign Development Investment (FDI) and generating revenue at an average of more than 300 billion rupees annually. Moreover, this sector is benefiting Government by contributing in GDP and society by providing employment and reliable communication services. Telecom industry of Pakistan and the associated suppliers were selected for this study.

Table 2: Respondent's Descriptive Statistics.

Category	Frequency	Percent
Gender		
Male	167	78.8
Female	45	21.2
Age		
20-30	127	59.9
31-40	57	26.9
41-50	21	9.9
51-60	5	2.4
Above 60	2	0.9
Department		
IT/Software Development	18	8.5
Marketing/Sales & Distribution/ Customer Services	32	15.1
Administration/HR/PM	38	17.9
Technical/Quality Assurance	64	30.2
Finance	48	22.6
Others	12	5.7
Experience		
0 - 5	108	50.9
6 - 10	92	43.4
above 10	12	5.7
Job Title		
Top	34	16
Middle	98	46.2
Lower	80	37.7
Employment status		
Permanent	142	67
Contract	70	33

Five CMOs, i.e. Telenor, Zong Ufone, Warid and Mobilink, and 22 suppliers were randomly selected for

the collection of data. The questionnaire was sent to a total of 350 employees and different stakeholders of telecom industry. Out of the 350 questionnaires, a total of 233 were returned with a response rate of 66.5%, 21 were excluded from the analysis due to missing data. The remaining sample hence consisted of 212(60.5%) respondents. Simple convenient sampling was used for the purpose of data collection. Out of 212 usable respondents used in final analysis, 18(8.5%) respondents held the titles of IT/Software Development, 32(15.1%) Marketing/Sales & Distribution/Customer Services, 38(17.9%) Administration / HR / PM, 64(30.2%) Technical / Quality Assurance, 48(22.6%) and 12(5.7%) others. Descriptive summary of the respondents is depicted in Table 2.

To test nonresponse bias, early and late response bias was checked by splitting the data into two groups, early received (153) and late received (59) the data. Thereafter, t-tests were performed on the mean responses of two groups on five randomly selected questions it was found that no significant difference exists among the two groups. Hence, data was free from potential no response bias (Armstrong & Overton, 1977). Moreover, Harman's one-factor test was also applied to examine the potential existence of common method variance and the analysis proposed the incidence of multi factors and the data was free from significant bias between variables (Podsakoff & Organ, 1986).

4. Data Analysis and Results

4.1 Data Preparation

The questionnaire prepared to measure the five constructs in the study comprised of a total of 79 items. To measure each construct at least three indicators were used. These items were wrapped to a manageable size and to meet the multiple group analysis (Hall, Snell, & Foust, 1999). Items are wrapped just by taking average of items in respective indicator.

4.2 Scale Reliability and Validity

The constructs of latent variables were subjected to the validity and reliability analysis prior to their deployment in the model. Validity tests were performed in four steps: unidimensionality and reliability, convergent validity, discriminant validity and criterion-related validity (Sila & Ebrahimpour, 2005).

4.2.1 Unidimensionality and Reliability

Unidimensionality measures the extent to which the different items in a construct measures the same construct (Jackson, Denzee, Douglas, & Shimeall, 2005).

Table 3: Unidimensionality, Convergent Validity and Reliability

Factor	Indicator	CFI	Factor Loading	Cronbach's alpha
TQM	ER	0.998	0.752	0.873
	LS		0.809	
	CR		0.793	
	PPM		0.833	
QP	SQ	0.973	0.912	0.948
	SD		0.961	
	PQ		0.862	
	SER		0.886	
IP	PdI	1	0.728	0.779
	PrI		0.652	
	ICI		0.831	
OP	HRR	1	0.678	0.832
	FP		0.855	
	NFP		0.842	
CS	CS	1	0.835	0.877
	OS		0.948	
	NCS		0.741	

Unidimensionality in this study was measured through Confirmatory Factor Analysis (CFA) and Comparative Fit Index (CFI). Significant factor loadings, which are good indicators of CFA (Demirbag et al., 2006) were calculated through standardized regression weights and it was noted that almost all the standardized regression weights were above 0.7 (or at least 0.96), and were satisfactorily high and statistically significant (Table 3). Similarly, CFI value of more than 0.90 for a construct shows an satisfactory unidimensionality of the data (Hatcher, 1994). Analysis of Table 3 shows that CFI values ranged from 0.973 to 1.00. CFI compares the proposed and null model with the assumption that no relationship exists among the measures. CFI values range from 0.973 to 1, indicating considerably good fit to the data. The reliability of the scales was measured by calculating the Cronbach's alpha value for each of the construct. The results as reported in Table 3 shows that the Cronbach's alpha value of all five constructs is more than the recommended value of 0.70 (Hair, Black, Babin, Anderson, & Tatham, 2005), thus showing considerable internal-consistency and reliability of the constructs.

4.2.2 Convergent Validity

The convergent validity of the scales can be assessed through CFA, i.e., the significant factor loadings of the indicators of the constructs show convergent validity of the constructs (Bagozzi & Yi, 1991). As shown in Table 3, all the factor loadings are significant while ranging from 0.652 to 0.961, thus indicating a strong convergent validity.

4.2.3 Discriminant Validity

Discriminate validity is the extent to which the different latent constructs in an instrument and their corresponding indicators/items are unique enough to be differentiated from the other constructs and their indicators/items (Hatcher, 1994). This type of validity can be confirmed if the square root of Average Variance Extracted (AVE) of a latent variable is greater than its correlation with other latent variables (Fornell & Larcker, 1981). Moreover, if AVE is greater than 0.50 it also shows good convergent validity. The square root of AVE are shown diagonally in Table 4 and value of all the constructs are greater than the absolute value of its correlation with other latent variables hence confirm discriminant validity. A CFA was also performed to assess the convergent as well as the discriminant validity of the multi-item construct. The results CFA show that the measurement model fits the data ($\chi^2 = 177.539$; $p < 0.001$; $df = 70$; $\chi^2/df = 2.536$; RMSEA = 0.079; RMR = 0.019; TLI = 0.94; CFI = 0.95; IFI = 0.96; NNFI = 0.79).

Table 4: Discriminant validity

	CR	AVE	IP	TQM	QP	OP
IP	0.79	0.55	(0.74)			
TQM	0.88	0.66	0.71	(0.81)		
QP	0.94	0.80	0.33	0.22	(0.89)	
OP	0.83	0.62	0.59	0.56	0.225	(0.79)

*AVE of each latent variable is shown in diagonal in parentheses. ^CR is composite reliability.

4.2.4 Criterion-related validity

This type of validity entails the correlation among the predictor variables and their pertinent criterion variable (Büttner, 1997). In this study, the three latent predictor variables of the model as reported in Table 1 have this validity if they have a high and positive correlation with the outcome variable, i.e., the OP. The latent variable of support of culture is not accounted for the criterion-related validity because it does not have a direct impact on the OP. The bivariate correlations between each of the three predictor variables and the OP are significant (Sila & Ebrahimpour, 2005), and thus indicates considerable criterion-related validity as shown in Table 5.

4.3 Assessment of Model Fit

The hypothesized model was tested using Amos 16 for analyzing the relationships between the latent constructs under the study.

Table 5: Correlations between latent variables

	TQM	QP	IP	OP
TQM	1			
QP	.867**	1		
IP	.233**	.184**	1	
OP	.633**	.509**	.170*	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed)

Table 6: Summary Statistics of The Model Fitness Indices

Fit Index	Recommended Value	Observed Value
χ^2/df	≤ 3.00	1.946
GFI	≥ 0.90	0.913
AGFI	≥ 0.80	0.872
NFI	≥ 0.90	0.928
CFI	≥ 0.90	0.963
RMSEA	≤ 0.080	0.067

GFI = goodness-of-fit index;
 AGFI = adjusted goodness-of-fit index;
 NFI = normed fit index;
 CFI = comparative fit index;
 RMSEA = root mean square error of approximation.

Six model fit indices (χ^2/df , GFI, AGFI, NFI, CFI and RMSEA) were employed to test the fitness of the model (Fotopoulos & Psomas, 2010; Jung, Wang, & Wu, 2009; Prajogo, McDermott, & Goh, 2008; Su et al., 2008). These indexes of the model fitness, on the basis of the structural model analysis, are summarized in Table 6. In practice, Chi-square / degrees of freedom should be less than 3, GFI, NFI, CFI should be greater than or equal to 0.9, AGFI should be more than 0.8, and RMSEA should be less than or equal to 0.08 are considered as indicators of good fit (Teo & Khine, 2009; Jackson et al., 2005). As shown in Table 6, all goodness-of-fit indices are in the acceptable range.

4.4 Hypothesis Testing

The model was tested by employing the data received from the 212 respondents. SEM path analysis was used to test the hypothesis therein. Figure 2 depicts the standardized regression coefficients of hypothesized paths and also the loadings of latent variable's indicators. H₁ postulated that TQM positively influences organizational performance. The level, with b = 0.33, hence H₁ is supported. Similarly H₂ and H₃ with a path coefficient of b = 0.35 and b =

0.75 are accepted. The standard path coefficient estimate from quality performance towards organizational performance b=0.09 (p=0.189) is not significant, hence, H₄ is not supported. On the other hand H₅ has significant path coefficient b=0.31 (p<0.05) and is accepted. H₆ and H₇ were tested by using sobel test and innovation performance significantly at (p<.001) partially mediates the path between TQM and organizational performance whereas quality performance does not mediate the link among the TQM and OP (Sobel, 1982; Venkatraman, 1989), primarily may be due to insignificant relationship between quality performance and organizational performance.

Finally, to verify H₈ regarding the moderating effect of support of culture, a two-group analysis was conducted. Concerning support of culture the sample was split as close as possible on the basis of means into two groups, the 'low culture of support' group consists of (89) and the 'high culture of support' group consists of (123) respondents(Bryde & Robinson, 2007). This technique to divide the data into two subgroups was used by for group analysis of the data. A t-tests for mean differences to detect if these thresholds statistically discriminate the sub-samples. The t-test for OP is, t = -16.001(p < 0.01). First the paths were calculated to be unconstrained across the two groups and then these paths were estimated to be constrained and unchanging across the groups. If the change in the chi-square value between the constrained and unconstrained multi-group SEM is statistically significant, it shows that the path loadings in different groups are significantly changed (Su et al., 2008). That is, the culture of support significantly moderates the relationships between TQM and OP. Table 7 shows the results of Multi-group SEM analysis. It is evident that both of the two models fitness is good, and the chi-square change of 18.1 with five degree of freedom is statistically significant at (p<0.01). Hence H₈ is accepted. standardized regression coefficient from TQM to OP is statistically significant at five percent significance

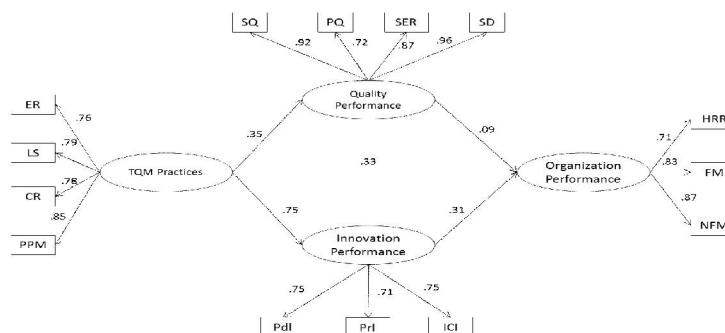


Figure 2: Results of Structural Model

Table 7: Results of Multi-Group Structural Model for Moderation Effect of Support of Culture

Model Description	χ^2	df	χ^2/df	CFI	RMSEA
Constrained	341.7	149	2.29	0.867	0.078
Unconstrained	323.6	144	2.24	0.876	0.077

5. Discussions

Existing literature supports the findings of this study. An analysis of the model provides that the TQM practices positively impacts the primary QP and IP and the OP. This provides an insight that the adoption and encouragement of TQM practices surely improves the performance of Telecom firms. The path diagram shows that TQM has a strong impact on IP of the organization as compared to the QP. This also confirms the old literature on quality management and its relationship with innovation (Anderson, Rungtusanatham, Schroeder, & Devaraj, 1995). Notwithstanding the QP and the IP are different from each other, the results shows that there exists a significant correlation among the two and they are interrelated with each other. This finding further endorses the theory that the exploration of new and state of the art technologies improves the product quality (Benner & Tushman, 2003). Similarly, the improvements in the product/service quality are also deemed effective in the development of new products (Prajogo et al., 2008). For example, the enhancement of new features in a product may require change and improvement in the technology. However there is a need for effective integration among the two in order to obtain the optimal business results. The relationship of QP and OP, though positive but not significant, suggests that there may exist a more complex relationship among the QP and OP. Therefore QP alone cannot significantly influence the organizational performance in the telecom sector. It might include other variables like marketing, sales and distribution, etc. IP has a strong and positive significant relationship with the OP showing the importance of innovation in improving the OP. Further, the maximum factor loading of the indicator product innovation among the other indicators of the latent construct of IP suggests that fostering innovation in the products in the form of new features and services significantly contributes in IP, which ultimately explains the OP of telecommunication firms and their suppliers. Despite the contrast between the QP and the IP as discussed earlier, both of these together are the intermediate performance outcomes of TQM. Together they positively and significantly mediate the relationship between the TQM practices and OP. This elucidates that the enhancement in the QP, IP is

essential to have the expected outcome of implementing TQM practices (Su et al., 2008).

This study also suggests that the environment of culture support moderates the relationship between TQM practices and the OP. This confirms the suitability of the contingency theory approach to the successful implementation of TQM (Joiner, 2007). The culture of support can promote the team work and creates a synergistic effect on the TQM/organizational performance relationship. In addition to the co-workers and the organizational culture of support, the role of national cultural also shows the importance of national cultural values and support for improvement of the quality and performance of organizations.

5.1 Implications

The investigation of this research arises several interesting implications for business, research and education. Four conceptual frameworks Salaheldin (2009), Prajogo and Sohal (2003), Su et al. (2008) and Joiner (2007) were adapted and modified with the addition and deletion of new indicators to develop a new model for measuring the TQM/organizational performance relationship. The model includes both the mediating and the moderating impact that influences the TQM/organizational performance relationship; both of these were not tested before in a single framework. Another major contribution of the study is the development of a research instrument, being validated by the experts of the area. The instrument comprehensively covers the concepts of the latent variables under the study and is also statistically validated. The findings show that TQM practice improves the quality performance, innovation performance and the organizational performance. Therefore the practice of TQM philosophy should be promoted in the Telecom industry.

Innovation performance can alone positively influence the organizational performance. This indicates to the managerial implications of promoting innovation and creativity in the products/services and processes that can ultimately improve the organization performance. The study also suggests that improvement in the product alone is not adequate for improved organizational performance, so other, variables along with the quality performance, should also be considered by the telecom sector for the improved organizational performance.

The study signifies the need to integrate the relationship among the quality and the innovation performance that can result in improved quality of the services and may bring more innovation in the products. Since the primary measures under this study mediate the TQM/organizational performance relationship, the telecom firms need to focus on the immediate impact of TQM practices to ensure its

secondary impact in the form of improved organizational performance, particularly on the innovation performance due to its direct and strong positive effect on the organizational performance. Culture of support, that moderates the TQM/organizational performance relationship, should also be encouraged at organizational and national level. This can help in the promotion of the quality culture, whilst bringing synergies and teamwork that ultimately shall affect the performance measures.

5.2 Limitations

We so acknowledge several limitations of the study. First, the present study is only limited to the telecom industry of Pakistan and hence it has less generalization. More significant results could have been achieved from the study by the comparison of different industries. Secondly, the sample size was limited due to time and financial constraints. Although the response rate was satisfactorily good, so it is believed that the non-response bias has not unsubstantiated the results of this study. Thirdly, the cross-sectional data was used, though the causal relationships have been achieved, but a longitudinal research could add strength to causality.

5.3 Future Recommendation and Conclusion

The proposed model has not been tested for its relevance and significance in different sectors. This can be explored in future research. The study can further be enriched by focusing on different geographic regions. The same size can also be increased to further improve the generalizability of the results. Further research can include other contextual and environmental factors to see how they can play a moderating or intervening role in the relationship of the TQM practices and the OP. This study concludes that the TQM practices (leadership, employee relation, customer relations and product/process management) positively and significantly influences the quality performance, innovation performance and the organizational performance. The positive correlation among the quality and innovation performance shows that these two aspects should be integrated and balanced to support and improve each other.

The insignificant impact of quality performance on organizational performance shows that quality performance alone is not sufficient to improve the overall organizational performance of telecom firms. On the other side, innovation performance in the telecom sector can itself positively and significantly impacts the organizational performance. Further, the immediate impact of TQM practices significantly mediates the secondary outcomes of TQM practices. Culture of support also moderates the TQM/organizational performance relationship.

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Appendix 1: Measurement scale

TQM

Employee Relations ((Jung et al., 2009)

1. We are authorized to inspect our own work (Ahire et al., 1996).
2. We are encouraged to find out and fix the problems/issues (Ahire et al., 1996).
3. Technical assistance is provided to us for solving the problems (Ahire et al., 1996).
4. We are recognized and rewarded for superior quality performance (Saraph et al., 1989; Sila and Ebrahimpour, 2005).
5. We are encouraged to give suggestions (Ahire et al., 1996).
6. There are no communication barriers between the departments (Terziovski and Samson, 1999)
7. The communication processes are not only “top-down” but “bottom-up” as well (Terziovski and Samson, 1999).
8. We are provided with the quality-related training (Saraph et al., 1989; Sila and Ebrahimpour, 2005).

Leadership (Jung et al., 2009)

1. Management takes the responsibility for quality performance (Saraph et al., 1989; Sila and Ebrahimpour, 2005)
2. Management views improvements in quality as a way to increase the profits (Saraph et al., 1989; Sila and Ebrahimpour, 2005).
3. Management offers incentives to achieve quality goals (Tabak and Jain, 1999).
4. Management ensures that each new product and service meets customer expectations (NIST, 2002).
5. Management uses quality performance as an incentive to recruit and retain staff (NIST, 2002).
6. Supervisors try to obtain the trust of employees (Tamimi and Gershon, 1995).
7. Supervisors promote the customer satisfaction (Stock and Hoyer, 2002).
8. Our top leaders stress the impacts that our organization has on the society (Kuei and Madu, 1995).

Customer Relations (Jung et al., 2009)

1. We assume that ensuring customer satisfaction is our major responsibility (Ross and Georgoff, 1991).

2. We determine our customers' satisfaction relative to the customers' satisfaction by the competitors (Black and Porter, 1996).
3. We link customer satisfaction with our internal performance indicators (Black and Porter, 1996).
4. We use Customer complaints as an input to improve our processes (Terziovski and Samson, 1999).
5. Customer requirements are communicated to us (Terziovski and Samson, 1999).
6. We use various methods to build relationships with customers and to increase repeat business and positive referrals (NIST, 2002).
7. We follow up with customers on products/services and transactions to receive prompt and actionable feedback (NIST, 2002).
8. We reset our standards whenever customer needs and expectations change (NIST, 2002).
9. We ensure that the data and information we provide to our customers on the internet are: reliable; accurate; timely; and secure (NIST, 2002).

Product/Process Management (Jung et al., 2009)

1. We emphasize the continuous improvement of quality in all work processes (Anderson et al., 1995).
2. We use statistical techniques to control processes (Saraph et al., 1989; Sila and Ebrahimpour, 2005).
3. Our product/service specifications are clear (Saraph et al., 1989; Sila and Ebrahimpour, 2005).
4. Systematic recording and analysis of the company's performance data is in place (Fotopoulos and Psomas, 2010).
5. Determination of areas and points for improvement are practiced (Fotopoulos and Psomas, 2010).
6. Standardized and clear work or process instructions are given to all of us. (Anderson et al., 1995).
7. We effort to prevent errors during the phase of process planning. (Fotopoulos and Psomas, 2010)
8. Our product/service specifications are clear (Saraph et al., 1989; Sila & Ebrahimpour, 2005).

Culture of Support

Co-worker Support(Joiner, 2007)

1. We willingly share our expertise with each other (Zhou and George, 2001).
2. We help out each other if someone falls behind in his/her work (Zhou and George, 2001).
3. We encourage each other when someone is down (Zhou and George, 2001).
4. We try to act like peacemakers when there are disagreements (Zhou and George, 2001).

Organizational Support (Joiner, 2007)

1. Creativity is encouraged at the company (Zhou and George, 2001).

2. Our ability to function creatively is respected by the leadership (Zhou and George, 2001).
3. The reward system here encourages innovation (Zhou and George, 2001).
4. Company publicly recognizes those who are innovative (Zhou and George, 2001).

National Cultural Support(Noronha, 2002)

1. Our national culture promotes honor and dignity (Noronha, 2003)
2. We experience harmony and piece in our nation (Noronha, 2003)
3. We have international harmony and integrity (Noronha, 2003)
4. Our cultural values encourage interdependence, support and affiliation (Noronha, 2003)
5. People are oriented to respect authority (Noronha, 2003)

Quality Performance

Service Quality (Curkovic et al., 2000)

1. Our services are reliable (Curkvoic et al., 2000; Su et al., 2008)
2. Our services conform to the specifications that we offer for that service (Ahire et al., 1996, Curkvoic et al., 2000)

Service Design (Curkovic et al., 2000)

1. Our services perform as per their intended use (Ahire et al., 1996; Curkvoic et al., 2000).
2. Our service features are up-dated and attractive (Garvin, 1987; Curkvoic et al., 2000).

Perceived Quality (Arumugam et al., 2008; Curkovic et al., 2000)

1. The quality of our services is superior as compared to the competitors (Flynn et al., 1995; Arumugam et al., 2008).
2. In general, our company's level of quality performance has been high as compared to the industry norms (Arumugam et al., 2008).
3. Our customers have been well satisfied with the quality of our services (Arumugam et al., 2008).
4. Our customer relations are superior as compared to the competitors (Flynn et al., 1995; Arumugam et al., 2008).

Serviceability (Curkovic et al., 2000)

1. We immediately solve our customer complaints/issues (Garvin, 1987, Curkvoic et al., 2000).
2. We are courteous in provision of customer services (Garvin, 1987, Churkvoic et al., 2000).
3. We are responsive in identifying potential customer needs (Churkvoic et al., 2000).

Innovation Performance

Product Innovation (Prajogo & Sohal, 2003)

1. The level of newness (novelty) of our new features/packages is high (Prajogo and Sohal, 2003).

2. We use latest technological innovations in new product/services development (Prajogo and Sohal, 2003).
3. Our speed of new product/service development is fast (Prajogo and Sohal, 2003).
4. There are wide number of new services that we introduce to the market (Prajogo and Sohal, 2003).
5. There are a number of new services that we introduce first in the market (Prajogo and Sohal, 2003).

Process innovation (Prajogo & Sohal, 2003)

1. We have technological competitiveness in our processes (Prajogo and Sohal, 2003).
2. The up-datedness or novelty of technology used in our processes is high (Prajogo and Sohal, 2003).
3. The speed of adoption of the latest technological innovations in our processes is fast (Prajogo and Sohal, 2003).
4. We have a high rate of change in our processes, techniques and technology (Prajogo and Sohal, 2003).

Innovation and Continuous Improvement (Sila & Ebrahimpour, 2005)

1. We emphasize the continuous improvement of quality in all aspects of work (NIST, 2002).
2. We observe continuous improvement in our job performance (Jung et al., 2009).

Organizational Performance

Human Resource Results (Sila & Ebrahimpour, 2005)

1. Employee turnover rate is low (Adam et al., 1997).
2. Low employee absenteeism (Mc Adam and Bannister, 2001).
3. High Employee job performance (NIST, 2002).

Financial Performance (Demirbag et al., 2006)

1. Revenue growth over the last three years (Demirbag et al., 2006).
2. Net profits (Hendricks and Singhal, 1997; Das et al., 2000).
3. Profit to revenue ratio (Demirbag et al., 2006).
4. Return on total assets (Sankar, 1995, Demirbag et al., 2006).

Non-financial Performance (Demirbag et al., 2006)

1. Capacity to develop a unique competitive profile (Kim et al., 2002).
2. New product/service development (Demirbag et al., 2006).
3. Productivity (NIST, 2002).
4. Market development (Demirbag et al., 2006).

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Influence of Home Visits Nursing on Activities of Daily Living in Stroke Patients

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Abstract: Objective Exploring the influence of the home visits nursing on stroke patients' activities of daily living(ADL). **Methods** 60 cases who suffered from stroke at the first time were grouped into two groups: intervention and control. 30 cases in control group received conventional finally discharge instructions, and in intervention group 30 cases received the home visits nursing on the basis of the conventional discharge instructions, including health education and training in activities of daily living. The improved Barthel indexes were used to evaluate their activities of daily living in one month and three months respectively before and after leaving hospital. **Results** Patients' ADL is stronger than that of the control group after leaving hospital in one and three months respectively, with a significant difference ($P < 0.01$). **Conclusions** Continuous home visits nursing after leaving hospital improves significantly stroke patients' ADL.

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Keywords: Stroke, home visits nursing, activities of daily living (ADL)

1. Introduction

Stroke is a common disease with higher disability rate, and 70%~80% stroke patients have consequent dysfunctions to some extents, which seriously impact their activities of daily living (ADL) and results in their poor self-care(Zhang and Liu,2009).The poor ADL not only disorders patients' and work seriously and gives patients some negative moods such as needless emotion and depression, but also poses a huge burden on patients' dependents and society. Due to long-term suffering, hospitalization costs and other causes for stroke patients, more and more patients come back home with physical disability for recovery after their conditions are stable in hospital. However, patients miss the best recovering opportunity and suffer from sequela because they and their dependents are lack of knowledge and skills of rehabilitation. Thus, patients should need instructions timely in continuous rehabilitation nursing after leaving hospital (Qian, 2011). In this study, we observed influences of home visits nursing on the stroke patients' ADL after just leaving hospital.

2. Methods

60 stroke patients who suffered from stroke for the first time were selected from the wards of the Neurology Departments in two third grade A-class hospitals in Zhengzhou from December 2011 to June 2012. They were separated into two groups randomly: intervention group and control group, 30 cases each. Inclusion criteria for patients: ① Conforming to diagnostic criteria revised in the 4th National

Cerebrovascular Disease Academic Conference (Qian, 2011), and diagnosed as brain stroke by the CT or MRI head inspection; ② Suffering from limbs dysfunctions to some extents; ③ Having clear consciousness and voluntarily cooperating with researchers; ④ Informed consent to participate in the research. Exclusion criteria: ① Bleeding in subarachnoid space; ② Severe cognitive and speech disorders; ③ The mentally disturbed. Exit criteria: ① Those who have to exit from the study as they suffered major accidents such as critical diseases, harms or death during the study; ② Patients and their dependents exit from the study actively; ③ Those who are unable to be visited due to all kinds of reasons.

Patients in two groups received the conventional intervention and nursing in hospital. ① Control group: Receiving conventional hospital leaving instructions before leaving hospital; ② Intervention group: Before stroke patients leaving hospital, researchers, doctors, nurses, and physical therapists in hospital as well as nurses in community jointly work out the home-visit plan, including time, content and executors for home visits.

The home-visit time: Seeing patients once every week in the first month; once every two weeks in the second month; and once in third month; 30-60 minutes at every turn; the visiting time lasted three months. The home-visit content: ① Health education: arrangements in home environment, requirements of diet nutrients, and precautions in daily ; ② Training of activities of daily living: Training patients how to

dress on and off themselves, feeding, going to toilet, brushing teeth, walking, going up and down stairs; instructing patients to use their affected hands to hold bowls, feeding with healthy hands; encouraging patients to carry a cup for drinking by crossing hands, put on the affected side first then healthy side when dressing up, while taking off healthy side first then the affected side; wringing out towels for washing their faces by taking a faucet as a fulcrum, and combing their hair with long-handle combs(Qi et al., 2009). The home-visiting executors: Qualified researchers and nurses in community by training jointly entered the patient homes for nursing and intervention.

The ADL of patients in two groups was evaluated in one month and three months when and after leaving hospital respectively by the modified Barthel index (MBI) (Wang and Chen,2011). Total ten ADL levels such as feeding, dressing, going to toilet, individual hygiene, bathing, bed and chair shifting, walking on flat ground, going up and down stairs, and bowel and urinary control. Each evaluating item was divided into 1-5 levels with total score of 100 points by complete dependence, maximum help, medium help, minimum help and complete independence. It is found that there is a positive correlation between self-care ability and scores, and the score per level for each item is different from another. The higher score indicates the better ADL. The patients who got more than and equal to 60 points were cared by themselves basically, those who got 41-59 points had moderate dysfunctions, those who got 21-40 points had severe dysfunctions, and those who got less than and equal to 20 points lived their life by depending on other completely. This Scale is applied widely at home and abroad, with better reliability and validity(Wang and Chen,2011).

Data were analyzed by SPSS (16.0 version) after collected. Differences of variables between two groups were compared at base level and timing points by the T-test, and data in abnormal distribution were analyzed by the rank sum test of non-parameter statistical method. The repetitive measurement deviation analysis was used to evaluate whether there was statistical difference of MBI scores at different timing points in two groups.

3. Results

There were 60 hospitalized stroke patients totally in the hospital, who were 30 in control group and another 30 in intervention group. After leaving hospital for one month, 2 patients in control group were not visited for no phone answering or voluntarily quitting the research participating; 2 patients in control group were rejected (rehabilitation) within three months after leaving hospital, and 3 in

intervention group were rejected (two were recovered and one was unable to be contacted). Rejected and un-visited cases were 7 cases, accounting for 11.7% of total patients. Finally, data from 53 patients were collected, of whom were 26 in control group and 27 in intervention group.

The research objects ranged from 36 to 77 years old, with an average of 63.41 ± 11.08 , of whom were 30 male and 23 female. Their educational levels: 25 graduated from junior middle schools and below, 18 graduated from senior schools or technical secondary schools, and 10 were from colleges or above; 28 patients retired, 15 were employed, and 10 were unemployed. Most research objects(44) had spouses. Main watchers for research objects were spouses (33), and their sons and daughters (15). In 53 research objects, 4 patients were lived alone, and most of them (49) lived with their family members(dependents). Clinical data for research objects indicated that 43 patients suffered from cerebral infarction, 8 suffered from cerebral hemorrhage, and 2 were others. 81.1% patients suffered from chronic diseases such as high blood pressure, diabetes and heart disease. 23 in all patients had only one chronic disease, 20 had two or more chronic diseases. All research objects (32) suffered from diseases for the first time.

With the statistical test performed for population demography and disease data for patients in intervention and control groups, the result shows no significance ($P>0.05$) between groups, with better comparability.

Through statistical comparison to indexes from control group(4) and intervention group(3) where some patients had been unable to be contacted or rejected before intervention, the result indicated it is of no statistical significance($P>0.05$) between two groups.

The MBI score for research objects in intervention and control groups were analyzed first to check its distribution was normal, then T-test of two independent samples (T: statistical magnitude) were done for normal distribution data, and non-parameter statistical Mann-Whitney U rank sum check (Z was statistical magnitude) for non-normal distribution data.

Results in Table 1 show that there is no significant difference of MBI scores ($P>0.05$) between intervention and control groups before intervening; after intervening, the comparison at two timing points shows that MBI scores in intervention group are higher than that of control group, with significant difference ($P<0.01$).

Table 2 shows that results of repetitive measurement data variance analysis of MBI scores for research objects in two groups. Factors between

groups goes by grouping (control and intervention), and factors within group goes by time(three timing points were respectively: before intervening, one month and three months after leaving hospital); their interaction is grouping \times time. The result shows: (1) The difference of MBI scores for intervention and control groups is significant($P < 0.01$), while the score for intervention group is higher than that of control group at two timing points after intervening; (2) The difference of MBI scores for stroke patients is statistically significant ($P < 0.01$) between different timing points, which mainly presents more remarkable increase of MBI scores for intervention group at two timing points after intervening than before intervening; (3) Grouping is interactive with time ($P < 0.01$), that is, changes of MBI scores are evidently different for intervention and control groups at different timing points before and after intervening; ADL for intervention group changes evidently at different timing points before and after intervening.

Table 1. Comparison of MBI index scores for stroke patients in two groups(Tp, Timing points ; Bi, Before intervening; Om, One month after leaving hospital; Tm, Three months after leaving hospital)

Index	Tp	Control group (N=26)	Intervention group (N=27)	t/Z
MBI	Bi	45.76 \pm 21.39	47.68 \pm 20.11	-1.728
	Om	55.68 \pm 19.45	77.89 \pm 15.57	-12.951**
	Tm	58.55 \pm 17.39	84.76 \pm 13.63	-8.046**

** $P < 0.01$, the difference is extremely significant.

Table 2 Repetitive measurement variance analysis on MBI index scores for stroke patients in two groups

Index	Factors between groups	Factors within groups	Interaction
	F	F	F
MBI	213.003**	118.821**	27.683**

** $P < 0.01$, the difference is extremely significant.

Table 3 Comparison pairwise to MBI index scores at different timing points for stroke patients in two groups (Cg, control group; Ig, Intervention group; Bi, Before intervening; Om, One month after leaving hospital; Tm, Three months after leaving hospital)

Index	Group	Bi:Om	Bi:Tm	Om:Tm
		MD	MD	MD
MBI	Cg	-5.166**	-4.021**	-0.916
	Ig	-33.081**	-25.821**	-5.754**

** $P < 0.01$, the difference is extremely significant.

Means of MBI scores at timing points for stroke patients in control and intervention group are compared pairwise by LSD multiple comparison. The results are shown in Table 3.

4. Discussion

The common physical disability after patients suffer from stroke is hemiplegia. All stroke patients' disabilities are not caused by the hemiplegia, and the ADL disorders may be also caused by such disabilities as spasm in posture, deformity in knuckles, contracture and muscular atrophy due to lack of necessary recovering nursing method and suspension of rehabilitation training from the acute to recovering period. For this reason, they live a poor life(Yang et al.,2010).

This study demonstrates the continuous home visits nursing the patients receive after leaving hospital can improve their ADL, and also shows through three months of home visits, the ADL in intervention group is better than that of control one, which implies the recovering training instructions and health education in the home visits exert a good effect to improve patients' ADL. In addition, the MBI scores at two timing points for patients in control group after leaving hospital are higher than before the health instructions, which is in agreement with previous similar results(Torres-Arreola et al.,2009). There are two reasons for that. On one hand, the central nervous system in structure and functions has compensation and functional reorganization, a spontaneous recovery after stroke(He et al.,2005). On the other hand, the stroke patients mainly receive treatment at the acute stage in hospital, while after leaving hospital, most of them still have various degrees of dysfunction. As a result, it may cause self-care disability in their daily life and their family burden, and patients and their dependents have to continue to seek for out-of-hospital supports. However, owing to lack of timely and accurate instruction, the effect of spontaneous health promotion from patients and dependents is evidently lower than that of continuous rehabilitation nursing instructions in the control group from the study. Thus, from the repeated measurement variance analysis on MBI scores for research objects in two groups, it is seen that changes at different timing points after intervention are different significantly.

The community-based rehabilitation is a continuation of the hospital-based rehabilitation, whereas home visits nursing is an important way for community-based rehabilitation, and also is one of rehabilitation nursing approaches from hospital-based to community-based. Through home visits, some problems of patients can be found timely, and poor nursing support for patients after leaving hospital can be improved effectively(Huang,2010;Feng et al.,2003). The rehabilitation in the home, a homely re-healthy training, means patients can blend the rehabilitation therapy into daily life, and their re-

healthy training is consolidated constantly. By this way, they reach rehabilitation to the hilt. Besides that, the rehabilitation in the home needs jointly participation of their dependents and supports of family members. Thus, nursing and other medical personnel should pay attention to roles of their dependents, who are indispensable in the whole intervention of community and family.

Stroke patients become more and more in China with improvement of living standard and ageing of society. Currently, rehabilitation instructions for stroke patients are most limited to hospitalized period, as a result, rehabilitation instructions after leaving hospital are restricted to great extent.

However, stroke patients' rehabilitation training is a long-term and continuous process. A great many practices have proved that recovery of motor functions for stroke patients can last over five years till the fixed harms come into being, and overall effect and importance of systematical rehabilitation exercise on stroke patients are recognized in the world. It is very necessary to carry out a long-term home-visiting system and dynamic and continuous rehabilitation instructions for stroke patients. Nurse clinicians have heavy daily work, so it is difficult to do home-visiting and rehabilitation instructions for the out-of-hospital patients in the long term. Thus, the community-based medical services should be strengthened, and community-based rehabilitation nursing services should be developed depending on the hospital. The clinical doctors, physical therapists and nurses should cooperate with the community-based service department, and instruct and train medical workers in communities to improve stroke knowledge and rehabilitation skills. Through correct instructions, patients can promote recovery of their limb functions, strengthen activities of their daily living, and improve their life quality.

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Effect of irrigation by contaminated water with cloth detergent on plant growth and seed germination traits of maize (*Zea mays*)

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Abstract: People are worried about effect of household cleaning products in the environment. One of the sources of detergent is sewage that is being used for irrigation of the crops. A laboratory experiment and a pot experiment were conducted in 2012 to determine the effect of irrigation with different doses of detergent on plant growth and seed germination traits of maize (*Zea mays*). The experiments included eight doses of cloth detergent (0, 0.00002, 0.0002, 0.002, 0.02, 0.2, 2, 20 g/L). Results showed that 20 g/L of detergent severely reduced seed germination and root length. 20 and 2 g/L of detergent reduced shoot length and seedling weight. 20 g/L of detergent produced the lowest leaf area, leaf weight, stem weight and total biomass. The results demonstrated that irrigating by the sewage contaminated by household cleaning products at high concentration should be avoided.

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Key words: Detergent; maize; seed germination; seed vigor; specific leaf weight

1. Introduction

People are worried about effect of household cleaning products in the environment. Sewage contains great deal of the product. Using sewage for irrigation is increasing for crop production. Jadia and Fulekar (2008) reported that the lower concentration of heavy metals increased root growth, shoot growth and biomass production of sunflower (*Helianthus annuus*). Contamination doses of 4 and 5 % of spent diesel fuel had 40% seed germination for maize (*Zea mays*) and 22% for peanut (*Arachis hypogaea*) respectively compared to control (Ehiagbonare et al., 2011). In alfalfa (*Medicago sativa*), 5 ppm of Cd(II) reduced shoot size by 16% compared to the control, but Cr(VI), Cu(II), Ni(II), and Zn(II) increased the shoot size by 14.0%, 60.0%, 36.0%, and 7.7%, respectively (Peralta et al, 2000). Seed germination percentage of ryegrass (*Lolium multiflorum*) was decreased with increasing concentrations of chlorpyrifos (pesticide) in the soil (Korade and Fulekar, 2009). Maize is one of the most important warm season crops in Iran. There is little information about effect of detergent on seed germination and plant growth, so the objective of this study was to determine maize growth and seed germination traits at different doses of detergent powder.

2. Materials and Methods

2.1. Experiment 1

The experiment included eight doses of detergent powder (T1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002, T8=0 g/L). Chemical ingredient of studied cloth washing powder included sodium alkyl benzen sulfonate, nonil phenol

etoxilate, sodium silicate, sodium carbonate, sodium sulphate, sodium toluene sulfonate, acrylate polymer, optical brightner, bleach, builder, essence.

The study was conducted as a randomized complete block design with three replications in 2012. Seeds of maize (*Zea mays*, C.V. S.C. 704) were gathered from maternal plants harvested in 2011. After harvesting seeds from maternal plants, they were stored at 25°C for six months. Before trial beginning, seeds were sterilized by sodium hypochlorite solution (1% active chlorine) for 10 minutes to avoid fungal contamination. Then each Petri dish received 20 seeds and 8 cc of solution was added to them. The control solution (0 g/ L) used in this study was distilled water. The Petri dishes were categorized by dose and each category was sealed with plastic wrap to keep moisture in. The temperature during experiment period was kept at 26 ± 1°C. Two millimeters growth of coleoptile and radical was the criterion for germination. The trial period was 7 days. Seed vigor was estimated by these equations (Sharifzadeh et al, 2006; Abasian et al, 2010):

$$\text{Seed vigor (\% cm)} = [(\text{Radicle length (cm)} + \text{Caulicle length (cm)}) * (\text{Germination percentage (\%)})]$$

$$\text{Seed vigor (\% g)} = [(\text{Radicle weight (g)} + \text{Caulicle weight (g)}) * (\text{Germination percentage (\%)})]$$

2.2. Experiment 2

Plant materials, experimental design and treatments: The pot experiment was conducted in 2012 at Faculty of Agriculture, University of Razi, Kermanshah, Iran. Maize seeds (*Zea mays*, CV S.C. 704) were planted in 24 pots (7 cm in diameter, 7.5 cm in depth) on Jun 26, 2009. The pots were filled with

clay soil. Seeds were densely sown 1 cm deep but after emergence seedling were thinned to five plants per pot. Plants were initially well-watered and treatments of irrigation with contaminated water were only imposed 8 days after sowing. 336 mg nitrogen per 1 kg of soil as urea was used for nourishing plants after 17 days from sowing. The study was involved a factorial experiment in a randomized complete block design (RCBD) with three replications. The treatments were different detergent doses. There were eight doses of contaminated water with cloth detergent for irrigation: T1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002, T8=0 g/L). At each irrigation event, enough water was allowed to be absorbed by the soil in each pot, and any excess water was allowed to drain.

Plant sampling and measurements: Leaf Area (LA) was measured using $LA = \text{Leaf Width} * \text{Leaf Length} * 0.75$ (Chaab et al., 2009). LA and Leaf Dry Weight (LDW) were used to calculate Specific Leaf Weight (SLW) as:
 $SLW = LDW/LA$

Measurement of dry weight, leaf to stem ratio, specific leaf weight and leaf dry weight was carried out by five plants while plant height, leaf number per plant and leaf area were measured by random selection of three plants per each pot. Harvest time for total dry weight was 23 days after sowing and plant samples were dried in a forced-air oven at 65 °C for 2 days.

2.3. Statistical analysis

Analysis of variance (ANOVA) was used to determine significant differences. The Multiple Range Test of Duncan performed the separation of means ($P < 0.05$). Correlation coefficients were calculated for the relationship between several crop parameters. All statistics were performed with the program MINITAB (version 14.0), SAS (version 9.1) and SPSS (version 16.0).

3. Results and Discussion

3.1. Experiment 1

Seed germination percentage: The highest dose of detergent (T1) reduced seed germination severely compared to other treatments (Table 1). Control (T8) had higher seed germination than T1 and T6. Seed germination percentage had a positive and

significant correlation with all traits (Table 2). It was reported that the seed germination of *Lolium multiflorum* was not affected by the anthracene amended in the soil (Korade and Fulekar, 2009). The results are compatible with findings of Ehiagbonare et al (2011), Barua et al (2011) and Ashraf and Ali (2007). Reduction in seed germination may be due to induced oxidative stress, resulting in lipid peroxidation and increase in cell membrane permeability to toxic ions (Hejazi Mehrizi et al, 2012).

Shoot length: High doses (T1, T2) of detergent reduced shoot length (Table 1). Shoot length had a positive and significant correlation with all traits (Table 2). Jadia and Fulekar (2008) reported that increasing doses of cadmium to sunflower grown at pot increased shoot length compared to control. Reduction in shoot length is probably due to oxidative stress.

Root length: The highest dose of detergent (T1) reduced root length severely compared to other treatments (Table 1). Root length had a positive and significant correlation with all traits (Table 2). Jadia and Fulekar (2008) reported that increasing doses of cadmium to sunflower grown at pot reduced root length compared to control. Plants under high osmotic potential cannot uptake water to initiate seed germination processes and other stresses such as heavy metal stress and salinity stress can increase root damage.

Seedling weight: T7 and T8 had higher seedling weight than T1 and T2 (Table 1). Seedling weight had a positive and significant correlation with all traits (Table 2). High osmotic potential due to high concentration of detergent does not let seed absorb required water for starting metabolic activities and probably production of oxygen free radical at the condition can damage cell membrane (Sharifzadeh et al, 2006).

Seed vigor: T1 had the lowest seed vigor (Table 1). Seed vigor had a positive and significant correlation with all traits (Table 2). Reduction in seed vigor due to high doses of detergent can be described by higher osmotic water potential, salinity and heavy metal stresses (Sharifzadeh et al, 2006; Jadia and Fulekar, 2008).

Table 1. Effect of detergent doses on maize seed germination traits.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling weight (g/plant)	Vigor (% g)	Vigor (% cm)
T1	11.67 d	0.79 d	1.23 b	0.0077 c	0.0010 c	0.240 b
T2	83.33 abc	2.93 c	11.76 a	0.0313 b	0.0262 b	12.348 a
T3	88.33 abc	4.49 ab	12.50 a	0.0360 ab	0.0319 ab	15.029 a
T4	95.00 a	4.47 ab	13.01 a	0.0387 ab	0.0367 a	16.612 a
T5	91.67 ab	5.29 a	12.95 a	0.0387 ab	0.0356 ab	16.806 a
T6	78.33 c	4.94 ab	11.33 a	0.0393 ab	0.0310 ab	12.773 a
T7	80.00 bc	3.52 bc	13.86 a	0.0420 a	0.0337 ab	13.921 a
T8	91.67 ab	5.13 a	11.73 a	0.0413 a	0.0379 a	15.540 a

^aT1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002, T8=0 g/L

Table 2. Pearson's correlation coefficients among studied traits in maize under different doses of cloth detergent

	Germination percent	Shoot length	Root length	Seedling weight	Vigor weight	Vigor length
Germination percent	1	.877**	.963**	.935**	.972**	.988**
Shoot length	.877**	1	.807*	.890**	.917**	.906**
Root length	.963**	.807*	1	.957**	.954**	.960**
Seedling weight	.935**	.890**	.957**	1	.981**	.945**
Vigor weight	.972**	.917**	.954**	.981**	1	.985**
Vigor length	.988**	.906**	.960**	.945**	.985**	1

*.Correlation is significant at the 0.05 level; **.Correlation is significant at the 0.01 level

3.2. Experiment 2

Plant height and leaf number per plant: High doses of detergent (T1 and T2) reduced plant height and leaf number per plant (Table 3). T1 had the lowest plant height and leaf number per plant. Plant height and leaf number per plant had a positive and significant correlation with most traits (Table 4). In rosemary, increasing salinity was associated with a significant increase in the electrolyte leakage and lipid peroxidation (Hejazi Mehrizi et al, 2012). Reduction in plant height is an obvious effect of salinity. This decrease in plant height may be attributed to intelligent response of plant to prevent shoot transpiration (Karam et al, 2003), reduction of cell size and internodes length and accumulation of Abscisic Acid (Sharp, 1996).

Leaf area and leaf weight: T1 produced the lowest leaf area and leaf weight (Table 3). T7 had higher leaf weight than T8. It is probably due to that under low dose of detergent, soil can be sterilized against microbes or maybe some elements present in detergent such as sulphate can be readily absorbed by plant. Increasing doses of cadmium to sunflower increased shoot length compared to control (Jadia and Fulekar, 2008).

Stem weight and total biomass: High doses of detergent (T1) reduced stem weight and total biomass compared to control (T8) (Table 3). T7 produced higher stem weight and total biomass. Like leaf

weight it may be attributed to sterilizing soil against microbes or absorbing some elements present in detergent such as sulphate. Total biomass had a positive and significant correlation with most traits except specific leaf weight (Table 4). Elevated salinity reduced water uptake by seeds, thereby inhibits root elongation (Rahimi et al, 2006). Presence of elements such as Na in contaminated water can inhibit activities of some enzymes and decrease availability of some nutrients (Al-Taisan, 2010). Lower biomass accumulation under higher doses of contaminated water by detergent powder can be explained by three stresses; salinity stress, water stress and heavy metal stress (Sharifzadeh et al, 2006; Jadia and Fulekar, 2008).

Leaf to stem ratio and specific leaf weight: T2 had higher leaf to stem ratio (Table 3). It is due to that under T2, maize saved its leaf weight, but its stem weight was reduced severely compared to leaf weight (Table 3). These data show that by increasing detergent dose, the leaf became thicker (higher specific leaf weight) and its leaf area was reduced (Table 3). Pace and Benicasa (2010) reported similar results. T1 had the highest specific leaf weight (Table 3). Similar result was reported by Alyemeny (1998). Save et al (1993) reported that water stress resulted in decreasing cell size and increasing solute concentration so specific leaf weight increases under high level of detergent stress.

Table 3. Effect of contaminated water by different doses of detergent powder on maize traits

Treatments	^b Plant height (cm)	Leaf number per plant	Leaf area (cm ² / plant)	Stem weight (mg/plant)	Leaf weight (mg/plant)	Leaf to stem ratio	Total biomass (mg/plant)	Specific leaf weight (mg/cm ²)
T1 ^a	12.2 c	2.3 d	6.44 b	16.0 d	33.87 c	2.11 b	49.87 c	4.53 a
T2	29.4 b	2.9 c	40.07 a	28.9 c	80.13 ab	2.88 a	109.07 b	2.04 b
T3	33.9 ab	3.2 abc	38.34 a	38.9 ab	83.00 ab	2.13 b	121.93 ab	1.81 b
T4	35.4 a	3.5 a	47.04 a	41.0 ab	85.60 ab	2.09 b	126.60 ab	1.51 b
T5	34.9 a	3.1 bc	46.41 a	36.8 bc	92.13 ab	2.53 ab	128.93 ab	2.05 b
T6	32.3 ab	3.4 ab	37.81 a	42.1 ab	83.20 ab	1.98 b	125.27 ab	1.90 b
T7	35.9 a	3.2 abc	45.85 a	46.7 a	98.93 a	2.11 b	145.68 a	2.16 b
T8	32.8 ab	3.4 ab	33.07 a	35.8 bc	75.73 b	2.12 b	111.53 b	2.31 b

^a T1, T2, T3, T4, T5, T6, T7 and T8 are different doses of contaminated water by detergent powder (T1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002 and T8=0 g/L respectively).

^b Means followed by the same letter within each column are not significantly different at P < 0.05 as determined by Duncan's Multiple Range Test.

Table 4. Pearson's correlation coefficients among studied traits in maize under different doses of contaminated water by detergent powder

	Plant height	Leaf number		Stem weight	Leaf weight	Leaf to stem		Specific leaf weight
		per plant	Leaf area			ratio	Total biomass	
Plant height	1	.896**	.954**	.922**	.965**	.023	.970**	-.948**
Leaf number per plant	.896**	1	.784*	.881**	.780*	-.273	.829*	-.882**
Leaf area	.954**	.784*	1	.850**	.973**	.218	.952**	-.938**
Stem weight	.922**	.881**	.850**	1	.909**	-.283	.958**	-.836**
Leaf weight	.965**	.780*	.973**	.909**	1	.136	.990**	-.900**
Leaf to stem ratio	.023	-.273	.218	-.283	.136	1	.000	-.118
Total biomass	.970**	.829*	.952**	.958**	.990**	.000	1	-.898**
Specific leaf weight	-.948**	-.882**	-.938**	-.836**	-.900**	-.118	-.898**	1

*.Correlation is significant at the 0.05 level; **.Correlation is significant at the 0.01 level

4. Conclusion and Suggestions

Cloth detergent powder in high concentrations (2 and 20 g/L) can reduce seedling weight, seed vigor and leaf number per plant of maize probably by means of high osmotic potential, oxidative stress, salinity stress and heavy metal stress. At early growth stage, most maize growth parameters showed a reduction initiated from 2 g/L of detergent powder. Germination stage was more sensitive to detergent than the next stage (early growth stage). However under lower doses (< 2 g/L), the adverse effects of detergent were not observed, it is need to test plant quality traits to suggest this dose. Due to little information about effect of detergent powder, it is recommended to study effect of detergent on wide range of crop plants to find the tolerant crops for irrigation with contaminated water.

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Nominal system in Buzābādi, one of the north-eastern dialects of central Iran

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Abstract: The article is intended to have a systematic study on the nominal system and conjugation of nominals in Buzābādi dialect. Since Buzābādi is a dialect of central Iran and is considered as a western Iranian language, its nominal system is studied from three aspects: numerals (singular – plural), definite – indefinite nouns and gender (masculine- feminine). The research was carried out through synchronic methodology (describing language in a specific period). Having studied the results of the researches done on the dialects of cenral Iran, the researcher started her field study in Buzābād area. The data were basically gathered through observation, experience, questionnaires and interviews. Findings: _ As in all ancient Persian – rooted languages, nouns in this dialect are conjugated. _ Adjectives are conjugated based on the gender (feminine-masculine). _ Pronouns are conjugated based on the gender. Numerals (singular – plural) are conjugated based on the gender _ Based on the gender, some verbs are conjugated differently. _ Definite / indefinite indicators are specific characteristic of the dialect.

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Key words: Nominal system, Buzābādi, Definite-indefinite- North-western dialect of central languages, Numerals (singular-plural), gender (masculine-feminine)

Introduction

In central parts of Iran, Persian has gradually replaced local dialects even though local languages are still spoken in certain areas. This group of Northeastern languages is known as Central dialects. Based on the phonological systems of these dialects, some Iranologists classify them as: Northwestern, Northeastern, Southwestern and Southeastern dialects.

North eastern dialects¹, which are rather numerous, are spoken in the areas between Kashan and Natanz. Kashani dialects, which are spoken in Kashan, and its neighboring dialect, Arani, are considered the most Northern dialects. In the mountains located in the west of Kashan-Natanz road, people speak the following dialects: to the east of the road Gohrudi, Jwšaqāni, Abyānei, Farizandi, Yarani, Meymei, Kešei, Tari, Natanzi are spoken and at the edge of the desert (Kavir) Buzābādi and Bādrudi are spoken.

The research was carried out through synchronic methodology (describing language in a specific period). Having studied the results of the researches done on the dialects of cenral Iran, the researcher started her field study in Buzābād area. The data were basically gathered through observation, experience, questionnaires and interviews.

Buzābād:

Buzābādi /(Abuzeydābād) is located in southeastern Kashan, with a distance of 30 kilometers to Kashan, in a low land desert. It is one of Aran & Bidgol's towns located in the province of Esfahan.

The town functions as the population centre of the following villages: Hosein Abad, Kaqazi, Muhammad Abad, Yazdelan. Concerning the geographical location of Buzābād (a desert dead-end), the dialect could survive changes and events. However, with recent language changes, this dialect, as any other local dialect, is exposed to extinction. Also, since Buzābādi is not a written language, extinction threatens it most. In this regard, to enrich and to keep Iranian culture, describing the structure and the grammar of this dialect, preparing a lexicon of the language as well as doing a comparative study on this language and other Persian Languages seem inevitable. Buzābād as the documents and discoveries- historical and linguistic- indicate is a very old town.

Some of the Conjugational Characteristics:

1. Nominal Conjugations in different cases: merda (addressing), merdā (with indefinite suffix), merde (in noun as modifier and in plural form)
2. Having commonalities with certain eastern languages- Soqdi and Karazmi- such as lack of /e/ (sound) after a noun in possessive and certain adjectival structures (noun as modifier): ketāb man / ketāb-m/ (my book).
3. Gendered conjugation of adjectives describing masculine or feminine nouns: žange gordē (big woman), merdā gordā (big man) .

4. Compared to other dialects, in Buzabadi, pronouns _ and demonstrative adjectives_

have always kept their masculine and feminine aspects.

	Singular			Plural		
	First person	Second person	Third person	First person	Second person	Third person
Buzābādi	Ma	te	na,nōn	hama	šama	nōnū

Nominal system in Buzābādi:

Despite similarities, there are linguistic differences between north-eastern dialects of central accents and Buzābādi. Hence, studying structural particularities of the linguistic system of Buzābādi is of great importance. One of the specific characteristics of this dialect is the conjugation of nouns, which has been studied from the following aspects:

- gender (masculine – feminine)
- definite-indefinite
- numerals (singular-plural)

Gender (masculine – feminine):

- Demonstratives are used both as adjectives and personal pronouns. They also show the gender of the noun: na pūr (that boy), nēm dōt (this girl)
- The noun itself does not show the gender. The gender is given to a noun through the addition of the prefix –a for a masculine noun esbā (dog) and the suffix –e for a feminine noun lāse (feminine dog). (Lecoq, 1976:18)
- The word for water ,ow , is feminine.
- The names of the animals whose sex is not clear are always considered feminine: mōljī (cat)
- Gender can also be shown through the suffixes indicating the small size: espa-ja (a small masculine dog), easpe-je (small feminine dog)
- The indicator connecting a noun to its adjective or to its determiner is –e. That is, a at the end of a noun will change to –e when it comes with an adjective or noun determiner: kara (butter) Kare tāze (fresh butter).

- The past form of third person singular shows the gender itself bešā (he went) būšta (she went)
- In present perfect and past perfect tenses, the gender is clear in the first and second person verbs. The past participle used in these two tenses are –e for the feminine and –a for the masculine:
be` tātā eb` ādōy I have run (masc.)
be` tāte eb` ōyād I have run (fem.)
bo` rejoyā yō I have been seen (masc.)
bo` rejoye yō I have been seen (fem.)

Definite-Indefinite

In middle Persian languages, a noun would become indefinite by ēw, which is left from aiwa- in ancient Iran. Today also, in some dialects as in Zoroastrian middle Persian and in Buzābādi it is used instead of ē and ēw.

- To make a noun definite, the suffix –a (-ya after a vowel) is used šū-ya (your husband)
- The suffix –a, in non-subject position, changes into –e: pūrē ma my son pūrā boy
- The suffix ey is the same as ham in Persian, which is used in Bizooiy dialect quite often: nemuney these too
nemey these asp-ey the hourse already talked about.
- Dfinite noun indicators are i (meaning one), -e suffix or both: i pūrē one boy pūrē a boy

Numerals (singular-plural)

- In Buzābādi Dialect, when the concept of plurality is already in the utterance, singular form is preferred: ma pōwam tar ān my feet are wet

To stress the concept of plurality they use the word *pāk* before a noun.

pāk šev all the nights

pāk gerē all the knots

Only the nouns ended with the vowel –a will be pluralized with the –e indicator: *esbā* dogs

→ *esbē* the dog

- The plural indicator for both genders is (stressed) –e. Some times –on as a plural indicator is equally used :
dote-doton girls
- In this dialect singular form is preferred over the plural one.
- The plural indicator –ē is Buzābādi has a lot of similarities with the dialects of central Iran- concerning used stressed for both genders as well as adjectives and nouns.

Therefore, since their nominal, verbal, pronominal and adjectival systems- it has its own special characteristics. In this essay “nouns” have been studied from the point of singular-plural, definite-indefinite, and gender aspects.

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Effect of defoliation intensity on maize yield, yield components and seed germination

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Abstract: A field experiment and a laboratory experiment were conducted in 2011 to determine the effect of intensity of defoliation on yield of maize (*Zea mays*). The field experiment included seven defoliation intensities (0, 2, 4, 6, 8, 10 and whole leaves per plant) from top to bottom leaves. Seeds of the field experiment were used for the laboratory experiment. In the laboratory experiment, germination traits of seed produced from maternal plant under defoliation treatment were tested. Results showed that defoliation had a significant effect on seed yield, rows number per ear, seed number on row, cob length, cob weight and harvest index ($P < 5\%$). Seed yield was reduced by increasing defoliation intensity. The results suggest that the two upper leaves should not be defoliated, because this treatment has a remarkable negative effect on the seed and biological yield. Severe removal of leaves (T7) increased seed germination percentage, rate and vigor providing evidence for maternal environmental effects on germination. [Hassan Heidari. **Effect of defoliation intensity on maize yield, yield components and seed germination.** *Life Sci J* 2012;9(4):1594-1598] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 243

Key words: Defoliation; harvest index; maternal effect; seed vigor

1. Introduction

There are many causes for defoliation such as herbivores, hailstorms, wind, insect, diseases, herbicides and farm machinery. Use of leaves for feeding animal or human consumption can be considered in poor country. Erbas and Baydar (2007) reported that sunflower (*Helianthus annuus* L.) yields were reduced by 42% when 25 leaves per plant were removed at the preflowering stage. In cowpea (*Vigna unguiculata*), defoliation at podding stage and at intensity below 50% is recommended (Ibrahim et al. 2010). Maturity in maize was significantly affected by defoliation treatments and soluble-solid content in the stem reduced quickly after leaf removal (Tollenaar and Daynard 1987). Luzuriaga et al. (2006) reported that in *Sinapis arvensis*, addition of nitrogen to maternal environment reduced germination rate of seeds. In other research, seed germination percentage reduced due to increasing maternal nutrient and light levels (Galloway 2001). In *Vicia sativa*, seeds produced by plants in different defoliation treatments had similar germination percentage and germination time (Koptur et al. 1996). Maize is one of the most important warm season crop in west of Iran. In The area, foliage loss from some insects, diseases and hail result in economical problems for farmers. There are a few studies about effect of material plant environment such as defoliation on seed germination of crop plant, so the objective of this study was to determine maize (*Zea mays*) seed yield and seed germination traits at different levels of artificial defoliation.

2. Materials and Methods

2.1 Experiment 1

1. Site, experimental design and cultural practices: The field experiment was conducted at Chamchamal plain, 47 km from Kermanshah, west of Iran in 2011 (Latitude 34° N, longitude 47° E, and altitude 1300 m above sea level). Average annual rainfall of the zone is 442 mm (IMO 2012). The study was conducted as a randomized complete block design (RCBD) with three replications. There were seven defoliation levels:

- T1: Control, no leaf removal
- T2: removal of 2 leaves
- T3: removal of 4 leaves
- T4: removal of 6 leaves
- T5: removal of 8 leaves
- T6: removal of 10 leaves
- T7: removal of all leaves

At summer after harvesting the wheat (*Triticum aestivum*), the soil was plowed by mouldboard plowing. Maize seeds (*Zea mays*, CV S.C. 704) were sown on April 18, 2011 using a pneumatic maize seeder. Row spacing and plant spacing within row were 75 and 17 cm, respectively. Seed emerged by rain water. Irrigation interval was 8-day and plants were irrigated 8 times. 625 kg ha^{-1} of urea fertilizer (46=N%, CO (NH₂)₂), was applied as split application and top dressing. 375 Kg ha^{-1} of triple super phosphate (P₂O₅%=46, Ca (H₂PO₄)₂) was applied as presowing.

Weeds were controlled by hand weeding and Nicosulfuron (Cruz) herbicide (3-Pyridimicarboxamide,2-[[[4,6-Dimethoxy-pyrimidin-2-yl) amino-carbonyl]aminosulfonyl]-N,N-dimethyl).

Plot size was 3 m wide and 3 m long. The distances between plots and between replications were both 1.5 m. Plants were well-watered during the growth season and defoliation treatments were imposed at silking stage (93 days after sowing).

2. Plant sampling and measurements: Plant samples were taken by selecting five plants per plot. Up to 50 cm of primer line and edge line were discarded. In order to measure the seed yield and total dry matter, five plants were cut and after drying, dry matter (biological yield) and seed yield were measured as gram per plant. Plants were harvested when they yellowed (140 days after sowing). Row number per cob, seed number per row and cob length were measured on three ears per plot by random selection. Harvest index was computed as the ratio of the grain to the aboveground dry matter at harvest. In this study, the effects of varying defoliation levels on stem and leaf weight, cob weight, ear skin weight, ear weight and 100-seed weight were also evaluated. These traits were measured by random selection of five plants per plot and they were weighed after drying.

2.2. Experiment 2

After harvesting seeds from maternal plants, they were stored at 25°C for three months. Seeds of the field experiment were used for the laboratory experiment. In the laboratory experiment, germination traits of seed produced from maternal plant under different defoliation treatments were tested to study the effect of maternal environment. The study was conducted as a factorial experiment in a Randomized Complete Block Design with three replications in 2011.

Before starting the trial, seeds were sterilized using sodium hypochlorite solution (1% active chlorine) for 10 min to avoid fungal contamination. Twenty seeds were then placed in each Petri dish and 10 mL of distilled water added. Temperature during experiment was kept at $26 \pm 1^\circ\text{C}$. Two millimeters growth of coleoptile was the criterion for germination. Following formula estimated germination rate (GR, Zareyan et al. 2010):

$$GR = a/1 + b/2 \dots + n/N$$

Where a, b, n are germinated seed number and 1, 2, N are day after trial beginning. Seed vigor estimated by these equations (Sharifzadeh, 2006; Abasian, 2010):

$$\text{Seed vigor (\% cm)} = [(\text{Radicule length (cm)} + \text{Caulicle length (cm)}) * (\text{Germination percentage (\%)})]$$

$\text{Seed vigor (\% g)} = [(\text{Radicule weight (g)} + \text{Caulicle weight (g)}) * (\text{Germination percentage (\%)})]$

The trial period was 7 days and germination percentage was recorded every day.

2.3. Statistical analysis

Analysis of variance (ANOVA) was used to determine significant differences. The Multiple Range Test of Duncan performed the separation of means ($P < 0.05$). Correlation coefficients were calculated for the relationship between several crop parameters. All statistics were performed with the program MINITAB (version 14.0), SAS (version 9.1) and SPSS (version 16.0).

3. Results and Discussions

3.1. Experiment 1

1. Stem and leaf weight: Defoliation did not have significant effect on stem and leaf weight of maize (Table 1). It is probably due to that stem and leaf weight growth was partially completed at silking stage and defoliation at this stage only had a negative effect on seed filling, because seed yield was reduced by increasing defoliation intensity (Table 1). Ahmadi and Joudi (2007) did not observe significant difference among grain yields of wheat (*Triticum aestivum*) under defoliation treatments.

2. Ear skin weight and ear weight: Control, no leaf removal had the highest ear skin weight (except compared to T2,) and the difference among other treatments was not significant (Table 1). T1 had the highest ear weight and T6 and T7 had the lowest ear weight (Table 1). The result shows that presence of two above leaves is important to form ear with thick and big skin. This skin photosynthesis and reserves had a remarkable effect on row number per ear, cob length, cob weight (Table 2). Barimavandi et al. (2010) reported that the upper leaves should not be defoliated, due to their negative effect on the seed yield. This leaves are more efficient in absorbing light than lower leaves.

3. Row number per ear and seed number per row: T1 had higher row number per ear than T5, T6 and T7 (Table 1). T6 had lower seed number per row than other treatments and the difference among other treatments was not significant (Table 1). Barimavandi et al. (2010) reported that the row numbers per ear only was affected by complete defoliation; it is due to that stem reserves can compensate insufficient photosynthesis from leaves. Row number per ear had a remarkable effect on seed yield (Table 2).

4. Cob length and cob weight: T1 had higher cob length than T6 and T7 (Table 1). With increasing defoliation intensity, cob weight was decreased. This negative correlation was reported by other researchers (Zewdu and Asregid 2001). Cob length and weight had an important role in increasing seed yield and harvest index (Table 2). Fasae et al. (2009) reported that defoliation at 12 and 16 weeks after maize planting had no significant effect on cob length.

5. *Seed yield and biological yield:* Seed yield and biological yield were reduced as defoliation increased. Control, no leaf removal had the highest seed yield (Table 1). This shows the importance of upper leaves in absorbing light. Hassen and Chauhan (2003) reported similar results. Some reasons for higher seed yield of T1 is increasing cob length and row number per ear (Table 1, Table 2). Reduction in leaf area reduces resources for grain filling (Koptur et al. 1996).

6. *Harvest index and 100-seed weight:* T1 had higher harvest index than T6 and T7 (Table 1). This shows that with severe removal of leaf, partitioning of assimilate changes and less assimilate moves from reserves such as stem toward seeds. Increasing of

100-seed weight, cob length, cob weight and row number per ear resulted in higher harvest index (Table 2). There was minor difference among defoliation treatments in terms of 100-seed weight and only removal of whole leaves reduced 100-seed weight. Maposse and Nhampalele (2009) reported that as the intensity of defoliation increased, 100-seed weight decreased. It seems that seed weight is more dependent on genetic factors than environmental factors and environmental stresses and cultural factors can not reduce seed weight a lot because the plant provides the least required nutrients for each seed by reducing the number of seed (Heidari Zolleh et al. 2009).

Table 1. Effect of defoliation treatments on maize traits

^a Treatments	^b Stem and leaf weight (g/plant)	Ear skin weight (g/plant)	Ear weight (g/plant)	Row number per ear	Seed number per row	Cob length (cm)	Cob weight (g/plant)	Seed yield (g/plant)	100-seed weight (g)	Biological yield (g/plant)	Harvest index (%)
T1	86 a	8.6 a	186.3 a	117.0 a	42.6 a	54.0 a	24.1 a	159.1 a	27.0 a	280.9 a	0.57 a
T2	83.5 a	6.66 ab	138.8 b	86.3 ab	37.0 a	45.3 ab	18.3 ab	117.8 b	30.0 a	228.9 b	0.51 ab
T3	76.7 a	4.86 b	120.1 b	82.3 ab	42.6 a	43.0 ab	15.4 bc	103.6 b	28.6 a	201.6 bc	0.51 ab
T4	91.6 a	5.06 b	100.7 b	83.0 ab	42.0 a	41.3 ab	12.3 bc	87.6 b	27.0 a	195.8 bc	0.51 ab
T5	82 a	5 b	95.4 b	65.6 bc	40.6 a	42.0 ab	12 c	83.2 b	29.0 a	182.4 cd	0.45 ab
T6	119.3 a	4.2 b	28.8 c	28.6 c	10.0 b	33.9 b	5.1 d	25.06 c	23.6ab	144.4 de	0.37 bc
T7	77.8 a	5.8 b	34.7 c	47.3 bc	32.0 a	38.5 b	6.1 d	30.32 c	17.6b	118.3 e	0.27 c

^a T1, T2, T3, T4, T5, T6, T7 are defoliation intensities (0, 2, 4, 6, 8, 10 and whole leaves per plant, respectively)

^b Means followed by the same letter within each column are not significantly different at $P < 0.05$ as determined by Duncan's Multiple Range Test

Table 2. Pearson's correlation coefficients among studied traits in maize under different defoliation treatments

	SLW	ESW	EW	RNE	SNR	CL	CW	SY	HSW	BY	HI
SLW	1	-.359	-.430	-.514	-.841*	-.497	-.421	-.434	-.142	-.239	-.182
ESW	-.359	1	.752	.762*	.442	.890**	.799*	.745	.119	.743	.455
EW	-.430	.752	1	.971**	.719	.953**	.995**	1.000**	.697	.979**	.908**
RNE	-.514	.762*	.971**	1	.808*	.952**	.961**	.973**	.583	.931**	.865*
SNR	-.841*	.442	.719	.808*	1	.711	.678	.727	.476	.583	.609
CL	-.497	.890**	.953**	.952**	.711	1	.965**	.952**	.473	.920**	.757*
CW	-.421	.799*	.995**	.961**	.678	.965**	1	.993**	.650	.977**	.874*
SY	-.434	.745	1.000**	.973**	.727	.952**	.993**	1	.700	.978**	.911**
HSW	-.142	.119	.697	.583	.476	.473	.650	.700	1	.705	.859*
BY	-.239	.743	.979**	.931**	.583	.920**	.977**	.978**	.705	1	.929**
HI	-.182	.455	.908**	.865*	.609	.757*	.874*	.911**	.859*	.929**	1

SLW, ESW, EW, RNE, SNR, CL, CW, SY, HSW, BY, HI are stem and leaf weight, ear skin weight, ear weight, row number per ear, seed number per row, cob length, cob weight, seed yield, 1000-seed weight, biological yield, harvest index.

*.Correlation is significant at the 0.05 level

**..Correlation is significant at the 0.01 level

3.2. Experiment 2

1. *Seed germination percentage and rate:* T7 had the highest germination percentage and rate (Table 3). This shows that severe removal of leaves increased seed germination and rate. Seed germination percentage and rate had a positive and significant correlation with whole traits except seedling weight (Table 4). Galloway (2001) reported that increasing maternal nutrient and light levels decreased seed

germination percentage and Luzuriaga et al. (2006) reported similar results. Koptur et al. (1996) reported that defoliation treatments on maternal plant did not have significant effect on days to germination in the common vetch (*Vicia sativa*). Increasing seed germination percentage and rate maybe due to that under severe defoliation, more light can penetrate in canopy that can increase evaporation from soil and

dry it. Water-stressed plants produce lower seed mass but with higher seed germination (Luzuriaga, 2006).

2. *Shoot length and root length:* Defoliation treatments had no significant effect on seedling shoot length, but T7 had the highest root length (Table 3). Shoot length and root length had a positive and significant correlation with whole traits except seedling weight (Table 4). Contreras (2007) reported that severe water stress during lettuce (*Lactuca sativa* L.) seed production on maternal plant increased seedling radical length.

3. *Seedling weight and vigor:* T2 and T7 had the highest seedling weight except compared to T6 (Table 3). T2 and T7 had the highest vigor based on weight and T7 had the highest vigor based on length (Table 3). Seedling weight had a positive and significant correlation with vigor based on weight (Table 4). Contreras (2007) reported that watering treatments during lettuce (*Lactuca sativa* L.) seed production on maternal plant did not affect seed vigor index. Removal of whole leaves (T7) produced the lowest 100-seed weight (Table 1) but with the highest seed germination percentage, seed germination rate, seedling root length and seed vigor. This shows that under environmental stresses such as defoliation, plant produces lower and lighter seed but with higher seed germination traits. It may be a mechanism for survival.

4. Conclusions and suggestions

Seed yield and biological yield were reduced as defoliation increased. No leaf removal had the highest seed yield, biological yield and ear weight. Removal of whole leaves (T7) had the lowest seed yield and biological yield, but had the highest seed germination percentage, rate, seed vigor and seedling root length providing evidence for maternal environmental effects on germination. Regarding few reports about maternal environment effects on seed traits, it is recommended to study effect of other environmental factors such as light by defoliation leaves under and at the top of ear, nutrients and water on seed germinability and storability.

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Table 3. Effect of defoliation treatments on maize seed traits

^a Treatments	^b Germination (%)	Germination rate (no/day)	Shoot length (cm)	Root length (cm)	Seedling weight (g)	Vigor (% g)	Vigor (% cm)
T1	43.33 bc	3.0300 bc	5.370 a	10.250 b	0.183 c	0.086 b	9.276 bc
T2	65.00 b	4.5533 b	6.253 a	11.153 b	0.297 a	0.192 a	11.184 b
T3	43.33 bc	3.1600 bc	5.460 a	7.093 b	0.187 c	0.078 b	5.266 cd
T4	43.33 bc	2.7000 bc	5.707 a	9.920 b	0.1737 c	0.078 b	8.197 bc
T5	48.33 bc	3.5933 bc	5.662 a	7.870 b	0.1837 c	0.096 b	9.328 bc
T6	25.00 c	1.5267 c	5.387 a	9.050 b	0.197 bc	0.052 b	3.672 d
T7	98.33 a	7.4033 a	7.660 a	17.967 a	0.2637 ab	0.259 a	25.186 a

^aT1, T2, T3, T4, T5, T6, T7 are defoliation intensities (0, 2, 4, 6, 8, 10 and whole leaves per plant, respectively)

^b Means followed by the same letter within each column are not significantly different at $P < 0.05$ as determined by Duncan's Multiple Range Test.

Table 4. Pearson's correlation coefficients among studied traits in maize seed under different defoliation treatments

	Germination percent	Germination rate	Shoot length	Root length	Seedling weight	Vigor (weight)	Vigor (length)
Germination percent	1	.996**	.962**	.874*	.713	.972**	.965**
Germination rate	.996**	1	.950**	.852*	.689	.960**	.961**
Shoot length	.962**	.950**	1	.933**	.714	.952**	.959**
Root length	.874*	.852*	.933**	1	.631	.873*	.940**
Seedling weight	.713	.689	.714	.631	1	.854*	.594
Vigor weight	.972**	.960**	.952**	.873*	.854*	1	.919**
Vigor length	.965**	.961**	.959**	.940**	.594	.919**	1

*.Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

6. References

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Assessment of Homocysteine Plasma Levels and Insulin Resistance among Obese Women with Anovulatory Infertility

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Abstract: This study was conducted to assess homocysteine plasma level and insulin resistance profile (blood glucose level, plasma insulin level, HOMA-IR, body mass index) among different groups (non-obese, over-weight and obese) of women with anovulatory infertility. This cross-sectional study was conducted in Suez Canal University hospital in the period from December 2011 to August 2012. Total of 150 women with anovulatory infertility were included in this study, divided equally into three groups: non-obese, over-weight and obese. Blood samples were collected in second or third day of menstrual cycle for laboratory work-up. Hormonal profile and insulin resistance profile were determined for each patient. Plasma level of homocysteine was determined using the commercially available ELISA kit. Results showed that there were statistically significant differences between the three groups regarding homocysteine plasma level, body mass index and HOMA-IR with p -value < 0.001 . There was a significant association between homocysteine plasma level and BMI. BMI and serum testosterone level were higher in obese and over-weight women in comparison to non-obese patients. Positive correlations were found between homocysteine plasma level with insulin level, HOMA-IR and LH/FSH ratio. In conclusion, homocysteine plasma level is positively correlated with BMI, insulin resistance, testosterone level and LH/FSH ratio in over-weight and obese infertile women. This highlights an interaction between high homocysteine level, insulin resistance and hyperandrogenemia, mimicking polycystic ovarian syndrome that could be responsible for the infertile state in these patients.

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Keywords: Homocysteine, Anovulation, Polycystic ovarian Syndrome, Insulin resistance, Body mass index

1. Introduction

Infertility occurs in 13 to 21% of married couples of reproductive age. One of the most common causes of infertility in women of reproductive age is chronic anovulation (ESHRE Capri, 2012). Polycystic ovary syndrome (PCOS) is responsible for 70% of cases of anovulatory infertility (Thanyarat *et al*, 2012).

Many authors showed that there is a strong correlation between plasma homocysteine concentrations and BMI (Elshorbagy *et al*, 2008; Zoppini *et al*, 2008; Elshorbagy *et al*, 2009) homocysteine levels may be elevated in patients with chronic anovulation and PCOS and may play a role in endothelial damage that occurs in these patients (Stefano *et al*, 2009).

Homocysteine is derived from the metabolic conversion of the essential amino acid methionine. Metabolism of homocysteine is via one or two pathways either trans-sulfuration or re-methylation. In the re-methylation pathway of homocysteine to methionine, vitamin B12 and folate act as cofactors (Fowler, 1997). One of the essential enzymes in the re-methylation process is methylene-tetra-hydro-folate reductase (MTHFR) (Goyette *et al*, 1994).

Hyperhomocystenemia occurs mainly due to genetic defect in this enzyme & other deficiencies in vitamin cofactors (folate Vitamin B 12). It may also be associated with certain chronic medical conditions and drugs such as fibrates and nicotinic acid (Cabarkapa *et al*, 2007).

Major determinants of plasma homocysteine levels are folate, vitamin B12 and B6 intake, renal function, and to a lesser extent cigarette smoking, arterial hypertension, hypercholesterolemia, physical exercise, coffee consumption, and alcohol consumption (Kazemi *et al*, 2006). In addition, individuals homozygous for the thermo-labile form of MTHFR also show higher levels of homocysteine, mainly in the presence of low folate (Rozen, 1997).

Hyperhomocystenemia is an independent risk factor for atherosclerotic vascular disease, cerebrovascular events and recurrent venous/arterial thrombo-embolism (Dierkes *et al*, 2004). The mechanisms by which hyperhomocystenemia may predispose to arterial thrombosis are not entirely clear but consist of endothelial cell damage (Blundell *et al*, 1996), inhibition of fibrinolysis (Bienvenu *et al*, 1993), activation of the coagulation cascade (Freyburger *et al*, 1997), impaired generation of

nitric oxide and prostacyclin (Stamler *et al.*, 1993), and enhanced collagen production by smooth muscle cells (Majors *et al.*, 1997).

Hyperhomocystenemia can induce insulin resistance (Welch and Loscalzo, 1998) leading to compensatory hyper-insulinemia, which may impair the activity of MTHFR and CBS enzymes leading to accumulation of homocysteine in plasma (Dicker-Brown *et al.*, 1999). Thus insulin levels have also been observed as a modulating factor of homocysteine as it inhibits hepatic cystathione β -synthase activity (McCarty, 2000). Insulin resistance or consuming a high insulinaemic index diet will tend to increase plasma homocysteine (Meigs *et al.*, 2001).

This study was carried out to evaluate homocysteine plasma level and its correlation with insulin resistance in non-obese, over-weighted and obese women with anovulatory infertility.

2. Methods:

This cross-sectional study was conducted in Suez Canal University hospital in the period from December 2011 to August 2012. This study was approved by the Ethics Committee of the Suez Canal University and carried out in accordance with the principles of Helsinki Declaration. One hundred and fifty infertile women were included in this study divided equally into three groups: non-obese, over-weight and obese.

Exclusion criteria included women with hyperprolactinemia, chronic diseases, endocrinal diseases, women on medications such as steroids, hypertension, diabetes mellitus, cardio-vascular diseases, any other medications known to affect plasma level of homocysteine.

An informed consent was obtained from each patient. All patients underwent physical and laboratory work-up. Physical examination included measurement of body weight, height and calculation of BMI. Patients were classified according to their BMI to non-obese (<25 kg/m²), over-weight (25-30 kg/m²) and obese (>30 kg/m²).

Blood samples were taken from each patient on the second day of the menstrual cycle. Fasting blood glucose was determined using fully-automated auto-

analyzer Hitachi 912 (Roche Diagnostics, Germany). Levels of fasting insulin, testosterone, TSH, FSH and LH were determined using chemiluminescent enzyme immune-assay techniques on Cobas e411 (Roche Diagnostics, Germany). Homocysteine plasma level was determined using commercially available kit DRG® Homocysteine (DRG International Inc., USA).

Insulin resistance was determined after using Homeostasis Model Assessment Insulin Resistance (HOMA-IR). HOMA-IR more than 2.5 was considered insulin resistant.

Anovulation was diagnosed clinically using folliculometry and low mid-luteal serum progesterone level. Trans-vaginal ultrasound was done for every patient on days 9, 11, and 13 of the menstrual cycle. Anovulation was diagnosed when there is failure of the ovaries to produce mature follicle in two successive cycles.

Collected data was analyzed using the Statistical Package for Social Sciences version 13 (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as means and standard deviations (SD). Comparison between study groups was done using analysis of variance (ANOVA). The means of continuous variables were compared by student 't' test. Association between characteristics and laboratory test results were assessed by Pearson's bivariate correlation analysis. Statistical significance was set at *p*-value less than 0.05.

3. Results:

This study included 150 infertile women divided equally into three groups with comparable age. However, there were significant differences between the three groups regarding BMI, homocysteine plasma level, insulin level and HOMA-IR with *p*-value < 0.001 (Table 1).

Hyperhomocystenemia was found in 75% of the obese women; however it affected only 31% of the over-weight and 3.5% of the non-obese women. This suggests a significant association between high homocysteine plasma level and high BMI. HOMA-IR was higher among obese women than among over-weighted and non-obese women with *p*-value <0.001 (Table 1).

Table 1: Comparison between the three study groups:

Character	Obese	Over-weighted	Non-obese	<i>p</i> -value
Age (years)	29 ± 7	28 ± 8	26 ± 7	0.687
BMI (kg/ m ²)	32.2 ± 0.94	27.6 ± 1.38	23 ± 0.96	< 0.001
FBS (mg/ dl)	92 ± 12	89 ± 15	91 ± 13	0.819
Insulin level (μU/ ml)	27.4 ± 9.91	15.6 ± 8.5	11.4 ± 2.8	< 0.001
Homocysteine (μmol/ L)	15.78 ± 4.34	10.19 ± 5.32	8.66 ± 2.14	< 0.001
HOMA-IR	6.02 ± 1.96	3.08 ± 0.98	2.12 ± 0.89	< 0.001

BMI: Body Mass Index, FBS: Fasting Blood Sugar, HOMA-IR: Homostasis Model Assessment Insulin Resistance

About 56% of over-weight and obese women had insulin resistance. When both obese and over-weight women (100 women) were categorized according to insulin resistance, homocysteine plasma level was significantly higher in those with insulin

resistance than those without insulin resistance with p -value < 0.001. In addition, there were statistically significant differences between both groups regarding BMI, Insulin level and HOMA-IR with p -value < 0.001 (Table 2).

Table 2: Categorization of obese and over-weighted women according to insulin resistance:

Character	With insulin resistance	Without insulin resistance	p -value
Age (years)	29 ± 5	28 ± 6	0.88
BMI (kg/ m ²)	30.2 ± 1.11	26.3 ± 1.09	< 0.001
FBS (mg/ dl)	93 ± 10	87 ± 11	0.461
Insulin level (μU/ ml)	29.21 ± 5.19	8.71 ± 3.22	< 0.001
Homocysteine (μmol/ L)	15.11 ± 3.66	9.22 ± 4.08	< 0.001
HOMA-IR	6.23 ± 1.61	1.88 ± 0.44	< 0.001

BMI: Body Mass Index, FBS: Fasting Blood Sugar, HOMA-IR: Homostasis Model Assessment Insulin Resistance

Regarding the hormonal parameters, comparisons between the three groups showed no significant differences in the level of TSH, FSH or

LH. However, a significant difference was detected in the testosterone level (p value < 0.001) (table 3).

Table 3: Sex hormone levels in the three study groups:

Item	Obese	Over-weighted	Non-obese	p -value
LH (IU/ ml)	12.44 ± 5.98	9.61 ± 3.76	7.54 ± 2.89	0.468
FSH (IU/ L)	6.81 ± 2.71	6.18 ± 3.01	4.89 ± 1.89	0.729
TSH (mIU/ L)	4.81 ± 2.38	4.21 ± 1.91	3.45 ± 2.02	0.382
Testosterone (nmol/ L)	2.26 ± 0.79	1.74 ± 0.82	1.27 ± 0.67	< 0.001

LH: Luteinizing Hormone, FSH: Follicular Stimulating Hormone, TSH: Thyroxin Stimulating Hormone

LH/FSH ratio was higher among obese and over-weight women with insulin resistance than those without insulin resistance with p -value 0.002 (Table 4). Although the level of total testosterone was also

higher among infertile women with insulin resistance than those without insulin resistance, but there was no significant difference between the two groups with p -value 0.191 (Table 4).

Table 4: Hormonal profile among obese and over-weight women with and without insulin resistance:

Item	With insulin resistance	Without insulin resistance	p -value
LH (IU/ ml)	13.39 ± 4.21	8.21 ± 3.02	0.002
FSH (IU/ L)	6.15 ± 3.19	6.78 ± 2.83	0.816
LH/FSH ratio	2.39 ± 1.54	1.42 ± 1.08	0.002
TSH (mIU/ L)	5.03 ± 2.19	3.78 ± 1.72	0.271
Testosterone (nmol/ L)	2.03 ± 0.78	1.81 ± 0.89	0.191

LH: Luteinizing Hormone, FSH: Follicular Stimulating Hormone, TSH: Thyroxin Stimulating Hormone

Correlation analysis was done between homocysteine plasma level and different demographic and laboratory parameters. Homocysteine plasma level was positively correlated with BMI, insulin level, fasting blood glucose level,

LH/FSH ratio, HOMA-IR and testosterone level. However, no correlation was found between homocysteine plasma level and patients' age, TSH level, LH level or FSH level (Table 5).

Table 5: Correlation analysis between homocysteine plasma level and different demographic and laboratory items:

Item	r	p
Age	- 0.256	0.411
Body mass index	0.521	< 0.001
Fasting blood glucose level	0.431	0.006
Insulin level	0.361	< 0.001
Luteinizing hormone level	0.295	0.318
Follicular stimulating hormone level	- 0.199	0.461
LH/FSH ratio	0.493	< 0.001
Thyroxin stimulating hormone level	- 0.271	0.384
Testosterone	0.417	< 0.001
Homostasis Model Assessment Insulin Resistance	0.562	< 0.001

4. Discussion:

Homocysteine is normally a sulfur amino acid that is formed by the trans-methylation of methionine amino acid. It can retransform into methionine amino acid by re-methylation, accompanied by folate and vitamin B12, as well as into cysteine amino acid by cystathionine- β -synthase enzyme mediated with vitamin B6. Cystathionine- β -synthase enzyme deficiency is associated with premature atherosclerosis and recurrent thrombo-embolic events in homocystinuria. In addition, nutritional (folic acid and vitamins B6 and B12), genetic (methylene tetrahydrofolate reductase gene mutations), and endocrine factors, as well as cancer, human immunodeficiency virus, and renal failure, have been claimed as conditions responsible for moderately high levels of homocysteine (Glowinska *et al.*, 2003).

Recently, there have been several studies showing an association between plasma level of homocysteine and obesity. However, some conflicting results were also reported. In this study, results revealed changes in the homocysteine levels according to BMI and insulin resistance among the three study groups.

The study revealed higher homocysteine levels as well as more insulin resistance (represented by HOMA-IR) among obese infertile women than among over-weight and non-obese infertile women. This noticed the association of increases in the insulin resistance with higher BMI. Homocysteine plasma levels were highly correlated with BMI and insulin resistance (HOMA-IR) among obese women, with no correlation with patient age. These results are in agreement with a study done by Howard *et al.* who concluded a significant interaction between increasing obesity and insulin resistance (Howard *et al.*, 2004). Conversely, Vivian Fonseca *et al.* (2003) concluded that there was no correlation between homocysteine levels and BMI.

Although patients in the three groups were of comparable age, homocysteine plasma levels were significantly different. However, Henry *et al.* concluded that plasma homocysteine levels showed an increasing trend with age (Henry, 2011).

In our study, results revealed higher insulin levels among obese women than among both over-weight and non-obese ones. In a prospective study on middle aged women, Guthrie *et al.* (2001) found that although increases in insulin levels were independent of age, they were positively associated with increases in BMI. Siegfried *et al.* (2000) concluded in their study that insulin is a main correlate of homocysteine in obese children and adolescents and suggested that hyperinsulinism may contribute to impairment of homocysteine metabolism in childhood obesity.

The coexistence of severe insulin resistance and hyper-insulinemia has been demonstrated, whereby hyperinsulinemia is considered secondary to the defects in insulin action but has also been implicated in the development and maintenance of excess obesity. In agreement with the assumption that hyperinsulinemia contributes to elevated homocysteine levels,

Jacobs *et al.* (1998) demonstrated an increased activity of trans-sulfuration enzymes and consecutive decreased homocysteine levels in rats with streptozotocin induced diabetes. This effect was reversible after insulin treatment.

In this study, results showed higher homocysteine levels among obese and over-weight women with insulin resistance than among those without insulin resistance. This finding was comparable to results of a study done by James *et al.* (2001) who had reported a positive association between levels of plasma homocysteine and some individual traits associated with insulin resistance.

Sex steroid hormones and androgens appeared to influence the metabolism of homocysteine and have been found to increase its plasma levels. In the present study, results revealed association between homocysteine levels and testosterone level as well as LH/FSH ratio. These findings might suggest the association of Polycystic Ovarian Syndrome (PCOS) with this state of hyper-androgenemia and high homocysteine plasma level. Similar results were obtained by Sachan *et al.* (2012). In contrast to this finding, George E *et al.* found that DHEAS and testosterone level were not related to homocysteine level (George *et al.*, 2006). However, Randolph *et al.* (2006) found, in his cross-sectional study, variations in all body hormonal assays, positively with testosterone level and negatively to all the others. A genetic study done by Maristella *et al.* highlighted that homocysteine metabolism may be involved in patho-physiology of these cases of un-explained female sterility (UFS) because of the association between hyperhomocysteinemia, low serum folate and TT genotype of MTHFR (Maristella *et al.*, 2007).

In the current study, results revealed positive correlations between homocysteine levels and BMI, insulin levels and HOMA-IR. Similarly, Gideon *et al.* (2007) also reported that homocysteine levels were higher in metabolic syndrome patients compared to patients without metabolic syndrome. But contrary to, the results of a study by Tanrikulu- Kilic *et al.* (2006) who reported that plasma homocysteine concentration was not related to insulin resistant.

Conclusion:

The study results revealed correlations between homocysteine plasma levels and BMI and HOMA-IR,

as well as with LH/FSH and testosterone level. This suggests that the interaction of high homocysteine level, insulin resistance and hyperandrogenemia may create a state mimicking polycystic ovarian syndrome that could be responsible for infertile state of these patients.

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Natural Radioactivity Levels in Environmental Samples (Iron and Copper) in the Arabian Shield, the Western Part of Saudi Arabia

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Abstract: The Arabian shield, in the western part of Saudi Arabia has significant iron and copper ore deposits, they have promising economic potential with their reserves. Nine rock samples were collected from different areas of it and prepared for analysis. XRD was applied to determine the mineral composition of the samples, which revealed that the major minerals are QUARTZ (mostly in all samples), MAGNETITE, HEMATITE, CORUNDUM, GOETHITE, MONTMORILLONITE, PYRITE, ANKERITE, BOEHMITE, SPINEL and ALBITE, with additional minor and trace minerals. Samples were analyzed for concentrations of ^{238}U , ^{232}Th , and ^{40}K by the gamma spectrometer based on hyper pure germanium detector "HPGe" crystal. For ^{238}U concentrations, the values in Bq/kg dry weight ranged from 152.68 to 264.73 (for iron ore), 156.37 and 329.98 (for copper ore). For ^{226}Ra , the activities of ^{214}Pb and ^{214}Bi in equilibrium with parent (^{226}Ra) were used to calculate the concentrations in Bq/kg dry weight, the average values ranged from 2.50 to 386.30 (for iron ore), 57.41 and 1048.01 (for copper ore). While the activities of ^{232}Th series were calculated from daughters ^{228}Ac , ^{212}Bi , and ^{208}Tl , the average concentrations in Bq/kg dry weight ranged from 1.50 to 183.90 (for iron ore), 43.66 and 44.41 (for copper ore). ^{40}K concentration values in Bq/kg dry weight ranged from 2.70 to 186.99 (for iron ore), 48.92 and 191.33 (for copper ore), and the ^{235}U concentrations in Bq/kg dry weight ranged from 8.35 to 13.70 (for iron ore), ND and 18.37 (for copper ore). the Ra_{eq} Bq/kg dry weight which ranged from 10.88 to 333.59 (for samples 1,2,3, 6,7,9), they are less than 370 the permissible value adopted by EPA and UNSCEAR (2000), while for samples 4, 5 were 610.63 and 662.56 which they are higher than the value 370, for sample 8 (Jabal Sayid) has the highest value 1114.21 (Bq/kg).

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Keywords: Arabian shield- iron ore and copper ore- MAGNETITE- HEMATITE- Ra_{eq} Bq/kg

1. Introduction

Saudi Arabia has a significant iron ore (Wadi Sawawin and Wadi Fatima) and copper ore (Jabal Sayid) deposits. These locations in the west central Arabian Shield in the Kingdom have promising economic potential with their reserves, as shown by several studies. Hematite (iron ore) from Wadi Fatima was analyzed by instrumental neutron activation analysis using ^{59}Fe ($^{241}\text{Am} - \text{Be}$) neutron irradiation facility. More than 35 gamma-ray lines were identified as well as the concentration of Mn in hematite was obtained to be about 0.332 (Hassan *et al.*, 1994). Chemical analysis of the ore shows that it has an average content of 41.27% iron (corresponding to about 58.96% hematite). X-ray analysis shows that the ore contains goethite in addition to hematite, The upgrading process consists of several steps to raise the hematite content of the ore to about 87.11 weight percent (Manieh, 1986).

Measuring the activity concentrations due to ^{226}Ra and ^{232}Th for hematite samples from two different locations (Abu Aggag and Um Gereifat) areas in the Eastern Desert of Egypt. The obtained results indicated that; samples from Um Gereifat area

have activity concentrations higher than that of Abu Aggag area (Ahmed *et al.*, 2007).

The Palaeoproterozoic Murphy Inlier is situated at the southern end of the McArthur Basin in Northern Australia. The inlier contains over uranium, copper, tin and base metal occurrences (Mernagh, 2011).

The ironstones of Wadi Al shemysi are mainly enclosed within the middle part of the fluvio-lacustrine siliciclastic succession which is consisted mainly of conglomerates, sandstones, siltstones, muddy, sandy and glauconitic ironstones, fresh water carbonates, tuffaceous mudstone and basalts in descending order. The most important diagenetic process in ironstone is the dehydration and recrystallization of the amorphous Fe-clays and formation of goethite and hematite cement (Mesaed *et al.*, 2012). The trace element contents in two copper minerals (brochantite and native copper) were determined using k_0 -NAA before and after quantitative removal of copper by electrolysis. This work confirmed that the content of some trace elements (Na, K, Rb, Cs, Sb, Pt and Zn) was higher after Cu removal in chalcopyrite (CuFeS_2) and chalcantite ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (Taseska *et al.*, 2012).

This study is aimed to determine the activity concentrations of naturally levels radionuclides in iron and copper ore samples that have been collected from different locations of the Arabian shield. The activity concentrations of ²³⁸U and ²²⁶Ra have been inferred from gamma-ray transitions associated with their decay progenies and measured using a hyper-pure germanium detector. Details of the samples' preparation are presented, the values of the activity concentrations and ²²⁶Ra equivalent are measured.

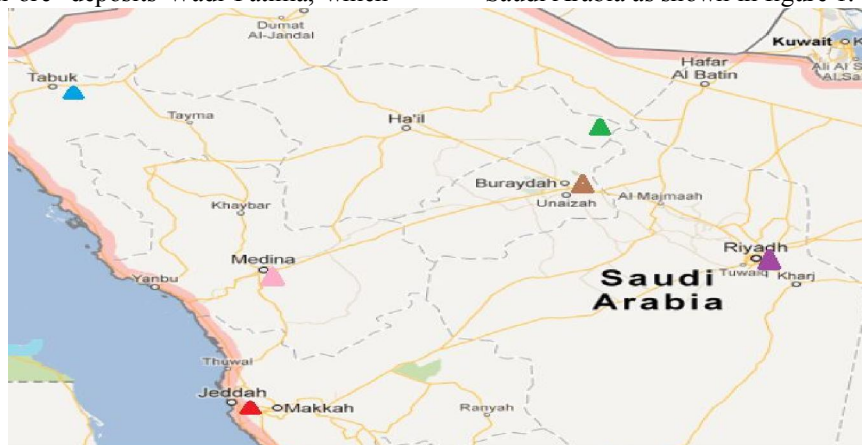
Geological setting

Many types of mineral deposits in Saudi Arabia are wide spread, the bulk of metallic mineral resources are contained in Precambrian rocks of the Arabian shield, in the western part of the country. For example deposits of base metals such as iron and copper. The largest known iron ore deposit in the Kingdom is Wadi Assawawin (extends over a belt measuring 15-20 by 25 km SW of Tabuk). This deposit holds reserves of 84 million tons at 42.5 % iron. Another iron ore deposits Wadi Fatima, which

is the largest NE-SW trending low topographic area in the west central Arabian Shield, Saudi Arabia (reserves 48.4 million tons at 45-48% iron). Also, Jabal Idsas (220 km SW of Riyadh) is an important iron ore deposit with reserves 105 million tons of 15 - 20% iron. The copper mineralization is the broader metals prevalent in rock belonging to the pre-Cambrian periods of Arabian Shield. Many reservoirs copper have been explored, with large reserves of copper metal. Jabal Sayid, (copper mine located at 315 km NE of Jeddah, about 40 km north of Mahd Althahab Mine) is the most important one discovered in the Kingdom, The mine under the surface contains proven reserves of up to 99 million tons of copper ore Of 2.68% copper metal. Also, there are many copper ore deposits such as Mibari at Alqassim (Saudi Geological Survey,2012).

Samples and measurements

Nine rock samples were collected from different areas of the Arabian shield, in the western part of the Saudi Arabia as shown in figure 1.



- ▲ Jabal Idsas-Alreyad (23^o.20, 45^o.10)
- ▲ Wadi Fadima-Makka (21^o.30 , 39^o.25).
- ▲ Alzobiera–Hail (25^o.80, 42^o.30).
- ▲ Wadi Sawawin –Tabouk (27^o.90, 35^o.40).
- ▲ Jabal Sayid- Al Madienah (23^o.90, 40^o.90).
- ▲ Mibari – Al Qassim (25^o.80,42^o.30).

Fig. 1 Map of the samples' locations

Table 1 shows the type of sample, its code, and sampling Location.

Table (1): Type of sample, its code and sampling Location.

Ore Name	Sample No.	Location (Lat. And Long.)
Iron Ore	1	Jabal Idsas-Alreyad (23 ^o .20 , 45 ^o .10)
	2	Wadi AsSawawin –Tabouk (27 ^o .90 , 35 ^o .40)
	3	Wadi AsSawawin –Tabouk (27 ^o .90 , 35 ^o .40)
	4	Wadi Fadima- east of Jeddah(Al shemysi) (21 ^o .30 , 39 ^o .25)
	5	Wadi Fadima- 45 km of Makkah) (20 ^o .30 , 39 ^o .25)
	6	Wadi Fadima- 45 km of Makkah

Copper Ore	7	(20 ^o .30 , 39 ^o .25) Alzobiera–Hail (25 ^o .80 , 42 ^o .30)
	8	Jabal Sayid- Al Madienah (23 ^o .90 , 40 ^o .90)
	9	Mibari – Al Qassim (25 ^o .80 , 42 ^o .30)

Samples were grounded, sieved by 1mm x 1mm, then dried to 95°C for 24 hours in order not to lose the volatile polonium or cesium. The dried fine grained samples were packed in polyethylene Marinelli beakers for gamma spectroscopy, and then stored for up to four months to reach secular equilibrium between ²³⁸U and ²³²Th and their progenies.

Ten grams of the dried sample were analyzed by XRD for the mineral constituents. Samples were analyzed for concentrations of U-238, Th-232 series and K-40 using the gamma spectrometer based on high pure germanium detector "HPGe" with relative efficiency of 20%, and FWHM 4.2 keV at 1461 keV, the measurements were done to twenty four hours. After analyzing the spectrum, count rates for each detected photopeak were used to calculate the specific activity (A) for each detected nuclide using the following equation:(Amrani and Tahtat,2001).

$$A = \frac{c}{M \beta \epsilon} \quad (1)$$

Where: c is the net counting rate of a specific gamma ray (count per second)

M is the mass of the samples (kg.)

β is the transition probability of gamma-decay energy.

ϵ is the detector efficiency at the specific gamma-ray energy.

Table 2 represents isotopes and photopeak energies used for gamma-ray measurements of ^{238}U decay series, ^{232}Th decay series, ^{235}U , and ^{40}K .

Table(2):Isotopes and photopeak energies used for gamma-ray measurements of ^{238}U , ^{226}Ra , ^{232}Th , ^{235}U and ^{40}K .

U-238, Ra-226 series		Th-232 series		U-35	K-40
Isotopes	E(keV)	Isotopes	E(keV)	E(keV)	EkeV
^{234}Th	63.29	^{228}Ac	338.42	143.80	1460.8
^{234}Th	92.78+ 92.35	^{228}Ac	911.16	185.70	
$^{234\text{m}}\text{p-}$	766.60	^{228}Ac	968.97		
$^{234\text{m}}\text{pa}$	1001.00	^{212}Bi	727.25		
$^{214}\text{Pb-}$	295.09	^{212}Bi	785.51		
^{214}Pb	351.87	^{208}Tl	583.10		
$^{214}\text{Bi-}$	609.31	^{208}Tl	860.40		
$^{214}\text{Bi-}$	1120.27	^{208}Tl	2614.5		
^{214}Bi	1764.49				

3. Results and Discussions

Table 3 gives the X-RD results, which revealed the major, minor and trace minerals. Results show that the major minerals are QUARTZ(mostly in all samples), MAGNETITE (Fe_2O_4 with 70% iron), HEMATITE(Fe_2O_3 with 70% iron), CORUNDUM, GOETHITE, MONTMORILLONITE, PYRITE, ANKERITE, BOEHMITE, SPINL, and ALBITE, with additional minor and trace minerals.

Table 4 represents the specific activity concentrations in Bq/kg dry weight for iron and copper ore samples.

There is disequilibrium in the ^{238}U - ^{226}Ra series, so for ^{238}U a 63.29KeV photopeak, which comes the decay of ^{234}Th , was used to find the concentrations in Bq/kg dry weight, the values ranged from 152.68 to 264.73 (for iron ore), 156.37and 329.98(for copper ore).

For ^{226}Ra , the activities of ^{214}Pb and ^{214}Bi in equilibrium with parent (^{226}Ra) were used to calculate the concentrations in Bq/kg dry weight, the average values ranged from 2.50 to 386.30 (for iron ore),57.41and 1048.01(for copper ore).

While the activities of ^{232}Th series were calculated from daughters ^{228}Ac , ^{212}Bi , and ^{208}Tl , the average concentrations in Bq/kg dry weight ranged from 1.50 to 183.90(for iron ore), 43.66 and 44.41(for copper ore).

^{40}K concentration values in Bq/kg dry weight ranged from 2.70 to 186.99 (for iron ore), 48.92 and 191.33 (for copper ore), and the ^{235}U concentrations in Bq/kg dry weight ranged from 8.35 to 13.70 (for iron ore), ND and 18.37 (for copper ore).

It is noticed that, copper – bearing samples (4,5,8) have ^{226}Ra much higher than ^{238}U (Tables 2,4) which means the leaching out of uranium during the processes of alterations. Samples with iron minerals show high uranium than radium, and these represent the disequilibrium in the ^{238}U -series (Table 4). Thorium was found to vary with variation of rock types and the same behavior of ^{40}K .

Exposure to radiation has been defined in terms of the radium equivalent Ra_{eq} which is calculated from equation (1) Tufail *et al.* (2006):

$$\text{Ra}_{\text{eq}} = A_{\text{Ra}} + (A_{\text{Th}} \times 1.43) + (A_{\text{K}} \times 0.077) \quad (2)$$

Where:

A_{Ra} , A_{Th} and A_{K} are concentrations Bq/kg for radium, thorium and potassium Bq/kg dry weight, respectively.

Table (5) represents the values of the Ra_{eq} Bq/kg dry weight which ranged from 10.88 to 333.59(for samples 1,2,3, 6,7,9), they are less than 370 the permissible value adopted by EPA and UNSCEAR (2000),while for samples 4, 5 were 610.63 and 662.56 which are higher than the value 370, for sample 8 (Jabal Sayid) has the highest value1114.21(Bq/kg) because it lies at 40 km of Mahd Althahab known with high radiation level (Saudi Geological Survey, 2012).

Table (3): The mineral constituents of iron and copper ore samples analyzed by XRD spectrometer (Leet *et al.*, 1982, and Mineral Data, 2012)

Sam.No.	Major	Minor	Trace
1	MAGNETITE($\text{Fe}^{3+}_2\text{Fe}^{2+}\text{O}_4$)	-----	GREENALITE ($(\text{Fe}^{3+}_3\text{Fe}^{3+})_2\text{Si}_2\text{O}_5(\text{OH})_4$)
2	QUARTZ(SiO_2) HEMATITE($\text{Fe}^{3+}_2\text{O}_3$)	CALCITE(CaCO_3)	-----
3	BOEHMITE ($\text{AlO}(\text{OH})$) HEMATITE($\text{Fe}^{3+}_2\text{O}_3$) GIBBSITE ($\text{Al}(\text{OH})_3$)	ANATASE (TiO_2)	-----
4	HEMATITE($\text{Fe}^{3+}_2\text{O}_3$) CORUNDUM (Al_2O_3) QUARTZ(SiO_2)	PERICLASE (MgO) LIME (CaO) KAOLINITE ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$) ILLITE($(\text{K}_2\text{H}_3\text{O})(\text{Al},\text{Mg},\text{Fe})_2(\text{Si},\text{Al})_4\text{O}_{10}(\text{OH})_{22}(\text{H}_2\text{O})$)	MANGANITE($\text{MnO}(\text{OH})$) ANATASE(TiO_2), ORSCHALLITE ($\text{Ca}_3(\text{SO}_3)_2(\text{SO}_4)\cdot 12(\text{H}_2\text{O})$)
5	GOETHITE ($\text{Fe}^{3+}\text{O}(\text{OH})$) MONTMORILLONITE ($\text{NaCaAl}_2\text{Si}_4\text{O}_{10}(\text{OH})_2(\text{H}_2\text{O})_{10}$) QUARTZ(SiO_2)	BEAVERITE($(\text{PbCu}^{2+}(\text{Fe}^{3+}\text{Al})_2(\text{SO}_4)_2(\text{OH})_6$) PARTHEITE ($\text{Ca}_2\text{Al}_4\text{Si}_4\text{O}_{15}(\text{OH})_2\cdot 4(\text{H}_2\text{O})$) BENAUITE($(\text{SrBaPbFe}^{3+}(\text{PO}_4)_2(\text{SO}_4)(\text{OH})_6$) SCHNEIDERHOEHNITE ($\text{Fe}^{2+}\text{Fe}^{3+}_3\text{As}_5\text{O}_{13}$) IRON (Fe) CHESTERITE ($(\text{MgFe}^{2+})_{17}\text{Si}_{20}\text{O}_{54}(\text{OH})_6$) MICROCLINE (KAlSi_3O_8) MARCASITE(Fe^{2+}S_2) PYRRHOTITE (Fe^{2+}S)	CHLORITE($(\text{MgFe}^{2+})_6(\text{AlFe}^{3+})\text{Si}_3\text{O}_{10}(\text{OH})_8$)
6	GOETHITE ($\text{Fe}^{3+}\text{O}(\text{OH})$) SPINEL (MgAl_2O_4) QUARTZ(SiO_2)	BEAVERITE ($\text{PbCu}^{2+}(\text{Fe}^{3+}\text{Al})_2(\text{SO}_4)_2(\text{OH})_6$) PARTHEITE ($\text{SrBaPbFe}^{3+}(\text{PO}_4)_2(\text{SO}_4)(\text{OH})_6$) BENAUITE ($\text{SrBaPbFe}^{3+}(\text{PO}_4)_2(\text{SO}_4)(\text{OH})_6$) SCHNEIDERHOEHNITE ($\text{Fe}^{2+}\text{Fe}^{3+}_3\text{As}_5\text{O}_{13}$) IRON (Fe) CHESTERITE ($(\text{MgFe}^{2+})_{17}\text{Si}_{20}\text{O}_{54}(\text{OH})_6$) MICROCLINE (KAlSi_3O_8) MARCASITE (Fe^{2+}S_2) PYRRHOTITE (Fe^{2+}S)	-----
7	QUARTZ(SiO_2) HEMATITE($\text{Fe}^{3+}_2\text{O}_3$)	CALCITE (CaCO_3)	-----
8	PYRITE (Fe^{2+}S_2) ANKERITE($\text{CaFe}^{2+}\text{MgMn}^{2+}(\text{CO}_3)_2$) QUARTZ(SiO_2)	CHABAZITE ($\text{CaNaKMgSrAlSiO}_{24}(\text{H}_2\text{O})$) CLINOCHLORE ($\text{MgFe}^{2+}\text{Si}_3\text{Al}_2\text{O}_{10}(\text{OH})_8$) CHALCOPYRITE ($\text{CuFe}^{2+}\text{S}_2$)	BIOTITE($(\text{K}(\text{MgFe}^{2+})_3\text{AlSi}_3\text{O}_{10}(\text{OH})_2$) HYDROTALCITE, MAGNETITE($\text{Fe}^{3+}_2\text{Fe}^{2+}\text{O}_4$) ROGGIANITE ($\text{Ca}_2\text{Be}(\text{OH})_2\text{Al}_2(\text{Si}_4\text{O}_{13})\cdot 2.4(\text{H}_2\text{O})$) NONTRONITE ($\text{NaFe}^{3+}\text{Si}_3\text{AlO}_{10}(\text{OH})_2\cdot 4(\text{H}_2\text{O})$) ARSENOPYRITE(Fe^{3+}AsS)
9	QUARTZ(SiO_2) ALBITE ($\text{Na}(\text{AlSi}_3\text{O}_8)$)	CLINOCHLORE ($\text{MgFe}^{2+}\text{Si}_3\text{Al}_2\text{O}_{10}(\text{OH})_8$) MICROCLINE (KAlSi_3O_8)	BIOTITE ($(\text{K}(\text{MgFe}^{2+})_3\text{AlSi}_3\text{O}_{10}(\text{OH})_2$) MAGNETITE($\text{Fe}^{3+}_2\text{Fe}^{2+}\text{O}_4$) ZIRCON(ZrSiO_4) SAPONITE($(\text{Ca}(\text{Mg},\text{Fe})_3((\text{Si},\text{Al})_4\text{O}_{10}(\text{OH})_2(\text{H}_2\text{O}))$) KOVDOORSKITE($(\text{Mg}_5(\text{PO}_4)_2(\text{CO}_3)(\text{OH})_2\cdot 4.5(\text{H}_2\text{O}))$)

Table (4): The specific activity concentrations in Bq/kg dry weight for iron and copper ore samples.

N D: not detected

Sample Code	Sample 1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9
U-238 s Series Nuclei									
U-238 s Series									
Th-234	183.46±0.8	167.46±0.77	ND	264.73±0.77	152.68±0.79	ND	ND	329.98±0.77	156.37±0.78
Ra-226s Series									
Pb-214	9.91±0.04	18.72±0.08	69.40±1.20	367.89±0.05	388.62±0.04	53.10±.90	LDL	1032.49±0.03	55.74±0.04
Bf-214	8.51±0.23	18.80±0.12	70.80 ± 1.02	365.09±0.11	383.97±0.06	57.500.40	2.5 0±0.20	1064.65±0.81104	59.07±0.11
Average	9.21±0.24	18.76±0.10	70.10±1.11	366.49±0.22	386.300.05	55.30±0.65	2.5 0±0.20	8.0±0.42	57.41±0.08
Th-232 Series									
Ac-228	1.04±0.11	14.24±0.11	181.20 ±2.00	167.65±0.14	184.47±0.12	27.50±1.00	6.9 0±1.20	ND	42.95±0.15
Bf-212	ND	14.86±0.34	184.70 ±6.00	164.61±0.71	181.76±0.71	28.20 ±2.90	LDL	45.75±0.17	45.73±0.73
TL-208	1.07±0.07	15.82±0.06	185.80 ±5.60	173.50±0.05	ND	30.40 ±2.60	4.30 ±0.90	41.57±0.05	44.54±0.08
Average	1.50±0.09	14.97±0.25	183.90 ±3.20	168.7±0.59	183.12±0.41	28.70±1.30	5.60±0.90	43.66±0.11	44.41±0.32
U-235	9.67±0.80	ND	ND	13.70±0.08	8.35±0.09	ND	ND	18.37±0.07	ND
K-40	39.14±0.65	15.22±0.65	6.30 ±0.10	32.61±0.10	186.99±0.08	2.70 ±0.10	4.80 ±0.10	48.92±0.08	191.33±0.06

Table (5) The radium equivalent Bq/kg for the samples.

Ore Name	Sample Code	Ra _{eq} Bk/kg
Iron Ore	1	14.36 ±0.16
	2	41.34±0.34
	3	333.59±1.3
	4	610.36±0.07
	5	662.56±0.06
	6	96.55±2.51
	7	10.88±0.91
Copper Ore	8	1114.21±0.04
	9	135.65±0.03

Conclusion

The X-RD results show that the major minerals are QUARTZ (mostly in all samples), MAGNETITE (Fe₂O₄ with 70% iron), HEMATITE (Fe₂O₃ with 70% iron), CORUNDUM, GOETHITE, MONTMORILLONITE, PYRITE, ANKERITE, and ALBITE. A state of disequilibrium between ²³⁸U and ²²⁶Ra is clear. Most of the analyzed samples have radioactive concentrations within the accepted range which gives radium equivalent less than 370 Bq/kg dry weight for samples 1, 2, 3, 6 and 7, 9 except samples 4, 5, and 8 which have high concentrations. There for, we can conclude that the mining process of iron and copper ore in these area caused enhancement of the exposure from natural radiation.

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Clinical Outcomes of Rectal Carcinoids: A Single-Institution Experience

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Abstract: To report clinical outcomes of rectal carcinoids through investigating patients with rectal carcinoid. Between December 2011 and January 2003, 16 consecutive patients with biopsy-proven rectal carcinoid were enrolled at our institution, including ten males and six females, with a median age of 49 years old (range 29 to 78 years). The median tumor size was 12.3mm, five lesions diameter were ≥ 20 mm, eight lesions diameter were ≤ 10 mm, three lesions diameter were 10mm-20mm. All rectal lesions were located within 10cm from the anal verge. 9 cases underwent transanal local excision; 3 cases had received anterior resection (Dixon); 2 cases underwent abdominaloperineal resection (APR); 2 cases underwent Endoscopic submucosal dissection (ESD). 2 of patients received postoperative chemotherapy or radiation therapy. Calculation of the 5-year overall survival (OS), recurrence-free survival (RFS) and cancer specific survival (CSS) were performed by Kaplan-Meier methodology. All patients were followed up for a median of 45.4 months (Range: 6 to 161 months), no patient was lost to follow-up. The 5-year OS, RFS and CSS were 85.2%, 93.8% and 90.9% respectively. Rectal carcinoids had a favorable prognosis, an adequate resection play key role in management of rectal carcinoid tumors, the extent of the surgical resection depend on its size, its anticipated stage and the specific patient needs.

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Key words: Clinical outcomes; Neuroendocrine tumors; Rectal carcinoids

1. Introduction

Carcinoid tumors are a group of neoplasms with neuroendocrine features, which originate from argyrophil cells in intestinal mucosal glands. Rectal carcinoids are relatively rare, but are on the rise as the degree of screening sigmoidoscopy and colonoscopy increases in the last few years, accounting for approximately 17%~25% of all gastrointestinal tract carcinoids and 1.3% of all rectal tumors [1]. Rectal carcinoids are most often small and confined to the submucosa, they tend to show nonfunctioning and asymptomatic and are more likely to be found incidentally when compared with carcinoids at other sites. Generally, Rectal carcinoid tumors have been recognized as having low malignant potential [2]. However, recent studies have shown that carcinoid tumors with metastasis are thought to be tumors with a malignant potential comparable to that of an adenocarcinoma [3]. Even there has been report of small-sized carcinoid tumors with considerable rate of lymph node metastasis [4]. In the recently revised the American Joint Council on Cancer (AJCC) cancer staging, carcinoid tumors are classified as a malignant tumor [5]. Surgical resection has been the standard treatment of patients with rectal carcinoids, but there are still some controversy. The purpose of this report was to demonstrate the treatment outcomes of patients with rectal carcinoids in our institution.

2. Patients and Methods

Between December 2011 and January 2003, 16 consecutive patients with biopsy-proven rectal carcinoid were enrolled at our institution. Tumors diameters at the time of diagnosis were measured from the colonoscopic examinations. The median tumor size was 12.3mm (range, 3mm to 23mm), five lesions diameter were ≥ 20 mm, eight lesions diameter were ≤ 10 mm, three lesions diameter were 10mm-20mm. All rectal lesions were located within 10cm from the anal verge, the median distance of rectal carcinoids from the anal verge was 5.9cm (range, 2 to 10cm). All patients underwent chest, abdomen, pelvic computed tomography (CT) scans with intravenous contrast agent and colonoscopy examinations prior to surgery. Lymph nodal involvement was considered for a lymph node with a minimal diameter more than 5mm on the preoperative CT sets, only one case was diagnosed lymph node metastasis at mesentery. All patients were clinically staged according to the AJCC Cancer Staging 7th edition, revised in 2010 [5]. The Ki-67 ratios of all patients were detected by immunohistochemical staining using formalin aceticacid-fixed, paraffin-embedded postoperative specimens tissue sections. In addition, one of the patients had an active double cancer in his left inferior lung, the lung lesion were pathologically proven as lung adenocarcinoma,

No patient presented with the carcinoid syndrome. The clinical characteristic was shown in Tab. 1.

Surgery were performed in 16 patients, Among 11 \leq 20mm-diameter rectal lesions: 9 lesions were resected by transanal local excision, 2 lesions were removed by endoscopic submucosal dissection (ESD), while the one (patient 16) with an alive double cancer received prescription dose 60Gy in 30 fractions to his lung lesion after ESD. 5 patients \geq 20 mm -diameter rectal tumors, 2 cases received Dixon, 2 cases underwent APR and 1 case underwent transanal local excision. Among the 16 patients, only 1 patient (patient 11) with narrow tumor-free (an inadequate resection) received postoperative radiation therapy with prescription dose of 50Gy in 25 fractions after local excision, 1 patient (patient 1) with positive lymph node (T2N1M0) received postoperative chemotherapy (oxaliplatin 200mg on Days1) every 3 weeks for two cycles. The remaining 14 patients did not receive any adjuvant therapy.

3. Follow-up and Statistical Analysis

Patients were usually followed up at 3 and 6 months after treatment and at one year intervals thereafter. The follow-up investigations were documented for each patient (e.g. physical examination, chest, abdomen and pelvic CT and colonoscopy examinations). The diagnosis of relapsing or metastatic disease was made in accordance with the results of biopsy findings or/and imaging examinations.

OS and CSS rates were calculated for the interval from the date of surgery until death because of any cause and rectal carcinoid, respectively. RFS was defined as the period from date of surgery to the date of first documented evidence of distal or local recurrent disease. Kaplan-Meier method were used estimate the OS, CSS and RFS. All statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL).

4. Results

16 rectal lesions were confirmed rectal carcinoid on the basis of the postoperative routine pathological examination, which led to visual description of 68.75% (11/16) tumors invading submucosal, 6.25% (1/16) invading mucosal, and 25% (4/16) invading muscular. Immunohistochemical staining: the Ki-67 labeling index of all lesions were below 4%.

Until February 2012, all patients were followed for a median of 45.4 months, with a range of 6 to 161 months, no patient was lost to follow-up. There were 3 cases for following up full for 5 years. At time of follow-up, 14 patients were alive, 2 patients had died. A 29 -year -old male patient (patient 4) who underwent transanal local excision relapsed in 6 months and died

in 18 months after initial treatment, because he refused to therapy again. And a 78 -year-old male patient (patient 16) with an active double cancer died from lung cancer progression in 9 months after ESD. No local recurrence or metastasis was found in the other 14 patients. The 5- year OS and CSS as well as RFS were 85.2 % , 93.8% and 90.9% respectively (Fig.1).

5. Discussions

Neuroendocrine tumors of the rectum are uncommon, however, the incidence rate of carcinoid tumors has increased substantially over the past five decades with the rapid development of screening endoscopy [6]. Jetmore AB et al. [7] has predicted that rectal carcinoids may become the most frequent human carcinoid tumor, and a Japanese study illustrated that approximately 90% of colorectal carcinoids were located in the rectum.

Rorstad [8] reported that patients with rectal carcinoids had a wide variability in 5-year survival rates which ranged from 62% to 100%. Small rectal carcinoids are known to have little risk of metastasis, rectal carcinoids without metastasis usually have been considered both associated with a favorable prognosis and a high 5-year survival rate (85– 99%) [4]. Similar data was found in the cohort, the 5- year OS and CSS as well as RFS were 85.2 % , 93.8% and 90.9% respectively. Generally, 86% of rectal carcinoid tumors size were less than 10mm [9]. Our study showed that 50% (8/16) patients tumor were \leq 10mm. As the disease stage advances, carcinoid tumors develop in the mucosal gland and gradually infiltrate to the deeper layer of the bowel wall and may be in the wake of lymph node or distant metastasis. TNM stage have been believed as predictors in the assessment of survival rate of rectal carcinoids [10], and current treatment modalities are mostly in the light of TNM staging. Ramage et al. [11] reported that for the tumors diameter \leq 10mm which had rarely lymphatics, muscularis propria invasion or lymph nodes metastasis, local excision is appropriate for the tumors in this size. In this study, there were 8 patients with tumor \leq 10mm. 6 cases underwent transanal local excision and 2 patients chose ESD. They were well alive except the one died of lung cancer. For the tumors \geq 20mm in size or along with muscularis infringement, the radical resection was the optimal therapy [11]. In the study, a patient (patient 4) with a 20mm -diameter tumor and muscularis infringement underwent transanal local excision depend on his needs. However, he died from recurrent primary tumor one year later. As for rectal carcinoids diameter were 10-20mm, there was no agreement in the studies concerning the optimal therapy for tumors of this size. Some people supported rectal resection while others held on local excision for selected patients. Ramage et al. [11] suggested local

excision was reasonable for any rectal carcinoid \leq 20mm. In the cohort, of 3 male patients with 10-20 mm -diameter lesions and no invading muscular layer and positive lymph nodes. 2 cases chose transanal local excision and 1 patient received Dixon. These 3 patients had not discovered local recurrence or distant metastasis until the time of the last follow- up visit. One of the patients underwent two cycles chemotherapy postoperatively because of lymph nodes involvement (T2N1M0). One of the patients (patient 11) with an inadequate resection underwent postoperative radiation therapy. Usually, rectal carcinoids are considered as well -differentiated tumors, adjuvant therapies should not be recommended. Additionally, active double cancer and the nuclear proliferation marker were considered to be important factors of affecting the rectal carcinoids survival rate. There are studies [12, 13] suggested that patients with colorectal carcinoid tumors had an increased risk of synchronous primary cancer. The rate of a carcinoid tumor with a second primary malignancy ranges from 12 to 46%. Tichansky et al. [12] also found that patients with colorectal carcinoids had a high susceptibility of second primary cancer in other sites, such as small bowel, esophagus, stomach, lung and bronchus simultaneously. When the second tumor

is a more malignant lesion, the prognosis may be correlated closer with the noncarcinoid cancer [13]. In our study, we had a patient with active double cancer died from lung cancer after ESD of rectal carcinoid. The monoclonal antibody Ki-67 had been believed to be the nuclear proliferation marker, providing a measurement for the growth fraction in several different tumors [14]. Hotta K et al. [15] reported that the Ki-67 ratio was an effective histological parameter to predict metastatic behavior of rectal carcinoid tumors. In our study, Ki-67 of all lesions were below 4%, which indicated a low cellular proliferative activity in rectal carcinoids. The outcome was in accordance with the report of Shimizu T [16].

In conclusion, rectal carcinoids have diverse biological characteristics and a favorable prognosis. An adequate resection play key role in management of rectal carcinoid tumor, the extent of the surgical resection depend on its size, its anticipated stage and the specific patient needs. In addition, the small sample size and a single institution are notable limit in this study, it is desirable to build multi-institution cooperation to explore the appropriate therapy modality for different patients with rectal carcinoids

Tab. 1. Patient characteristics

Pat no.	Sex	Age (y)	D(mm)	DTI	Ki-67(%)	LNM	DAV	OM	AT	Staging
1	M	48	21	Muscularis	3.5	yes	2	Dixon	Chemo	T2N1M0
2	M	42	7	Submucosa	2.0	no	7	LE	no	T1aN0M0
3	M	40	15	Mucosa	2.5	no	7	LE	no	T1bN0M0
4	M	29	21	Muscularis	1.5	no	8	LE	no	T2N0M0
5	M	57	22	Muscularis	3.0	no	8	APR	no	T2N0M0
6	M	37	11	Submucosa	2.0	no	5	LE	no	T1bN0M0
7	F	42	5	Submucosa	1.0	no	6	LE	no	T1aN0M0
8	F	42	23	Submucosa	1.0	no	5	Dixon	no	T1bN0M0
9	F	65	5	Submucosa	1.5	no	3	LE	no	T1aN0M0
10	M	58	21	Submucosa	0.3	no	10	APR	no	T2N0M0
11	F	41	8	Submucosa	2.0	no	4	LE	RT	T1aN0M0
12	F	47	8	Submucosa	1.0	no	5	LE	no	T1aN0M0
13	F	46	6	Muscularis	2.6	no	6	LE	no	T2N0M0
14	M	50	16	Submucosa	1.2	no	7	Dixon	no	T1bN0M0
15	M	61	5	Submucosa	0.4	no	6	ESD	no	T1aN0M0
16	M	78	3	Submucosa	1.7	no	5	ESD	no	T1aN0M0

Abbreviation: Pat= patient; F = female; D=Diameter; DTI =depth of tumor invasion; LNM = lymph node metastasis; DAV = distance from the anal verge; OM = operative method; LE = Local excision; AT = adjuvant treatment. Chemo = chemotherapy; RT = radiotherapy.

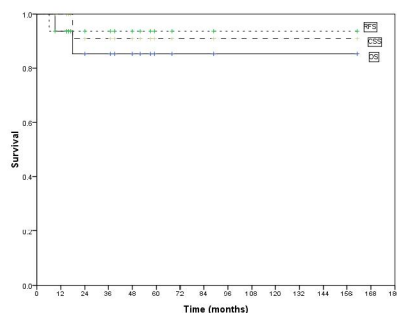


Fig.1 *Abbreviation:* Overall survival (Solid line), Recurrence-free survival (dotted line) and Cancer-specific survival (dashed line) of 16 patients.

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Effect of Weak Electro Magnetic Field on Grain Germination and Seedling Growth of Different Wheat (*Triticum aestivum* L.) Cultivars

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Abstract: Growth parameters data were used in this study for the evaluation of nine wheat (*Triticum aestivum* L.) cultivars Giza168, Tabouki, Kaseemi, Yamanei, Madini, Nagrani and Seds 12 at the University of King Abdul Aziz in season 2011. Grains of wheat different cultivars were exposed in batches to weak electric magnetic fields (3000 gauss = 0.3T of magnetic force) for 30 min. Then, the magnetic treated grains were placed in Petri dishes between two layers of moist germination paper by magnetic water. They were placed in the germination incubator at 20°C in an upright position. In order to estimate the rate of germination and percentage of germination. After 21 days, different plant growth parameters were tested such as shoot length, root length, shoot / root length, seedling length, seedling fresh and dry weight based on normal seedlings and effect of magnetic treatments on number of protein bands in wheat seedling. The results showed that all magnetic field treatments increased the rate and percentage of germination, all growth parameters and number of protein bands based on normal seedlings in wheat cultivars. The higher increments observed when grain exposed to weak electric magnetic field strengths 0.3 T at 30 min and dipp in magnetic water compared with control and cultivar Sakha93 showed decreased in the percentage of germination, all seedling growth parameters and numbers of seedling protein bands when exposed to all magnetic field treatments compared with controls, while Masr1 cv. No effected when treated compared with control. Magnetic field and water application gave best results in all seedling parameters compared to unexposed control.

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Key words: Weak Electro-Magnetic Field - Wheat (*Triticum aestivum* L.) - Grain Germination - Seedling Growth.

1. Introduction

The major crops in the Kingdom of Saudi Arabia include cereals (wheat, sorghum, barley and millet), vegetables (tomato, watermelon, eggplant, potato, cucumber and onions), fruits (date-palm, citrus and grapes) and forage crops (alfalfa). These crops are cultivated over an area of nearly 1.1 million hectare which represents about 81 percent of the total cultivated area. In 2009 year, wheat was cultivated over an area of about 744 422 hectare (i.e. 55 percent of the total cultivated area), and production was about 3.5 million tones. (FAOSTAT, 2009).

Electric field is one kind of stress, which can affect directly or indirectly on the plant. Different plant species in their sensitivity and response to environmental stresses because they have various capabilities for stress perception, signaling and response. Over many years, the effects of magnetic fields on plant life have been subjected to several studies. As early as **Savostin (1930)** reported a 100% increase in the rate of elongation of wheat seedlings under the influence of a magnetic field.

Several researches tried to define the effect of such field on the growth rate of the wheat plant.

Influence of magnetic field (MF) on the early growth processes in wheat plant grains was studied and the stimulating of MF on the early growth processes, plant grains is attenuated when the ratio between the periods of exposure and intervals between them (the on – off time ratio) increases (**Es, Kov and Darkov, 2003**). **Hanafy et al (2006)** indicated that the electric magnetic field of both systems showed a high frequency of chromosomal abnormalities and the treated wheat flower buds showed a marked increase in the frequency of the nonviable pollen grains. They also reported that the changes in the morphological characters where the stem length increased but the spike weight and the number of grains in the spike decreased. Furthermore, their data showed an increase in the total chlorophyll of leaf content and the total carbohydrates in the grains. On the other hand, molecular structure of the extracted Water soluble protein changed the amount of protein in the bands of exposed grains decreased and their molecular weights changed. **Hozayn and Abdul Qados (2010)** reported that the growth parameters and yield components of wheat plants is concomitantly increased when wheat plants irrigated

with magnetic water with increasing photosynthetic pigment; endogenous total indole; total phenols and protein synthesis. Earlier studies on the effects of

static fields on germination of other plants are summarized in table 1.

Table (1): Summary of previous researches involving static magnetic fields

Seed	Magnetic field strength and period	Effect of exposure	Ref.
Soybean <i>Glycine max</i> L. Merrill	Exposed to 2.9-4.6 mile Tesla for 2.2,6.6 and 19.8 seconds periods	Shoot and root formation, fresh weights and chlorophyll quantities were increased in all magnetic field experiments.	Atak, <i>et al.</i> , 2003
Tobacco <i>Nicotiana tabacum</i> L.	Magnetic field with induction of 0.15 Tesla, at expositions 10, 20 and 30 min.	The germination energy and the germination were increased.	Aladjiyan and Yaieva, 2003
Maize <i>Zea mays</i>	Exposed to one of two magnetic field strengths, 125 or 250 mT for different periods of time	Rate of germination was increased.	Fl'orez, <i>et al.</i> , 2007
Chick-Pea <i>Cicer arietinum</i> L.	Magnetic water prepared using permanent magnets (0.32T)	Magnetized water has very affective effects on seeds. The crop production and plant length increase noticeably. Treating water with static magnetic field	Nasher, 2008
Snow pea and Celery <i>Pisum sativum</i> var. <i>Apium graveolens</i>	Magnetic field in the range of 3.5-136 mT was used for the magnetic treatment of irrigation water.	Treatments were increases in plant yield and water productivity.	Maheshwari and Singh Grewal, 2009
Date Palm <i>Phoenix dactylifera</i>	Seedlings were treated with static magnetic field at three levels of (10, 50 and 100 mT) and different durations (30, 60, 180, 240 and 360 min). with alternating magnetic field at 1.5 T for different durations (1, 5, 10 and 15 min).	Results indicated that pigments content (chlorophyll a, chlorophyll b, carotenoids and total pigments) was significantly increased under static magnetic field.	Faten Dhawi and Al-Khayri, 2009
Rose coco beans <i>Phaseolus vulgaris</i>	Seeds were exposed to field generated by Helmholtz coil, North Pole or the South Pole with constant magnetic fields of 5 mT, 10 mT, 30 mT and 60 mT. The exposure period was fixed at 3, 4.5 and 6 h and exposed after 12 h incubation.	Maximum seed germination occurred when exposed to South Pole field inducing percent germination of approximately 73% compared to 52% of the control at field strength of 30 mT at exposure period of 4.5 h.	Odhiambo <i>et al.</i> , 2009
Chickpea (<i>Cicer arietinum</i> L.)	Seeds of different varieties of chickpea were exposed in batches to static magnetic fields (1500 X10 ⁻⁴ T of magnetic force) for 30, 50 and 70 min.	The results showed that magnetic field application enhanced seed performance in terms of laboratory germination and among the various duration exposures, 50 and 70 min. exposures gave best results.	Tahir and Hama Karim, 2010
Tomato (<i>Lycopersicon esculentum</i> Mill) cv. Castlrock	Exposed to different magnetic strengths (0.1, 0.15 and 0.2 Tesla) for periods of 1, 5, 10 and 15 minutes.	The best results were found by magnetic seed treatment with 0.1 Tesla for 15 min.	Abou El-Yazied, <i>et al.</i> , 2011

The main objective of this work is to quantify the possible effect of magnetic field strengths (0.3 T at 30 min) treatment on the wheat plant performances such as, germination %, shoot length, root length, shoot L./ root L., seedling length, seedling fresh weight, seedling dry weight and relative water content of different wheat plant cultivars. Effect of magnetic field and irrigation by magnetic water treatments on many of protein patterns in different wheat leaves cultivars were observed.

2. Materials and methods

Plant material

The plant material comprised of nine cultivars of wheat (*Triticum aestivum* L.) including Giza168, Sakha 93, Masr1 and Seds 12 were obtained from Agronomy Research Department, Field Crops Institute, Agriculture Research Centre, Giza, Egypt and Tabouki, Kaseemi, Yamanei, Madini and Nagrani

were obtained from Agronomy Research Department, Field Crops Institute, Agriculture Research Centre, Jeddah, Kingdom of Saudi Arabia.

Magnetic treatment

Grains without visible defect, insect damage and malformation were selected and divided into four groups in a complete randomized design. Each group consists of three replicates (a replicate is one Petri dish containing 20 healthy grains). The namely of the groups was as follows, group 1: Exposed to magnetic field and dipping in magnetic water; group2: Exposed to magnetic field and dipping in tap water; group3: Not exposed to magnetic field and dipping in magnetic water, and group 4: Not exposed to magnetic field and dipping in tap water (Control). Drought grains were exposed for 30 minutes to a constant of pulsed magnetic field by placing them between the poles of an electromagnet (58 mm in diameter, located 30 mm apart) with the longitudinal

(body) axis oriented along the magnetic lines of force at magnetic field strengths, 0.3 T.

Grain germination was achieved in three replications each with 20 grains placed on two layers of moist filter paper in Petri dishes (imbibed with 15 ml of magnetized water exposed at magnetic field strengths, 0.3 T.). They were placed in the germination incubator at 20 °C in an upright position. After 6 days, germinated seeds were grouped as normal, abnormal seedling, fresh ungerminated and dead grains. Germination percentage was calculated based on normal seedlings of plant research.

Growth parameters

This research was carried out in 2010 - 2011 season at Faculty of Science, North Jeddah Branch - Department of Biological Sciences - University of King Abdul Aziz as to determine the impact of magnetic application on nine wheat cultivars grown under optimum conditions. A complete randomized design with three replications was used. Each replicate consist of 20 grains were sown in a plastic pots (19 cm height, 15 cm diameter) of soil containing mix (2 soil: 1 peat moss). The four groups of each wheat grains cultivar are selected with 60 grains for each cultivar under each treatment. Group 1: Grain exposed to magnetic field and treated (pping & irrigation) by magnetic water; Group2: Grain exposed to magnetic field and treated (dipping & irrigation) by tap water; group3: Grain exposed to magnetic field and treated (dipping & irrigation) by magnetic water, and group 4: Grains not exposed to magnetic field and treated (dipping & irrigation) by tap water (Control). Irrigation was provided as and when required. The plastic pots were maintained in greenhouse under natural light. After three weeks from planting the growth parameters, including, shoot length, root length, shoot L./ root L., seedling length, shoot and root fresh weight and shoot & root dry weight of different wheat plant cultivars were measured and relative water content was calculated according to Henson *et al.* (1981) by the following equation: $100 \times (\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight}$.

Protein patterns analyses

Fifty mg dry tissues of nine wheat cultivars leaves which treated by magnetic field and magnetic water compared with un treated control leaves were ground to flour in a mortar by using liquid nitrogen. Total soluble proteins were extracted in SDS reducing buffer, (store at room temperature) composed of Deionized water (38 ml), 0.5 M Tris -HCl -pH 6.8 (10 ml), Glycerol (8 ml), 10 % (w/v) SDS (16 ml), 2-mercapto-ethanol (4ml) and 1% (w/v) Bromophenol blue (4ml) until became total volume 80 ml. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 10% acrylamide slab

gels following the system of (Laemmli, 1970). Separating gels composed of 0.75M Tris – HCl pH8.8, 10% SDS, 0.025% of N,N,N,N-tetramethylenediamine (TEMED) and 30% ammonium persulfate. Stacking gels contained 0.57M Tris-HCl pH6.8, 10% SDS, 0.025% TEMED and 30% ammonium persulfate. Electrode buffer contained 0.025M Tris, 0.192M glycine, 0.1% SDS and pH8.3. Electrophoresis was carried out with a current of 25 mA and 130 volts per gel until the bromophenol blue marker reached the bottom of the gel after 3hrs. After electrophoresis, the Commassie Brilliant R250 staining method was used for protein bands and polypeptides.

Statistical analyses

All data were subjected to analysis of variance (ANOVA), and means were compared by two conventional methods of analysis. The LSD values for significant mean differences at levels $P < 0.05$ and 0.01 were separated. All statistical tests were carried out using Costat software.

3. Results and Discussions

Data in (Table 2) Pointed out that all magnetic field treatments increased the percentage of germination and rate germination in all wheat cultivars but higher increments observed when grain exposed to weak magnetic field strengths 0.3 T at 30 min and dipped in magnetic water compared with control. Wheat cultivars such as Giza168, Tabouki, Kaseemi, Yamanei and Madini were observed 100% germination percentage compared with control. The magnetic field stimulates the development of the germ and leads to increasing the germination energy and germination. A hypothesis about the explanation of the results obtained has been proposed, especially about the stimulating effect of the magnetic different grain wheat cultivars treatment depended on the dose of magnetic field and the time of exposure used, these results are in agreement with those reported by Es,Kov and Darkov (2003); Hanafy *et al.* (2006) and Hozayn and Abdul Qados (2010), but, Gusta *et al.* (1978) who reported that the exposure of dry seeds of wheat, barley and wild oats to a magnetic field had no effect on germination and seedling growth.. On the other hand, Apasheva *et al.* (2006) reported that the statistically increase significant results demonstrating the effect of alternating electromagnetic field with different duration of exposure on the rate of seed germination depending on seed state (dry or moistened). Cultivars Sakha93 and Masr1 showed decreased in the percentage of germination and germination rate when exposed to all magnetic field treatments compared with controls, is shown to depend on the extent of membrane stretching and release of peripheral protein from membranes this result garmented with Aksyonov *et al.* (2007).

Table (2): Effect of magnetic treatments on germination rate in different wheat grain cultivars after 6 days from grains treatment

Treatments	Percentage of germination end after 6 days								Mean	LSD
	Exposure + Dipping Magnetic water		Exposure +Dipping Tap water		No exposure + Dipping Magnetic water		No exposure + Dipping Tap water			
	Germination Rate	Germination %	Germination Rate	Germination %	Germination Rate	Germination %	Germination Rate	Germination %		
Cultivars										
Giza168	60/60	100	50/60	83.33	54/60	90	25/60	83.33	a 94.17	6.78
Sakha 93	40/60	66.67	46/60	76.67	54/60	90	58/60	96.67	ab 90.84	
Tabouki	60/60	100	56/60	93.33	46/60	76.67	46/60	76.67	ab 90.84	
Kaseemi	60/60	100	60/60	100	56/60	93.33	50/60	83.33	abc 89.17	
Masr 1	46/60	76.67	36/60	60	42/60	70	56/60	86.67	bcd 86.67	
Yammanei	60/60	100	60/60	100	46/60	76.67	52/60	86.67	cd 83.34	
Madini	60/60	100	52/60	86.67	58/60	96.67	48/60	80	cd 82.50	
Nagrani	56/60	86.67	48/60	80	54/60	90	46/30	76.67	d 81.67	
Seds 12	52/60	83.33	48/60	80	52/60	86.67	46/60	76.67	e 73.34	

In general, growth parameters including: shoot length, root length, shoot length / root length and seedling length were better with magnetic field treatments comparing with control treatment. The data reported in (Tables 3 and 4) showed that the increase in stimulation rate of many different wheat cultivars (Giza168, Tabouki, Kaseemi, Yamane, Madini, Nagrani and Seds) in all seedling growth parameters.

Maximum shoot length, root length, shoot length / root length and seedling length parameters was obtained when magnetic grain treatment and magnetically treated water were jointly applied as compared to control treatments. While Sakha93 cultivar observed negative stimulating for magnetic field and magnetic water treatments therefore, recorded decreased significant in all seedling growth parameters that were measurements.

On the other hand, the magnetic treatment doesn't effects on seedling growth parameter for Masr1cv.

than those of the control treatment. Similar result was noticed by **Ibrahim and Khafagi (2004)** who found that seedlings from *Pergamum harmala* L. seeds treated with magnetic field were higher significant increasing compared with control. **Hanafy et al. (2006)** indicated that the electric magnetic field of both systems showed that the changes in the growth characters where the stem length increased and **Hozayn and Abdul Qados (2010)** who reported that the growth parameter and yield components of wheat plants are concomitantly increased when wheat plants irrigated with magnetic water.

On the other hand, **Kordas (2002)** who mentioned that the effect of a constant magnetic field on the root system and green tops, as well as on yield of spring wheat and in all cases there was observed a slight stimulating effect of the factors examined. Moreover, the growth dynamics were weakened and the plants were shorter, and so were their culms and ears.

Table (3): Effect of magnetic treatments on plant performance (Shoot length and Root length) in different wheat grain cultivars after 21 days from grains treatment.

Treatments	plant performance (cm)										LSD 0.05	LSD 0.05
	Shoot length					Root length						
	Exposure MF		Un exposure MF		Mean	Exposure MF		Un exposure MF		Mean		
	Magnetic water	Tap water	Magnetic water	Tap water		Magnetic water	Tap water	Magnetic water	Tap water			
Cultivars												
Giza168	20.11± 0.808332	15.17± 0.823165	17.83± 0.944646	12.67± 0.566647	a 18.46	13.17± 0.094163	10.66± 0.276446	11.41± 0.183848	9.81± 0.304083	a 15.61	8.03	1.85
Sakha 93	10.20± 0.642979	14.65± 0.629921	19.02± 0.044969	21.73± 0.348935	b 16.73	8.36± 0.151511	11.61± 0.430426	13.03± 0.024386	13.11± 0.045792	ab 15.16		
Tabouki	21.75± 0.349221	18.97± 0.430581	16.2± 0.571859	10.017± 0.671532	c 16.67	10.27± 0.185532	13.97± 0.469207	12.63± 0.409145	4.47± 0.173269	bc 13.69		
Kaseemi	14.79± 0.644429	20.57± 0.236972	20.13± 0.233381	9.1± 0.268494	d 16.45	15.01± 0.008165	16.99± 0.067987	16.15± 0.469065	12.48± 0.15195	cd 12.79		
Masr 1	19± 0.379327	9.07± 0.102089	14.92± 0.433667	21.53± 0.648811	d 16.45	15.89± 0.123648	10.20± 0.258371	9.85± 0.247117	15.20± 0.08165	de 11.53		
Yammanei	21.22± 0.089938	18.8± 0.286744	14.67± 0.173077	10.48± 0.278248	e 16.4	12.36± 0.214009	10.70± 0.339935	8.09± 0.143836	4.86± 0.092736	de 11.26		
Madini	20.83± 0.34322	18.07± 0.037712	20.77± 0.374789	14.17± 0.369955	f 16.29	15.75± 0.087305	13.82± 0.302692	17.78± 0.044969	7.42± 0.073485	ef 10.34		
Nagrani	19.82± 0.396569	14.68± 0.090921	21.68± 0.365361	9.6± 0.083799	g 16.15	11.08± 0.08165	9.45± 0.061644	10.43± 0.008165	5.06± 0.132749	f 9.01		
Seds 12	20.40± 0.357802	17.30± 0.137356	18.83± 0.206074	10.15± 0.365908	h 16.13	18.07± 0.03559	16.01± 0.012472	17.33± 0.24931	11.01± 0.012472	f 8.98		
Mean	18.68 a	18.23 ab	16.36 b	13.27 c		13.33 a	12.96 a	12.60 a	9.269 b			
LSD 0.05	2.01					1.23						

Table (4): Effect of magnetic treatments on plant performance (Shoot length / Root length & seedling length) in different wheat grain cultivars after 21 days from grains treatment

Treatments	plant performance													
	Shoot length / Root length					Seedling length (cm)								
	Exposure MF		Un exposure MF		Mea n	LSD 0.05	Exposure MF		Un exposure MF		Mea n	LSD 0.05		
Magnetic water	Tap water	Magnetic water	Tap water	Magnetic water			Tap water	Magnetic water	Tap water					
Giza168	1.53± 0.102307	1.42± 0.020548	1.56± 0.012472	1.29± 0.04899	a 1.87	0.18	33.28± 0.19754	25.83± 0.065997	29.24± 0.08165	22.48± 0.040277	a 32.28	4.63		
Sakha 93	1.22± 0.012472	1.26± 0.024495	1.46± 0.024495	1.66± 0.008165	a 1.83		18.56± 0.138884	26.26± 0.041899	32.056± 0.015755	34.84± 0.026247	a 32.15			
Tabouki	2.12± 0.016997	1.36± 0.024495	1.28± 0.012472	2.24± 0.029439	a 1.75		32.02± 0.043205	32.94± 0.109646	28.83± 0.053541	14.487± 0.005907	ab 31.31			
Kaseemi	0.99± 0.101434	1.21± 0.016997	1.25± 0.024495	0.73± 0.028674	b 1.45		29.8± 0.331696	37.56± 0.161314	36.28± 0.115854	21.58± 0.2585	abc 28.92			
Masr 1	1.196± 0.002055	0.889± 0.004497	1.51± 0.020548	1.42± 0.026247	bc 1.43		34.89± 0.086023	19.27± 0.030912	24.77± 0.024944	36.73± 0.179877	abc 27.91			
Yammani	1.72± 0.037417	1.76± 0.024495	1.83± 0.020548	2.16± 0.088066	bc 1.40		33.58± 0.073182	29.5± 0.08165	22.67± 0.012472	15.34± 0.462265	abc 27.71			
Madini	1.32± 0.020548	1.31± 0.012472	1.17± 0.01633	1.91± 0.020548	c 1.26		36.58± 0.326633	31.89± 0.179134	38.55± 0.161107	21.59± 0.100333	bc 27.07			
Nagrani	1.79± 0.012472	1.55± 0.016997	2.08± 0.043205	1.897± 0.003091	d 1.06		30.9± 0.014142	24.13± 0.061283	32.11± 0.063421	14.66± 0.12083	c 25.45			
Seds 12	1.13± 0.028674	1.081± 0.003742	1.09± 0.041737	0.922± 0.012037	d 1.05		38.47± 0.30576	33.31± 0.127105	36.16± 0.258242	21.16± 0.258242	c 25.27			
Mean	1.58 a	1.47 ab	1.45 b	1.32 c			32.01 a	31.19 a	28.97 a	22.54 b				
LSD 0.05	0.12							3.08						

The data reported in Tables (5,6) reveal that the treatment with magnetic water significantly increased the seedling fresh and dry weight and relative water content percentage in these wheat cultivars (Giza168, Tabouki, Kaseemi, Yamanei, Madini, Nagrani and Seds) when magnetic grain treatment and magnetically treated water were jointly applied as compared to control treatments. While Sakha93 cultivar observed negative stimulating for magnetic field and magnetic water treatments therefore recorded decreased significant in seedling fresh and dry weight and relative water content percentage compared with control treatment. The interactive effect of grain and water magnetic treatments, reveal significant interaction where the highest seedling fresh and dry weight and water content were obtained from those resulted from magnetically treated grains grown in magnetized water. Relative water content percentages at 21 days have shown a significant increase in response to exposure to magnetic field and irrigation with magnetic water. This increment may be attributed to increasing ions mobility and ions uptake improved under magnetic treatments which

leads to a better water content stimulation in positive stimulate wheat cultivars Moreover, magnetic field has the ability to change water properties. The above results mentioned to the better role of irrigation with magnetize water on seedling growth, whereas in general the magnetic grain and water treatment surpassed the control treatment. These results coincide with those of **FI'orez et al. (2007)** who reported that maize seedling treated with magnetic field were significantly heavier than the control, **Souza et al (2005)** indicated that the pre sowing magnetic treatment of tomato seeds, that led to significant increase in seedling root and stem fresh weight. **Abou El-Yazied et al. (2011)** who mentioned that in the nursery experiment, applying the optimal magnetic tomato seeds treatment (0.1 T for 15 min) and/or irrigation with magnetized water gave significant increases in transplant stem length, stem diameter, leaf area and fresh and dry weight than those in the control treatment which grew by untreated seeds and irrigated by ordinary (untreated water) water.

Table (5): Effect of magnetic treatments on seedling fresh and dry weight in different wheat grain cultivars after 21 days from grain treatment

Treatments	Seedling fresh and dry weight (gm)												
	Seedling fresh weight					Seedling dry weight							
	Exposure MF		Un exposure MF			Me an	L S D	Exposure MF		Un exposure MF		Me an	L S D
	Magnetic water	Tap water	Magnetic water	Tap water	Magnetic water			Tap water	Magnetic water	Tap water			
Giza168	0.723± 0.029691	0.5617± 0.011878	0.6257± 0.028912	0.487± 0.039064	a	0.11	0.0423± 0.009631	0.0303± 0.001349	0.0317± 0.003027	0.0223± 0.001347	a	0.01	
Sakha 93	0.231± 0.01271	0.385± 0.035462	0.5017± 0.032605	0.7127± 0.03045	ab		0.0213± 0.001271	0.0313± 0.001271	0.0417± 0.003027	0.054± 0.003682	a		
Tabouki	0.6223± 0.015976	0.6707± 0.038688	0.5073± 0.044577	0.1953± 0.045331	abc		0.0417± 0.003126	0.0401± 0.001702	0.0315± 0.002123	0.0157± 0.003197	ab		
Kaseemi	0.4853± 0.043799	0.7217± 0.01635	0.6497± 0.029303	0.2183± 0.020655	abc		0.0317± 0.00297	0.0557± 0.003197	0.0550± 0.002944	0.0221± 0.003023	abc		
Masr 1	0.7233± 0.020428	0.5047± 0.046073	0.667± 0.033905	0.7853± 0.043514	abc		0.0541± 0.003679	0.0221± 0.008552	0.0253± 0.015698	0.0551± 0.002642	abcd		
Yammanei	0.7553± 0.02284	0.687± 0.039064	0.547± 0.030576	0.4297± 0.038337	bcd		0.0423± 0.001382	0.0317± 0.001855	0.0223± 0.001837	0.0185± 0.003472	bcd		
Madini	0.6533± 0.022113	0.6013± 0.018557	0.7253± 0.021234	0.5183± 0.034728	cd		0.0550± 0.004288	0.0413± 0.002393	0.0577± 0.00641	0.0221± 0.005504	cd		
Nagrani	0.6137± 0.012684	0.4587± 0.035531	0.6806± 0.033946	0.229± 0.038404	cd		0.0320± 0.007608	0.0253± 0.014143	0.0417± 0.004036	0.0173± 0.003681	d		
Sods 12	0.7963± 0.060943	0.6497± 0.038396	0.711± 0.021453	0.2383± 0.033918	d		0.0567± 0.010748	0.0423± 0.001452	0.0551± 0.002082	0.0220± 0.00132	d		
Mean	0.62 a	0.62 a	0.58 a	0.42 b			0.042 a	0.044 ab	0.04 b	0.028 c			
LSD	0.08						0.01						
0.05													

Table (6): Effect of magnetic treatments on % of relative water content in different wheat grain cultivars after 21 days from grains treatment

Treatments	Relative water content %					L S D
	Exposure +Dipping MW	Exposure +Dipping TW	No exposure + Dipping MW	No exposure + Dipping TW	Me an	
Giza168	94.15± 0.532812	94.61± 0.325611	94.93± 0.537463	95.42± 0.148997	a	0.87
Sakha 93	90.78± 0.147045	91.89± 0.155134	91.69± 0.176824	92.42± 0.975329	ab 94.78	
Taboky	93.29± 0.577485	94.02± 1.829432	93.79± 0.975329	91.96± 0.176824	bc 94.33	
Kassem	93.47± 1.414269	92.28± 0.975329	91.54± 0.176824	89.88± 1.122567	cd 93.89	
Masr 1	92.52± 0.583229	95.62± 1.230239	96.21± 0.648194	92.98± 0.671764	d	
Yammany	94.39± 0.975329	95.39± 0.671764	95.92± 0.862915	95.69± 0.842312	de 93.12	
Madany	91.58± 0.47204	93.13± 0.84626	92.04± 0.763428	95.74± 0.990252	ef 92.35	
Nagrani	94.79± 0.519123	94.48± 0.546036	93.87± 0.501487	92.45± 0.910641	f	
Sods 12	92.88± 0.789472	93.49± 0.476725	92.25± 0.869572	90.77± 0.47204	f	
Mean	93.88 a	93.58 ab	93.09 b	93.03 b		
LSD	0.58					
0.05						

Table (7): Effect of magnetic treatments on number of protein bands in different wheat grain cultivars after 21 days from grains treatment.

Treatments	Number of protein bands			
	Exposure +Dipping MW	Exposure +Dipping TW	No exposure + Dipping MW	No exposure + Dipping TW
Giza168	20	12	14	8
Sakha 93	9	10	14	17
Taboky	14	13	14	5
Kassem	19	7	15	5
Masr 1	15	6	15	15
Yammany	12	12	6	9
Madany	13	12	15	8
Nagrani	15	10	12	8
Sods 12	12	8	16	8

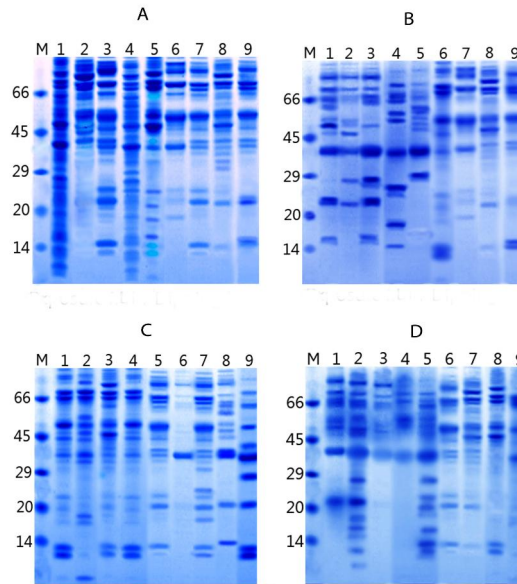


Fig (2): Sodium dodcyle sulphate– polyacrylamide gel electrophoresis (SDS-PAGE) For treated and un treated nine cultivars of wheat seedling cultivars with magnetic field.

(A). Exposed to **magnetic field + Dipping in magnetic water.**

(B). Exposed to **magnetic field + Dipping in tap water.**

(C). No Exposed to **magnetic field + Dipping in magnetic water.**

(D). No Exposed to **magnetic field + Dipping in tap water.**

Lanes 1- 9 (Different wheat cultivars seedling)

Lane M = SDS-Marker.

L1: Giza168, L2: Sakha 93, L3: Tabouki, L4: Kaseemi, L5: Masr1, L6: Yamanei, L7: Madini, L8: Nagrani and L9: Seds 12.

The changes in protein electrophoretic pattern of wheat seedlings treated with magnetic field and water are analyzed and recorded in Table (7) and illustrated in (Figure 2). In the control seedling cultivars (Giza168, Tabouki, Kaseemi, Yamanei, Madini, Nagrani and Seds), the separation of 8, 5, 5, 9, 8, 8, 8 protein bands (PBs) were appeared respectively, while, Sakha93 cv recorded 17 PBs and Masr1 recorded 15 PBs their molecular weights ranged between 78 K Da. and 10 K Da. Magnetic field and water treatments of wheat seedling cultivars (Giza168, Tabouki, Kaseemi, Yamanei, Madini, Nagrani and Seds12) showed an increase in the number of protein bands to 20, 14, 19, 12, 13, 15, 12 PBs were appeared respectively, while, Sakha93 cv recorded 9 PBs and Masr1 recorded 15 PBs their molecular weights ranged between 85 K Da. and 9 K Da. respectively. Therefore, wheat cultivars (Giza168, Tabouki, Kaseemi, Yamanei, Madini, Nagrani and Seds12) gave height increase in protein bands number when grain exposed to magnetic field and irrigated by magnetic water and gave high stimulation rate of novel protein bands forms compared to control treatments. While Sakha93 cultivar observed negative stimulating for magnetic field and magnetic water treatments. On the other

hand, the magnetic treatments don't effects on number of protein bands for Masr1cv. than those of the control treatment. These results indicate that the wheat seedling treated with magnetic field and water characterized by disappearance of certain bands and the appearance of new ones as compared with that of the control plant. Similar result was noticed by **Hozayn *et al.* (2010)** who found that the magnetic water treatment of other wheat cultivars showed an increase in the number of protein bands to 16 bands and the formation of new protein bands in wheat plants treated with magnetic water was accompanied with increasing growth parameters and total indole acetic acid in treated plants. **Shabrangi and Majd (2009)** reported that magnetic field is known as an environmental factor which affects on gene expression, therefore, by augmentation of biological reactions like protein synthesis. **Balouchi *et al.* (2007)** confirmed that MF influences the structures of cell membrane, and increases their permeability and ion transport, which then affects some metabolic pathways. **Moon and chunge (2000)** reported that magnetic field treatments influencing the biochemical processes involve free radicals by stimulating the activity of proteins and enzymes. On the other hand, **Hanafy *et al.*, (2006)** indicated that the data indicated

that the molecular structure of the extracted WSP changed the amount of protein in the bands of exposed grains decreased and their molecular weights changed.

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Soluble Receptor for Advanced Glycation End Products: a new biomarker in diagnosis of Diabetic Nephropathy

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Abstract: Background: Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>300 mg/d or >200 mcg/min). The interaction of advanced glycation end products with their cellular receptor (RAGE) is implicated in the pathogenesis of diabetic vascular complications. RAGE has a circulating secretory receptor form, soluble RAGE (sRAGE), which, by neutralizing the action of advanced glycation end products, might exert a protective role against the development of cardiovascular disease. Objective: to study the serum levels of sRAGE in type 2 diabetic patients and to clarify the possible association with urinary albumin excretion as an early marker of microvascular damage. Patients and Methods: Eighty subjects divided into two groups; group I (patients group) included 60 type 2 diabetic patients. They were subdivided into 2 subgroups: twenty normo-albuminuric diabetic subgroup and forty micro-albuminuric diabetic subgroup. Group II (control group) included 20 apparently healthy individuals of matched age and sex. All cases were subjected for estimation of sRAGE by sandwich ELISA technique together with routine laboratory investigations including fasting blood glucose, s. creatinine, cholesterol, triglycerides, HDL-C, LDL-C, HbA1C and Microalbumin. Results: sRAGE was significantly lower in microalbuminuric diabetic than normoalbuminuric diabetic and control groups ($p<0.05$). There was a positive significant correlation between sRAGE and HDL-cholesterol and a negative significant correlation between sRAGE and creatinine, total cholesterol, triglycerides, LDL-cholesterol HbA1C and microalbumin. Conclusion: The present study found that sRAGE blood levels are lower in diabetic patients who have renal complications, supporting the hypothesis that sRAGE, by limiting the interaction of AGE with cell membrane RAGE, can protect vessels against AGE toxicity. Thus, stimulation of sRAGE production should be considered as a potential therapeutic target in diabetes and AGE-related vascular disease.

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Key words: sRAGE, diabetic nephropathy, microalbuminuria.

1. Introduction

Globally as of 2010 it was estimated that there were 285 million people with type 2 diabetes making up about 90% of diabetes cases⁽¹⁾. This is equivalent to about 6% of the world's adult population⁽²⁾. In screening for diabetic nephropathy, early testing for glucose intolerance and diabetes are recommended to identify patients who are at risk for developing microalbuminuria, particularly if they have other risks for type 2 diabetes, such as hypertension, lipid abnormalities, or central obesity, approximately one third of type 2 diabetics are believed to be undiagnosed. Once the diagnosis of diabetes has been made, check urinary protein levels to guide therapy and prognosis⁽³⁾.

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>300 mg/d or >200 mcg/min) that is confirmed on at least 2 occasions 3-6 months apart, a relentless decline in the glomerular filtration rate (GFR), and elevated arterial blood pressure. The main feature of diabetic glomerulosclerosis is excess accumulation of extracellular matrix leading to mesangial matrix

expansion as well as glomerular basement membrane thickening, which then becomes targeted by advanced glycation end product (AGE) modification. At a cellular level, release of transforming growth factor-beta (TGF-beta) is the main trigger of this process⁽⁴⁾.

Early in the course of the disease, mesangial cells may undergo a phase of limited proliferation, but then they typically arrest in the G₁ phase of the cell cycle to produce extracellular matrix⁽⁵⁾.

Furthermore, mesangial cells exposed to AGE-albumin at concentrations comparable to those found in human pathology showed increased collagen IV and TGF- β expression as well as activation of protein kinase C, the mesangial cells also produced monocyte chemotactic protein-1, which stimulated prostacyclin production by endothelial cells. This pathophysiological sequence may serve as a model for the events leading to chronic glomerular injury in the diabetic kidney *in vivo*⁽⁶⁾.

The exact cause of diabetic nephropathy is unknown, but various postulated mechanisms are hyperglycemia (causing hyperfiltration and renal injury), advanced glycosylation products, and

activation of cytokines. Hyperglycemia increases the expression of TGF-beta in the glomeruli and of matrix proteins specifically stimulated by this cytokine. TGF-beta may contribute to the cellular hypertrophy and enhanced collagen synthesis observed in persons with diabetic nephropathy⁽⁷⁾.

High levels of AGE accumulate in diabetic patients, specifically within the vascular intima, the nodular and diffuse lesions of the glomeruli and within hyaline deposits of arterioles, thus again demonstrating the importance of AGE in the pathogenesis of diabetic nephropathy⁽⁸⁾. Receptor for advanced glycation endproducts [RAGE] is a member of the immunoglobulin superfamily, encoded in the Class III region of the major histocompatibility complex⁽⁹⁾.

The interaction of advanced glycation end products, including Nε-(carboxymethyl) lysine-protein adducts (CML) and S100A12 protein, with their cellular receptor (RAGE) is implicated in the pathogenesis of diabetic vascular complications. RAGE has a circulating secretory receptor form, soluble RAGE (sRAGE), which, by neutralizing the action of advanced glycation end products, might exert a protective role against the development of cardiovascular disease⁽¹⁰⁾. Although several hyperglycemia-elicited metabolic and hemodynamic derangements have been implicated in the pathogenesis of diabetic vascular complication, the process of formation and accumulation of advanced glycation end products (AGEs) and their mode of action are most compatible with the theory 'hyperglycemic memory'. Further, there is a growing body of evidence that AGEs and their receptor (RAGE) axis is involved in the pathogenesis of diabetic vascular complication. Indeed, the engagement of RAGE with AGEs is shown to elicit oxidative stress generation and subsequently evoke inflammatory responses in various types of cells, thus playing an important role in the development and progression of diabetic micro- and macroangiopathy. These observations suggest that down-regulation of RAGE expression or blockade of the RAGE downstream signaling may be a promising target for therapeutic intervention in diabetic vascular complication⁽¹¹⁾.

The aim of the work is to study the serum levels of sRAGE in type 2 diabetic patients and to clarify the possible association with urinary albumin excretion as an early marker of microvascular damage.

2. Subjects and Methods:

The present study was conducted on 80 subjects divided into two groups; group I (patients group) included 60 type 2 diabetic patients. They were 22 males and 38 females, subdivided into 2

subgroups: Subgroup Ia (normo-albuminuric diabetic group) which included 20 type 2 diabetic patients with normo- albuminuria (8 males and 12 females). Their ages ranged from 45 to 62 years with a mean age of (52.9±6 years) and subgroup Ib (micro-albuminuric diabetic group) which included 40 type 2 diabetic patients with micro-albuminuria (14 males and 26 females). Their ages ranged from 48 to 65 years with a mean age of (51.5±5.5 years). Diabetic patients were selected from those attending Diabetes Outpatient Clinic in Benha University Hospital. Group II (control group) included 20 apparently healthy individuals of matched age and sex. They were 8 males and 12 females. Their ages ranged from 45-62 years (50.8±10.9). The control group consists of healthy volunteers without a history of arterial hypertension, neoplastic, cardiovascular, inflammatory, lung, endocrinal or central nervous system disorder. Exclusion criteria were inflammatory conditions, renal failure patients, cardiac disease and liver disease. All subjects were subjected to:

- I. Full history taking (age, sex, duration of diabetes).
- II. Through clinical examination.
- III. Laboratory investigations:

Blood samples were drawn from all subjects after overnight fasting (10-16 hours) by venipuncture:

- 1- One ml of blood on 15 µL EDTA for determination of HbA1C.
- 2- Two milliliters were anticoagulated using sodium fluoride for determination of fasting blood glucose level.
- 3- Four milliliters were placed in plain tubes and allowed to clot for 30 minutes in water bath at 37°C and then centrifuged for 15 minutes at 1000 xg. Serum was then subdivided into two aliquots:
 - a- The first aliquot was used for determination of creatinine and lipid profile assays.
 - b- The second aliquot was used for RAGE assay. This aliquot was kept at - 70°C for subsequent assay.

Second morning urine samples were voided after rising for estimation of microalbumin.

Methodology:

- 1- Fasting blood glucose, creatinine, cholesterol, triglycerides and HDL-cholesterol were performed by automated enzymatic methods (Cobas Integra 400 analyzer, Roche, Germany). LDL-cholesterol was calculated according to Friedwald formula:

$$\text{LDL-cholesterol} = \text{Total cholesterol} - \text{HDL-cholesterol} - \text{TG}/5.$$
- 2- Glycated hemoglobin (HbA1C) level was assessed through HPLC technique using Bio – Rad, Hercules, USA kits.

- 3- sRAGE level was assessed through enzyme linked immunosorbent assay (ELISA) supplied by RayBiotech Incorporation, Norcross, GA, USA. The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for RAGE (extracellular domain) has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any RAGE present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for RAGE (extracellular domain) is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of RAGE bound in the initial step. The color development is stopped and the intensity of the color is measured using a microplate reader at 540 nm.
- 4- Microalbumin in urine was assessed by using Micral test strips.

Statistical Analysis:

The data were coded, entered and processed on an IBM-PC compatible computer using SPSS (version 11). The level $p < 0.05$ was considered the cut-off value for significance. Descriptive statistics of the different studied groups were done using the mean and standard deviation.

Student "t" test was used for the comparison between each 2 groups according to all measured parameters. Correlation analysis was used for assessing the strength of association between two variables. The correlation coefficient denoted symbolically r , defines the strength and direction of the linear relationship between serum levels of sRAGE and other variables among cases.

3. Results:

Parameters including fasting blood glucose, S. creatinine, total cholesterol, triglycerides, LDL-cholesterol and HbA_{1c} were significantly higher in diabetic than control group while HDL-cholesterol and sRAGE were significantly lower in diabetic than control group.

Table (1): Comparison of different laboratory parameters between the control group (GI) and the diabetic 2 patients group (GII)

Parameter	Control group (N=20) Mean ± SD	Type 2 diabetic patients (N= 60) Mean ± SD	t	p
Age (years)	50.8 ± 10.9	51.9 ± 5.7	0.58	>0.05
Fasting Blood Glucose (mg/dl)	86.3 ± 8.8	168.3 ± 29.2	19.3	<0.001
S. Creatinine (mg/dl)	0.6 ± 0.1	0.95 ± 0.2	7.5	<0.001
Total cholesterol (mg/dl)	98.3 ± 8.6	198.2 ± 47.7	15.49	<0.001
Triglycerides (mg/dl)	76.7 ± 7.8	131.6 ± 60.6	6.86	<0.001
HDL-cholesterol (mg/dl)	74.1 ± 17.5	33.6 ± 4.3	10.49	<0.001
LDL-cholesterol (mg/dl)	49.1 ± 4.4	98.5 ± 24.8	14.75	<0.001
HbA1C(%)	4.9 ± 0.5	10.7 ± 0.8	38.6	<0.001
sRAGE (pg/ml)	1452.1 ± 854.7	940 ± 680	2.42	<0.05

p value >0.05 is considered non significant. p value <0.05 is considered significant.

p value <0.01 is considered highly significant.

Table (2): Comparison of different laboratory parameters between control group and normoalbuminuric diabetic group

Parameter	Control group (N=20) Mean ± SD	Normoalbuminuric diabetic group (N= 20) Mean ± SD	t	p
Age (years)	50.8 ± 10.9	52.9 ± 6.0	0.96	>0.05
Fasting Blood Glucose (mg/dl)	86.3 ± 8.8	165.6 ± 26.1	10.8	<0.001
S. Creatinine (mg/dl)	0.6 ± 0.1	0.6 ± 0.1	-----	-----
Total cholesterol (mg/dl)	98.3 ± 8.6	163.9 ± 27.4	10.4	<0.001
Triglycerides (mg/dl)	76.7 ± 7.8	90.2 ± 30.2	1.95	<0.05
HDL-cholesterol (mg/dl)	74.1 ± 17.5	38.1 ± 10.1	8.0	<0.001
LDL-cholesterol (mg/dl)	49.1 ± 4.4	82.1 ± 13.5	4.95	<0.001
HbA1C(%)	4.9 ± 0.5	8.0 ± 0.7	20.6	<0.001
sRAGE (pg/ml)	1452.1 ± 854.7	1050 ± 410	1.9	<0.05

Parameters including fasting blood glucose, total cholesterol, triglycerides, LDL-cholesterol and HbA_{1c} were significantly higher in normoalbuminuric diabetic than control group while

HDL-cholesterol and sRAGE were significantly lower in normoalbuminuric diabetic than control group.

Table (3): Comparison of different laboratory parameters between control group and microalbuminuric diabetic group.

Parameter	Control group (N=20) Mean ± SD	Microalbuminuric diabetic group (N= 40) Mean ± SD	t	p
Age (years)	50.8 ± 10.9	51.5 ± 5.5	0.33	>0.05
Fasting Blood Glucose (mg/dl)	86.3 ± 8.8	173.8 ± 35.4	13.2	<0.001
S. Creatinine (mg/dl)	0.6 ± 0.1	0.97 ± 0.2	7.8	<0.001
Total cholesterol (mg/dl)	98.3 ± 8.6	215.4 ± 46.6	11.1	<0.001
Triglycerides (mg/dl)	76.7 ± 7.8	152.3 ± 61.5	5.5	<0.001
HDL-cholesterol (mg/dl)	74.1 ± 17.5	27.1 ± 9.2	11.2	<0.001
LDL-cholesterol (mg/dl)	49.1 ± 4.4	106.8 ± 25.1	10.1	<0.001
HbA1C(%)	4.9 ± 0.5	11.2 ± 0.8	42	<0.001
sRAGE (pg/ml)	1452.1 ± 854.7	858.3 ± 488.7	5.1	<0.05

Parameters including fasting blood glucose, S. creatinine, total cholesterol, triglycerides, LDL-cholesterol and HbA_{1c} were significantly higher in microalbuminuric diabetic than control group while

HDL-cholesterol and sRAGE were significantly lower in microalbuminuric diabetic than control group.

Table (4): Comparison of different laboratory parameters between normoalbuminuric and microalbuminuric diabetic subgroups.

Parameter	Normoalbuminuric diabetic group (N= 20) Mean ± SD	Microalbuminuric diabetic group (N= 40) Mean ± SD	t	p
Age (years)	52.9 ± 6.0	51.5 ± 5.5	0.88	>0.05
Fasting Blood Glucose (mg/dl)	165.6 ± 26.1	173.8 ± 35.4	0.92	>0.05
S. Creatinine (mg/dl)	0.6 ± 0.1	0.97 ± 0.2	9.55	<0.001
Total cholesterol (mg/dl)	163.9 ± 27.4	215.4 ± 46.6	5.37	<0.001
Triglycerides (mg/dl)	90.2 ± 30.2	152.3 ± 61.5	5.24	<0.001
HDL-cholesterol (mg/dl)	38.1 ± 10.1	27.1 ± 9.2	4.07	<0.001
LDL-cholesterol (mg/dl)	82.1 ± 13.5	106.8 ± 25.1	4.95	<0.001
HbA1C(%)	8.0 ± 0.7	11.2 ± 0.8	21.3	<0.001
sRAGE (pg/ml)	1050 ± 410	858.3 ± 488.7	1.61	<0.05

Parameters including S. creatinine, total cholesterol, triglycerides, LDL-cholesterol and HbA_{1c} were significantly higher in microalbuminuric diabetic than normoalbuminuric diabetic groups while HDL-cholesterol and sRAGE were significantly lower in microalbuminuric diabetic than normoalbuminuric diabetic groups.

There was a positive significant correlation between sRAGE and HDL-cholesterol.

There was a negative significant correlation between sRAGE and creatinine, total cholesterol, triglycerides, LDL-cholesterol HbA_{1c} and microalbumin.

Table (5): Correlation coefficients (r) & probability value (p) between sRAGE and other parameters among type 2 diabetic patients group:

Parameters	sRAGE	
	r	p
Age (years)	0.0496	>0.05
FBS (mg/dl)	0.084	>0.05
S. creatinine (mg/dl)	-0.349	<0.05
Total cholesterol (mg/dl)	-0.804	<0.001
Triglycerides (mg/dl)	-0.656	<0.01
HDL-C (mg/dl)	0.719	<0.001
LDL-C (mg/dl)	-0.817	<0.001
HbA1C(%)	-0.554	<0.01
Microalbumin (mg/l)	-0.545	<0.01

4. Discussion:

Advanced glycation is one of the pathways by which cellular injury is induced in diabetes and lead to formation of advanced glycation end products (AGEs). There is substantial evidence to support the involvement of advanced glycation end-products (AGE) binding to its receptor (RAGE) in the development of diabetic microvascular complications as atherosclerosis and nephropathy⁽¹²⁾.

The comparative study of serum creatinine among the studied groups showed a significant increase in the serum level of creatinine in type 2 diabetic group as compared to the control group ($p < 0.001$), and significant increase in the serum level of creatinine in microalbuminuric diabetic group as compared to normoalbuminuric diabetic group ($p < 0.001$) and control group ($p < 0.001$). These results showed increased serum level of creatinine with the progression of diabetic nephropathy but it was within normal range in normoalbuminuric and control group.

The results of creatinine among the studied groups were in agreement with many investigators^(13,14). They demonstrated that, increased serum level of creatinine in microalbuminuric diabetic patients as a marker of diabetic nephropathy than normoalbuminuric diabetic patients.

In this study, there is a significant difference in serum levels of lipid profile in microalbuminuric diabetic group compared to normoalbuminuric diabetic group ($p < 0.001$) and control group ($p < 0.001$).

The results of lipid profile among the studied groups were in agreement with many investigators⁽¹⁵⁻¹⁸⁾. They demonstrated that diabetic dyslipidemia is characterized by an elevation of TG and a reduction in HDL-C in type 2 diabetes mellitus. Also they reported that serum total cholesterol and LDL-C may be elevated in type 2 diabetic patients and are considered as cardiovascular risk factors in type 2 diabetes mellitus.

In the present work, comparative study of HbA1C as a marker of glycemic control among studied groups showed poor glycemic control state in microalbuminuric diabetic patients compared with normoalbuminuric diabetic patients.

The present study showed significant decrease in serum level of sRAGE in diabetic group compared to control group ($p < 0.001$). This is in agreement with *Basta et al.*⁽¹⁹⁾ who reported that plasma sRAGE levels were diminished in type 1 and type 2 diabetes and correlated inversely with intima-media thickness, suggesting a protective role of high sRAGE levels in the development of late vascular complications.

These findings go on line with another study performed by *Grossin et al.*⁽²⁰⁾, they found that

patients with renal and retinal complications had significantly lower blood levels of sRAGE compared with patients without complications.

Glomerular hyperperfusion and renal hypertrophy occur in the first years after the onset of DM and are reflected by an increased glomerular filtration rate (GFR). Before the onset of overt proteinuria, there are various renal functional changes including renal hyperfiltration, hyperperfusion, and increasing capillary permeability to macromolecules then diabetic nephropathy develops⁽²¹⁾. In this study, the patients with type 2 diabetes were subdivided into 2 subgroups (normoalbuminuric and microalbuminuric diabetic patients) according to urinary microalbumin excretion, and the study showed a significant negative correlation of sRAGE with microalbuminuria in patient with type 2 diabetic nephropathy at the early stage.

The comparative study of sRAGE among the studied groups showed significant decrease in sRAGE in microalbuminuric diabetic patient ($p < 0.001$) than normoalbuminuric diabetic patient, and decrease in sRAGE level in microalbuminuric diabetic patients than control group.

These findings go online with many studies as *Tan*⁽²²⁾ who found that serum sRAGE levels and circulating AGEs are associated with the severity of nephropathy in type 2 diabetic patients, and *Bruno et al.*⁽²³⁾ also reported that low sRAGE with high CML-protein levels in diabetic patients developed severe diabetic complications and patients with higher sRAGE levels did not exhibit vascular complications.

Another study made by *Humpert et al.*⁽²⁴⁾ showed that plasma sRAGE was associated with albumin excretion in type 2 diabetic patient. Hence, they reported that plasma sRAGE levels might represent an early marker of microvascular dysfunction and diabetic nephropathy in type 2 diabetes. Also previous reports stated an inverse correlation of intima-media thickness with sRAGE levels in type 1 and type 2 diabetes⁽²⁵⁾.

This study showed that sRAGE was negatively correlated with HbA1C and its level was significantly higher ($p < 0,05$) in good glycemic control diabetic patients compared with lower level of sRAGE in poor control glycemic patients. These results revealed that sRAGE is inversely associated with HbA1c as a marker of glycemic control in diabetic subject. This is in agreement with *Nakamura et al.*⁽²⁶⁾ who found that sRAGE was inversely associated with HbA1c in their diabetic subjects.

On the other hand *Yamagishi et al.*⁽²⁷⁾ suggested that endogenous sRAGE may capture and eliminate circulating AGEs and decrease its serum levels. However, AGEs up-regulate tissue RAGE

expression and endogenous sRAGE could be generated from the cleavage of cell surface RAGE, so sRAGE may be positively associated with circulating AGEs and HbA1c (as one of the early glycation products) by reflecting tissue RAGE expression.

Basta et al.⁽¹⁰⁾ also found that circulating soluble receptor for advanced glycation end products is inversely associated with glycemic control and S100A12 protein as plasma level of sRAGE is down-regulated in chronic hyperglycemia; among its ligands, S100A12 protein.

The present study also showed that, low serum sRAGE levels were associated with hyperlipidemia as there was a significant negative correlation with s.cholesterol, s.triglyceride and LDL-C, and a significant high positive correlation with HDL-C among diabetic group. **Lehmann et al.**⁽²⁸⁾, demonstrated that type 2 diabetes patients with a state of chronic hyperglycemia, and glucose-dependent processes are likely to be involved in the pathogenesis of diabetic complications, including nephropathy. Glucose-induced tissue injury may be mediated by generation of advanced glycated proteins which have been implicated in nephropathy.

In conclusion, the present study found that sRAGE blood levels are lower in diabetic patients who have renal complications, supporting the hypothesis that sRAGE, by limiting the interaction of AGE with cell membrane RAGE, can protect vessels against AGE toxicity. Thus, stimulation of sRAGE production should be considered as a potential therapeutic target in diabetes and AGE-related vascular disease.

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Frequency, Temperature and Composition Dependence of Dielectric Properties of Nd³⁺ Substituted Cu-Zn Ferrites

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Abstract: The frequency, temperature and composition dependence of ac resistivity ρ_{ac} , dielectric constant ϵ' and dielectric loss ϵ'' of $\text{Cu}_{0.5}\text{Zn}_{0.5}\text{Nd}_x\text{Fe}_{2-x}\text{O}_4$ ferrites (where $x=0.0, 0.02, 0.04, 0.06, 0.08$ and 0.1) have been studied at low frequency range. For all samples, ρ_{ac} , ϵ' and ϵ'' are found to decrease with increasing the frequency. The composition dependence of ρ_{ac} , ϵ' and ϵ'' shows that, generally, ρ_{ac} increases while both ϵ' and ϵ'' decrease with increasing x . The obtained results are satisfactorily explained using the non uniform model of Koops.

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Key words: ferrites, dielectric properties

1. Introduction

Ferrites are well known dielectric materials which are useful in microwave applications. It is known that the intrinsic properties of ferrites depend on their chemical composition, heat treatment and type of additive or substituted ions [1]. The dielectric properties depend on the doping level as well as the valence of substituted ions. The effect of substitution and addition of different ions on the magnetic and electrical properties of Cu-Zn ferrite have been studied by several authors [2, 3]. Moreover, the influence of Nd oxide substitution on the magnetic properties of Cu-Zn ferrite was studied by members of our lab [4]. As an extension of these studies, this paper aimed to study the effect of Nd³⁺ ion substitution with different concentrations on the dielectric properties of Cu-Zn ferrite. As we aware, such a work was not previously studied.

2. Experimental techniques

Ferrite samples with the chemical formula $\text{Cu}_{0.5}\text{Zn}_{0.5}\text{Nd}_x\text{Fe}_{2-x}\text{O}_4$ ($x=0.0, 0.02, 0.04, 0.06, 0.08$ and 0.1) were prepared by the usual standard ceramic method. X-ray diffraction identification, the density and porosity were performed. More details about the samples preparation and characterization are given elsewhere [4]. For measuring the electrical resistivity, the sample's surfaces were rubbed with silver paste as a contact material. Parallel capacitance (C_p) was measured using PM 6304 LCR meter. The real part of the dielectric constant (ϵ') was

calculated using the formula [5] $\epsilon' = \frac{C_p d}{\epsilon_0 A}$, where

d is the thickness, A is the cross-sectional area of the sample and ϵ_0 is the permittivity of free space ($\epsilon_0 = 8.85 \times 10^{-12}$ F/m). The ac resistivity (ρ_{ac}) was measured using the two probe method and hence the dielectric loss tangent ($\tan\delta$) and the imaginary part

of the dielectric constant ϵ'' were obtained from the relations $\rho_{ac} = \frac{1}{\epsilon' \epsilon_0 \omega \tan \delta}$ and $\epsilon'' = \epsilon' \tan \delta$,

where ω is the angular frequency = $2\pi f$. The parameters ρ_{ac} , ϵ' and ϵ'' were measured in the frequency range (100 Hz to 100 kHz) from room temperature up to 500 K.

3. Results and Discussion

X-ray diffraction patterns showed that all investigated samples have cubic spinel phase [4]. Although we replace Fe³⁺ ion (radius=0.64 Å) by the larger ion Nd³⁺ (radius=1.08 Å), the lattice parameter remained nearly constant. This was attributed to the change of the oxygen parameter by the Nd³⁺ substitution, such that the ionic radius of the octahedral B-site seems to increase at the expense of the tetrahedral A-site [4].

3.1 Frequency dependence of the dielectric properties

3.1.1. ac resistivity

The variation of the ac resistivity (ρ_{ac}) with frequency (f) is illustrated in Figure (1) for $\text{Cu}_{0.5}\text{Zn}_{0.5}\text{Nd}_x\text{Fe}_{2-x}\text{O}_4$ samples. It is obvious that ρ_{ac} decreases with increasing f . Similar trend has been reported for different ferrites [5, 6]. This behavior could be explained in view of Koops's model [9]. According to this model, the polycrystalline ferrite is considered to be composed of two layers; grains and grain boundaries. The grains are large and of low resistive material, ρ_1 . The grain boundaries are thin and of high resistive material, ρ_2 . Following Koops's model, the total impedance ρ could be written as

$$\rho = \rho^\infty + \left[\frac{\rho^0 - \rho^\infty}{1 + \omega^2 \tau^2} \right]$$

where the superscripts 0 and ∞ refer to low and high frequency values respectively and τ is a relaxation time.

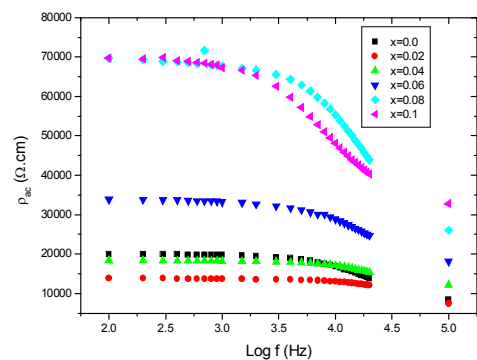


Fig. (1) Variation of the ac resistivity (ρ_{ac}) with frequency (f).

According to Koops's assumptions that $\rho_2 \gg \rho_1$, $h \ll 1$ (where h is the ratio of the grain boundary thickness to the grain thickness) and $h\rho_2 > \rho_1$, one can write the total impedance as

$$\rho \cong \rho_1 + \frac{h\rho_2}{1 + (b\rho_1\rho_2\omega^2 / h)}$$

where b is a constant [8]. Thus, at very low frequency, the impedance ρ^0 is given by

$$\rho^0 = \rho_1 + h\rho_2$$

According to the assumption that $h\rho_2 > \rho_1$, then the impedance at low frequency results mainly from the resistivity of the grain boundaries which have high resistivity. According to the above discussion, it is clear that Koops's model explains satisfactorily the frequency dependence of the resistivity of our investigated samples.

On the other hand, it was suggested that the conduction mechanism in ferrites is due to electron hopping between Fe^{2+} and Fe^{3+} [5]. The increase in frequency enhances the electron hopping frequency and hence increases the conductivity i.e. decreases the resistivity.

3.1.2 Real part of the dielectric constant

Figure (2) shows the frequency dependence of the real part of dielectric constant ϵ' for $Cu_{0.5}Zn_{0.5}Nd_xFe_{2-x}O_4$ samples. Increasing the frequency, it can be seen that ϵ' initially decreases by a small rate at low frequencies then it decreases by a rapid rate at high frequencies within our range. In fact, the decrease of ϵ' with increasing frequency was reported by several authors for different ferrites [9-11]. Moreover, the similarity between the frequency dependence of both the resistivity and the real part of the dielectric constant allows supposing that both parameters have the same origin [5, 12]. This means that the dielectric properties are mainly governed by the conduction mechanism in ferrites [5], wherein the electron hopping takes place. The electron

exchange between Fe^{3+} and Fe^{2+} gives local displacements of electron which induces polarization in ferrites. Therefore, at low frequency, where the electron hopping can match the frequency of the applied field, the dielectric constant has a maximum value. By increasing the frequency of the applied field, the electron exchange cannot follow the alternating field and so the dielectric constant decreases [13].

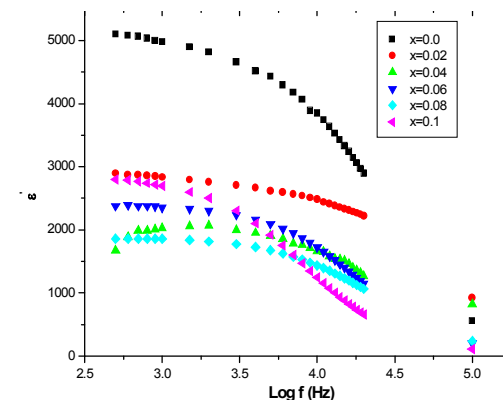


Fig. (2) Frequency dependence of the real part of the dielectric constant ϵ' .

3.1.3 Imaginary part of the dielectric constant

The variation of the imaginary part of the dielectric constant ϵ'' (which represents the dielectric loss) with frequency f is shown in Figure (3). It can be seen that ϵ'' decreases continuously with increasing the frequency. Such a decrease in ϵ'' could be discussed as follows. The electric dipole loss which results from the dipole orientation (relaxation) decreases, especially at high frequencies, as the dipole orientation can not follow the applied field frequency.

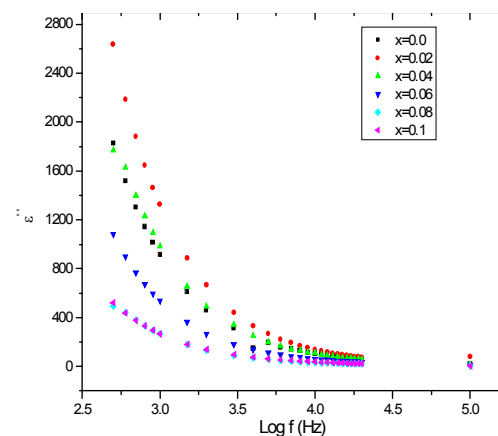


Fig. (3) Variation of the imaginary part of the dielectric constant ϵ'' with frequency.

3.2 Composition dependence of the dielectric properties

Figure (4) represents the composition dependence of ρ_{ac} along with the porosity P%, ϵ' and ϵ'' (at a frequency=10 kHz) for $\text{Cu}_{0.5}\text{Zn}_{0.5}\text{Nd}_x\text{Fe}_{2-x}\text{O}_4$ samples. This figure shows that ρ_{ac} generally increases with Nd concentration (x). The main factors that affect the resistivity in ferrites are the amount of Fe^{2+} ions and the porosity. It was found by many authors that the electrical resistivity is inversely proportional to the amount of Fe^{2+} ions as the decrease of Fe^{2+} ion concentration limits the hopping probability between Fe^{3+} and Fe^{2+} ions [14, 15]. Furthermore, the resistivity is directly proportional to the value of porosity because the increase of porosity hinders the motion of charge carriers [16]. For our samples, it can be seen that the increase of Nd content is on the expense of the iron concentration. So, as the Nd content increases, there is a continuous reduction of Fe^{2+} ion content, i.e. the decrease of the carrier concentration. This leads to increase the resistivity. Moreover, the increase of porosity causes the mobility of the carriers to decrease which enhances the resistivity.

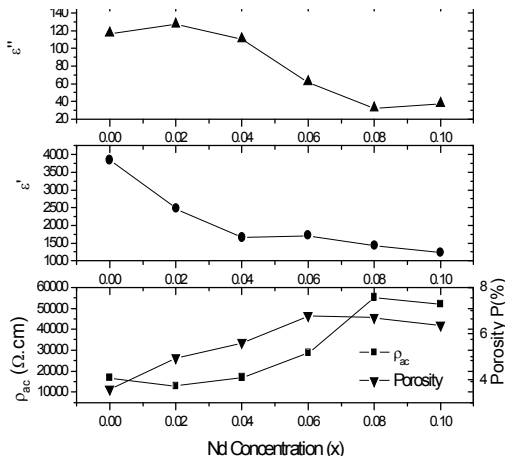


Fig. (4) Composition dependence of ρ_{ac} , ϵ' and ϵ'' ($f=10$ kHz).

On the other hand, ϵ' and ϵ'' have almost a reverse trend to ρ_{ac} which has been reported for Li-Mg [17], Li-Cd [18] and Li-Ti [19] ferrites. This reverse trend of ϵ' with ρ_{ac} for our investigated samples could be explained on the basis of the relation between the mobility μ of the electron hopping and resistivity $\rho = \frac{1}{ne\mu}$. Increasing the Nd

content decreases the electron exchange between Fe^{2+} and Fe^{3+} ions, i.e. the mobility becomes small and then this leads the resistivity to increase. Meanwhile such a decrease in the electron hopping causes the polarization to decrease i.e. ϵ' decreases.

Furthermore, the reverse behavior of ϵ'' with ρ_{ac} is expected as the increase of resistivity decreases the loss ϵ'' and vice versa which is in a good

agreement with the relation [5] $\rho_{ac} = \frac{1}{\epsilon'' \epsilon_o \omega}$

3.3 Temperature dependence of the dc resistivity

Figure (5) illustrates the variation of the dc resistivity ρ_{dc} with the temperature for all samples. It is obvious that the electrical resistivity decreases with increasing temperature, i.e. the resistivity exhibits a normal semiconducting behavior. This could be described by the well known relation [20].

$$\rho = \rho_o \exp\left(\frac{E_p}{KT}\right)$$

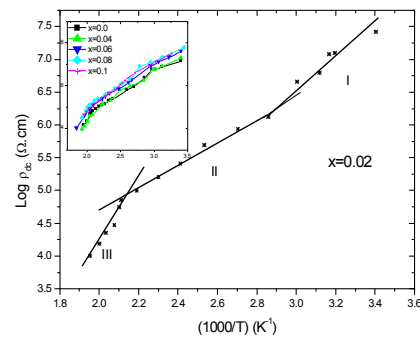


Fig. (5) Temperature dependence of the dc resistivity ρ_{dc} for the sample of $x=0.02$. (other samples ρ_{dc} are plotted inside the inset)

where ρ_o is a constant, E_p is the activation energy of the resistivity, K is Boltzmann's constant and T is the absolute temperature. Moreover, one can notice that each curve could be divided into three regions. Each region has different activation energy (E_p). The first region ranged from room temperature up to nearly 360 K. The conduction phenomenon in this region is attributed to the presence of impurities i.e. extrinsic conduction mechanism [21-23]. The formation of such impurities is due to the oxygen loss during the sintering process. The loss of oxygen leads to the formation of Fe^{2+} ions on the account of Fe^{3+} ions for charge compensation. These Fe^{2+} ions act as donor centers [24]. On the other hand, the transition temperatures, T_p , between the second and third regions have values that agree well to the determined values from the magnetic measurements T_c [4]. Therefore, the change in the activation energy at T_p could be attributed to a magnetic transition from the ferrimagnetic to the paramagnetic state. The effects of the magnetic transitions on the electrical properties of ferrites were reported by many authors [21-23].

3.4 Temperature dependence of the dielectric properties

3.4.1. ac resistivity

Figure (6) illustrates the variation of the ac resistivity ρ_{ac} with the temperature at different frequencies for the unsubstituted sample as an example. It is obvious that ρ_{ac} has similar behavior as ρ_{dc} . So, the temperature dependence of ρ_{ac} could be discussed on the same way as ρ_{dc} . In fact, our all investigated samples show the same trend. This can be noticed from the inset of Figure (6) that shows the temperature dependence of the ac resistivity for all investigated samples at $f=10$ kHz. Moreover, generally, the dispersion of ac resistivity decreases with increasing the temperature for all samples. This behavior was detected for many other ferrites [25].

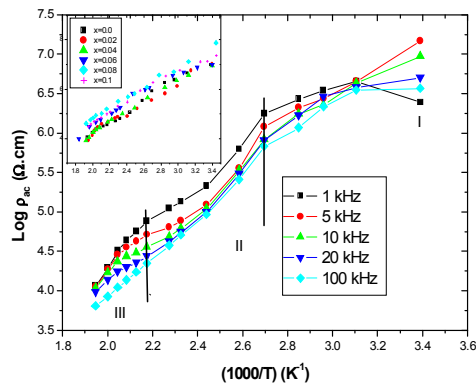


Fig. (6) Temperature dependence of the ac resistivity ρ_{ac} at different frequencies for the sample $\text{Cu}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$. (the inset shows the temperature dependence of the ac resistivity ρ_{ac} , at $f=10$ kHz, for all samples.)

3.4.2. Real part of the dielectric constant

The temperature dependence of ϵ' is shown in Figure (7) for all samples (at $f=10$ kHz). From the graph, it can be seen that ϵ' initially increases up to a certain transition temperature beyond which the value decreases. Such a temperature variation of ϵ' was reported earlier for many ferrites [26]. This behavior can be explained as follows: at relatively low temperature, the charge carriers on most cases can not orient themselves with respect to the direction of the applied field, therefore, they possess a weak contribution to the polarization and ϵ' . As the temperature increases, the bound charge carriers get enough excitation thermal energy to be able to obey the change in the external field more easily. This in turn enhances their contribution to the polarization leading to an increase of ϵ' [25]. Moreover, the values of the transition temperature are in good agreement with those obtained for Curie temperatures from the dc resistivity measurements. This suggests that these changes are accompanied with the magnetic transition from the ferrimagnetic

(ordered) state to the paramagnetic (disordered) state [27].

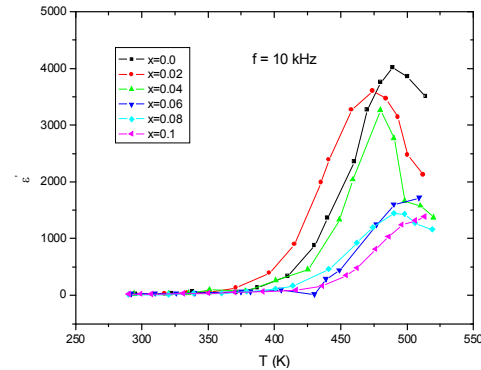


Fig. (7) Temperature dependence of ϵ' (at $f=10$ kHz) for all samples.

3.4.3. Imaginary part of the dielectric constant

The variation of ϵ'' with temperature for all samples (at $f=10$ kHz) is illustrated in Figure (8). One can notice that ϵ'' increases continuously with increasing temperature. This result is in a good agreement with the decreasing resistivity with temperature on the same way of the above discussion in section (3.2).

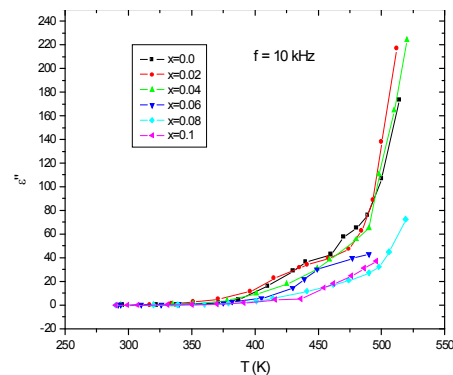


Fig. (8) Temperature dependence of ϵ'' (at $f=10$ kHz) for all samples.

Conclusion

1. ρ_{ac} , ϵ' and ϵ'' decrease with increasing frequency for all samples.
2. ϵ' and ϵ'' have almost a reverse trend of ρ_{ac} with increasing the Nd concentration.
3. The substitution with Nd ions generally improves the dielectric properties of Cu-Zn ferrite which are promising results for different applications.

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Estimation of Genetic Parameters and Inbreeding effects of Economic Traits in native chicken under Short Term Selection

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Summary: Iranian native hens are a valuable genetic resource, due to their adaptability to harsh conditions of husbandry and environment in rural area. The genetic parameters for various traits of economic importance were studied in an Iranian Native chicken population under short term selection for egg production and body weight for over 2 years. The parameters studied were body weight at day old (BW1), 8 weeks (BW8) and 12 weeks (BW12), the weight of first egg (EGGW1) and egg weight at 30 weeks of age (EGGW30), the average number of stock eggs per day (EGG/DAY). They showed mostly moderate to high heritability estimates. Higher heritability estimates were obtained for body weight traits. Therefore, selection for body weight traits before mature age will result in gain in egg weight traits and it will be useful for breeding plans. The average inbreeding coefficients for all birds were and ranged from zero to 0.15. In this population, 34.5% of birds were inbred, with a mean inbreeding coefficient of 0.28. Inbreeding as variable has no significant effect on EGGW1; however, age of sex maturity as variable has significant effect ($p < 0.001$) on EGGW1.

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Keywords: Genetic parameters, Inbreeding, selection, Native chicken breeding program

Introduction

Increased rates of inbreeding in selection programmes may have an important effect on medium and long-term response to selection and reproductive performance through reduction in genetic variability, inbreeding depression and reduced probability of fixing favorable genes. Artificial selection generally results in larger variances of family size and reduced effective population sizes. This can be particularly important in small highly selected nucleus populations and when selection is based on estimated breeding values from index or best linear unbiased predictions (BLUP) with the animal model (Caballero *et al.*, 1996).

Crossbreeding and selection are two based alternative approaches in genetic improvement of livestock and poultry. Crossbreeding leads to the creation of more heterozygotes and in consequence to greater genetic variation of population. By contrast, selection determines both genetic gain and inbreeding rate. The inbreeding effects include increased homozygosity, a higher risk for the incidence of lethal or deleterious recessive alleles, and decrease in performance and fitness traits (Szwaczkowski *et al.*, 2003).

Native chickens are important in some rural areas. They usually produce meat and eggs without extra feed, only picking food. Improving their economical traits, such production efficiency would save these genetic resources. Since chickens under rural production systems are kept both for meat and egg

production selection for genetic improvement of local chickens should seek to improve the two traits simultaneously (Nasr *et al.*, 2012).

Identification of genes determining the expression of economically important traits of plants and animals is a main research focus in agricultural genomics. Most of these traits are characterized by a wide variability of the expression of genes at certain loci called quantitative trait loci (QTL). Characterization of the chromosomal regions carrying QTL can be applied in marker-assisted Selection (MAS) to improve breeding efficiency (Alexei *et al.*, 2010).

The usefulness of molecular genetics tools as; microsatellites in estimating genetic relatedness and diversity in chickens have been demonstrated in a number of native breeds, inbred strains and in commercial lines. Shahbazi *et al.* (2007) represents the first results from the selection and evaluation of five polymorphic microsatellite markers for the genetic assessment of five native chicken populations (*Gallus domesticus*) in Iran. The lowest heterozygosity is found in the Isfahan population (62%) and the greatest in the populations from West Azerbaijan and Mazandaran (79%). The results showed that the genetic diversity of Isfahan native chicken population was richer than the other populations. Thus, molecular linkage maps in combination with powerful statistical methods facilitate the genetic dissection of complex traits, and the chicken

is ideally suited for this task due to the relatively short life cycle and large number of progeny.

Age at sexual maturity, number of eggs, egg weight and body weight at 8th week of age are among most important traits for improving economical efficiency of Iranian native fowl (Kianimanesh & Nejati, 2009). Although natural selection is the great evolutionary force that fuels genetic change in all living beings, selection at the long term has important effects such as increased inbreeding and decreased genetic variation. However, changes in average of production traits and population response in breeding programmers' after a few generations of selections depended on accuracy and intensity of selection, effective population size and the rate of inbreeding (Bahmanimehr, 2012).

Kamali et al. (1995; 2007) has been used, different methods of selection index for selection of economic traits in Iranian indigenous hens. On the other hand, most of the estimates have been obtained through traditional estimation methods, but only very few of these were based on restricted Maximum Likelihood (REML) methodology and animal models. Best Linear Unbiased Prediction (BLUP) in animal model has been used more and more for selection in most species of farm animals. For this purpose estimates of genetic parameters in the base population are required.

The aim of this study is to estimation of inbreeding effects by using genetic parameters as; genetic, phenotypic and environment correlations variation, heritability, and correlation among several important economic traits and inbreeding effects on selection of these traits under short term selection in the native chickens of the Fars province of Iran.

Materials and Methods

In this project, data on 14,250 Iranian native chicken, belonging to The Breeding Center of Fars Native Chicken, from 7th to 11th generation were used to estimate the genetic parameters of six economic traits. All laying native birds were from a small population selected for individual phenotypic value of body weight at 12 weeks of age (BW12) and egg numbers (EN) during the first 12 weeks of laying period. In the first generation, eggs were randomly collected from rural areas and hatched to constitute the base population. Parents were selected on the basis of BW12 and EN. Cocks were selected on the basis of BW12 and the production of their sisters.

Selection procedures were continued for the next generations based on estimated breeding value and calculated genetic and environmental parameters using best linear unbiased prediction (BLUP) in an animal model. A pedigree file of 14,250 birds were used to calculate genetic and environmental parameters on some economically traits as; body weight at day old

(BW1), body weight at 8 weeks (BW8), body weight at 12 weeks (BW12), the weight of first egg (EGGW1) and egg weight at 30 weeks of age (EGGW30) as well as the average number of stock eggs per day (EGG/DAY). The birds were maintained under uniform management conditions as far as possible.

A pedigree file collected on birds was used to calculate the inbreeding coefficient and their influence on these traits. The number of inbred individuals and mean inbreeding coefficients over generations are presented in Table 3. A null inbreeding level in the 7th generation was influenced by available pedigree information (birds from generation 7 were treated as base). Generally, in the next generations, the inbreeding rates increased, as the number of inbred individuals increased. Estimated genetic parameters on this population of a previous study of ours (Bahmanimehr, 2012) has used mixed model least squares, animal model and maximum likelihood whereby the variance components were partitioned into those of the traits and generations to design the fitted models of the inbreeding effects. The genetic and phenotypic correlations between six traits were re-estimated from variance and covariance component analysis. Also according to regression coefficient of inbreeding on generation in different traits Statistical models for inbreeding effects on body weight traits and egg production traits were designed:

$$Y_1 = A + b_1(F) + b_2(In)$$

$$Y_2 = A + b_1(F) + b_2(In) + b_3(Age)$$

Where Y_1 – vector of observed values of the weight traits, Y_2 – vector of observed values of the egg traits, A – fixed effects on traits, b_1 , b_2 and b_3 – regression coefficient of generation, inbreeding and age of sexual maturity respectively.

Results

The heritability for economic traits in the animal model studied was reported in a previous study (Bahmanimehr, 2012) are presented in Table 3. The regression coefficient (b_2) of age at maturity on first egg weight was 0.164; meaning that each day increase in age of sex maturity corresponded to 0.164 g gain on the first egg weight. In addition, the regression coefficient of age of sex maturity on egg weight at 30 weeks of age was 0.051, i. e. each day increase in age of sexual maturity corresponded to 0.051 g increase on the egg weight at 30 weeks of age.

The genetic, phenotypic and environmental correlation between economic traits of this population studied on previous paper (Bahmanimehr, 2012) is presented in Table 2. There was a positive genetic correlation between weight traits and egg weight traits was also reported (Bahmanimehr, 2012). Higher

estimates were obtained for BW1 and EGGW30 (0.64). However, the genetic correlation between body weight traits and number of eggs was negative. Also the moderate to high positive correlation estimates obtained between BW8 and BW12 agreed with the general observation that body weight at all ages is highly heritable and are positively correlated (Ghorbani *et al.*, 2007).

The positive correlation observed between BW1 and EGGW30 and negative genetic and phenotypic correlations obtained between EGGW30 and EGG/DAY, indicated that chickens that attain higher body weight at the first day would lay bigger eggs. However, the negative genetic and phenotypic correlation obtained between number of eggs per day and all body weight traits suggest that the relationship could become more antagonistic during the process of selection. From the total 14520 birds in this population, 9510 birds were not inbred. Among them 2731 birds with zero inbreeding belonged to 7th generation due to uncertain parents and common ancestors as base population.

The number of inbred birds of this population was 5010. The inbreeding coefficients in inbred birds was between 0.0019 and 0.15 that average inbreeding coefficients in total population 0.0096 and in inbred birds 0.028 estimated.

Inbreeding coefficients from 7th to 11th generations is presented in the Table 4. In the 7th generation inbreeding was estimated to be zero due to selected as base population. Despite the increased inbreeding coefficient from 7th to 11th generations, the inbreeding was low because only 34.5% percent of the population was inbred (inbreeding between 0 and 0.15). It led to worry of planners from increasing the inbreeding that forced them to avoid the mating of relatives.

The average inbreeding coefficient of first six generations of this population of the breeding programme was 0.048, estimated and reported by Ghorbani *et al* (2007). They also demonstrated 1% increasing in inbreeding in the population, causing 0.50 and 0.51 g decrease respectively in BW12 and egg production also increase 0.3 in age of sex maturity and 0.03 in egg weight of and for each bird.

Regression coefficients of inbreeding and BW1, BW8, BW12 were estimated to be -0.09, 0.6 and 1.018 respectively. This regression coefficient was estimated -0.03 and -0.12 respectively for egg weight traits as EGW1 and EGGW30 while in contrast regression coefficient of inbreeding and EGG/DAY was estimated 0.00016 that despite of positive is so low.

These results demonstrate 0.09 and 0.12 g decrease respectively in BW1 and BW8 per one percent increasing in inbreeding. Generally this increasing and

decreasing is extremely low due to very small change of inbreeding in each generation also small numbers of inbred birds in the population. Present of production records of large numbers of non inbred birds (65.5 percent of total population was non inbred due to uncertain parents and common ancestors) probably caused estimation of low regression coefficient of inbreeding and production traits.

Conclusion

In designing a breeding programme, the number of individuals producing the next generation could affect stock performance. In addition, in evaluating of breeding programme some one should calculate the effect of inbreeding rate on performance traits in order to avoid misjudgments on the amount of progress in economical traits.

For improving the economic traits, the selection protocol is the main and first point of attention. Positive genetic correlation between body weight traits and egg weight traits (table 3) is an evidence of genetic potential of broilers to weight gain. Also negative correlation between body weight traits and egg number likewise is evidence to genetic potential of layers to produce more egg per year.

Inbreeding could use for determining the undesirable genes by low frequency in the mixed flocks. These undesirable genes almost always are recessive and their effects have covered by dominant alleles. Except of sex influenced traits, recessive alleles had not phenotypic expression while they are heterozygous thus, for their expression should appear homozygous genotype and it could removed from the population by increasing the inbreeding.

Inbred mating has been used in most domestic species as a way to increasing the uniformity in the breeds particularly for a traits that has simple inheritance as feather color in the birds. Genetically relative individuals have equal breeding value due to common genes and further homogeny among them comparing the non relatives. Regression coefficient estimated for breeding value (BV) under fix effect of generation for economical traits in this population showed that, significant genetic improvement was obtained under selection for these traits.

In conclusion, this study showed, selection for body weight traits before mature ages will cause gain in egg weight traits and it will be useful in breeding plans.

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Table 1. Estimated heritability and different Variances in the economic traits (Bahmanimehr, 2012)

Traits	* $\delta^2 a$	** $\delta^2 e$	*** $\delta^2 p$	**** h^2	Log L
BW1	4.482	3.498	7.98	0.56 ± 0.012	-16460.6
BW8	2090.82	5254.23	4736.05	0.44 ± 0.02	-64634.6
BW12	5536.86	5254.83	5254.83	0.51 ± 0.02	-71395.5
EGGW1	3.36	13.61	16.97	0.2 ± 0.2	-20882.8
EGGW30	5.57	4.36	9.93	0.56 ± 0.022	-16413.5
EGG/DAY	0.19	0.11	0.12	0.15 ± 0.025	10893.8

*Genetic (Additive) Variance; **Environmental Variance; ***Phenotypic Variance; ****Heritability

Table 2. Genetic, phenotypic and environmental correlations between the economic traits

Traits	BW1			BW8			BW12			EGGW1		
	r_a	r_e	r_p	r_a	r_e	r_p	r_a	r_e	r_p	r_a	r_e	r_p
BW1	1	1	1									
BW8	0.34	0.09	0.22	1	1	1						
BW12	0.32	0.03	0.18	0.94	0.56	0.74	1	1	1			
EGW1	0.51	-0.13	0.094	0.4	0.05	0.16	0.34	0.07	0.19	1	1	1
EG/DAY	-0.07	-0.014	-0.031	0.02	-0.07	-0.04	-0.05	-0.07	-0.06	-0.11	0.02	-0.05

r_a : Genetic Correlation; r_e : Environmental Correlation; r_p : Phenotypic Correlation;

Table 3. Inbreeding coefficient in different generations of chickens

Generation	No. of records	Average	Min	Max	Standard deviation (Sd)
G7	2731	0	0	0	0
G8	2817	0.062	0	12.5	0.88
G9	2997	0.285	0	12.5	1.408
G10	2985	1.76	0	7.03	1.55
G11	2981	2.6	0	15.04	1.58

Table 4. Average of Traits in different Generations of chickens, parallelly to increased level of inbreeding

Generation	BW1	BW8	BW12	EGGW1	EGGW30	EGGDAY	Inbreeding(F)
G7	.	585.22	919.67	35.43	44.04	.	0
G8	31.806	596.175	996.43	34.64	43.46	.	0.0621
G9	34.673	609.94	955.52	33.43	43.5	0.7765	0.285
G10	33.07	582.172	952.653	34.93	43.42	0.8007	1.756
G11	32.273	646	1070.1	35.55	45.82	0.8086	2.598

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A correlation study between metabolic syndrome and chronic kidney disease among populations older than 40 years

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Abstract: Aim: This study aimed to investigate epidemiological features of chronic kidney disease (CKD) and explore the correlation between CKD and metabolic syndrome (MS) among individuals ≥ 40 years old in urban populations of Henan Province, China. The broad purpose of the study was to improve the prophylaxis and treatment of CKD, reduce and defer the occurrence of end-stage renal disease, and provide evidence to support national public health and medical insurance strategies. **Methods:** This field epidemiology cross-sectional study followed a multistage stratified cluster random sampling strategy. The sampling frame consisted of urban residents ≥ 40 years old, who resided in the cities of Zhengzhou, Jiaozuo, and Pingdingshan in Henan Province, China. Epidemiological data pertaining to CKD were collected by questionnaires, physical examinations, kidney damage tests, blood glucose and lipid measurements for all subjects and were analyzed by statistical methods. **Results:** A total of 4156 adults took part in the investigation and 3981 (95.7%; 40-89 years old) valid samples were obtained, including 2178 males and 1803 females (the male to female ratio was 1.21:1). The overall prevalence of hypertension and diabetes in the 3981 subjects was 15.04% and 5.76%, respectively. Participants with MS had higher prevalence of albuminuria and decreased estimated glomerular filtration rate (eGFR) than those without MS. Participants with hypertension had higher prevalence of albuminuria and prevalence of decreased eGFR than those without. Participants with abnormally high triglyceride (TG) levels had a higher prevalence of decreased eGFR than those without. Participants with abnormal carbohydrate metabolism had a higher prevalence of albuminuria than those without. Of those subjects who exhibited signs of individual MS components, i.e. hypertension, low high-density lipoprotein cholesterol (HDL-C), high TG, fasting blood glucose ≥ 5.6 mmol/L, and abnormally large waist circumference, the prevalence of CKD was 18.27%, 11.49%, 15.89%, 31.03% and 12.24%, respectively. In addition, participants with hypertension, high TG, or fasting blood glucose ≥ 5.6 mmol/L had higher CKD prevalence than those without. The prevalence of CKD increased as the number of MS components increased. **Conclusions:** MS is a basic risk factor for CKD, and the risk of acquiring CKD increases with the increase of the number of MS components.

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Keywords: chronic kidney disease; metabolic syndrome; correlation

1. Introduction

Chronic kidney disease (CKD) and end-stage renal disease (ESRD) arising from CKD have become significant public health problems worldwide due to their high incidence, poor prognosis and high costs of treatment. According to the 2007 annual report of the United States Renal Data System, about 85,000 people die from ESRD annually and kidney disease is the ninth leading cause of death in the United States (Miniño et al., 2007). In Asia, an epidemiological report showed that the CKD prevalence among adults older than 40 years in Beijing was 9.4%, and the associated risk factors were similar to the pattern observed in western countries (Zhang et al., 2007).

The contribution of diabetes and hypertension to the development of CKD was early recognized (Bombardier et al., 2010; Fakhrazadeh et al., 2009). Recent studies have provided evidence that metabolic

syndrome (MS) and its abnormal components, including obesity, hyperlipidemia and hyperuricemia, affect the generation and progression of CKD (Bombardier et al., 2010; Fakhrazadeh et al., 2009).

The present study followed a multistage stratified cluster random sampling strategy. The sampling frame consisted of urban residents aged 40 years or older, who resided in the cities of Zhengzhou, Jiaozuo, Pingdingshan and Kaifeng, all in Henan Province. Epidemiological data obtained by questionnaires, physical examinations, kidney damage tests, blood glucose and lipid measurements for all subjects were analyzed by statistical methods. The prevalence, awareness rate and associated risk factors of CKD were obtained in order to design future strategies aimed at national prevention and control of pandemic of CKD.

2. Subjects and methods

Study population

The target population consisted of urban residents ≥ 40 years old, of Zhengzhou, Jiaozuo, Pingdingshan and Kaifeng in Henan Province from May 2007 to October 2009. Informed consent was obtained from all participants. The required statistical sample size (n) was calculated using following equation (HU,2006):

$$n = \left[\frac{57.3t_{\alpha}}{\text{Sin}^{-1}(\delta\sqrt{P(1-P)})} \right]^2$$

in which, the test standard, $\alpha = 0.05$, $t_{\alpha} = 1.96$; the allowable error, $\delta = 2\%$; $P = 15.0\%$, as the estimated prevalence rate of CKD, according to literature analysis and the general population CKD prevalence rate (10.1% ~ 11.3%). Following the formula, sample size should be 4156 adults, of those 3981 the effective cases. For each participant, demographic characteristics and health history was collected using a questionnaire and an overall physical examination and laboratory tests were performed.

Physical examination

The physical examination included measurements of height, weight (in light clothing without coat, hat and shoes), and blood pressure (BP; using calibrated electronic and mercury sphygmomanometers). BP was first measured by an electronic sphygmomanometer. If the two measurements were higher than the diagnostic criteria, the mercury sphygmomanometer was used after 15 minutes of rest with the participant in a seated position. The body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in square meters).

Laboratory tests

After an overnight fast, venous blood samples were collected to determine the levels of blood glucose, total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), using oxidase tests and colorimetry.

Quality assurance

A repeat survey was performed on 5% of the study population; the results were used to verify the representativeness and reliability of samples. The microbiological examination conformed to the laboratory quality control standard.

Diagnostic criteria for MS

MS was defined according to the diagnostic criteria of the International Diabetes Federation (IDF) (Motta et al., 2009) as central obesity (waist circumference ≥ 90 cm in men and ≥ 80 cm in women), together with the presence of two or more of the following risk factors: fasting TG ≥ 1.7 mmol/L or under relevant medical treatment; fasting HDL-C < 0.9 mmol/L in men or < 1.1 mmol/L in women or under relevant medical treatment; systolic BP ≥ 130 mmHg (1 mmHg = 0.133 kPa) or diastolic BP ≥ 85 mmHg or

under relevant medical treatment; fasting blood glucose level ≥ 5.6 mmol/L or under relevant medical treatment.

Diagnostic criteria for CKD

CKD was defined according to the definition and classification recommended by Kidney Disease Quality Outcome Initiative (K/DOQI) (Levey et al., 2005) as any of the following: (1) albuminuria, defined as urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g in urine specimens, including microalbuminuria (ACR = 30-299 mg/g) and macroalbuminuria (ACR ≥ 300 mg/g); (2) hematuria. Urinary specimens with red blood cells of 1+ or greater were centrifuged and the urinary sediment was microscopically examined. Three or more red blood cells by high power field were considered positive (excluding contamination and women during menstruation); (3) decreased glomerular filtration rate (GFR), defined as estimated GFR (eGFR) < 60 mL/min/1.73 m². The eGFR was calculated from the Modification of Diet in Renal Disease Study equation calibrated with Chinese CKD patient data (Ma et al., 2006).

Statistical methods

Data entry was performed using EpiData 3.0 software (EpiData Association, Odense, Denmark) by professional data entry clerks and repeated twice to assure accuracy. All statistical analyses and calculations were performed using Statistical Package for the Social Sciences (SPSS) 10.0 software (SPSS Inc., Chicago, IL). Prevalence rates were standardized according to demographic data of the Henan Province Fifth Census (<http://www.stats.gov.cn/tjgb/rkpcgb/>). Measurement data are presented as mean \pm standard deviation. Differences between groups were compared using Student's t-test, one-way analysis of variance, and the Mann-Whitney rank sum test. Categorical data were compared using the chi-square (χ^2) and chi-square for trend tests. $P < 0.05$ was considered statistically significant.

3. Results

Basic information of study population

A total of 4156 residents took part in the investigation and 3981 (95.7%; 40-89 years old) were included in this study, including 2178 males and 1803 females (the male to female ratio was 1.21:1). The prevalence of hypertension and diabetes were 15.12% (602/3981) and 5.83% (232/3981), respectively. The prevalence of hypertension and diabetes standardized according to the demographic data of Henan Province Fifth Census were 15.04% and 5.76%, respectively. The diabetes prevalence in women was higher than that in men (7.38% vs. 4.55%, $\chi^2 = 14.407$, $p < 0.01$). Compared with men, the levels of urea nitrogen, serum creatinine and serum uric acid in women were lower ($p < 0.01$), and the levels of TC, HDL-C and LDL-C were higher ($p < 0.05$), as shown in Table 1.

Table 1. Basic information of study population

Item	Male (n = 2178)	Female (n = 1803)	t value	p value
Age (year)	50.28 ± 16.41	53.39 ± 13.28	0.965	0.341
BP				
Systolic BP (mmHg)	131.65 ± 15.42	129.47 ± 17.56	1.602	0.116
Diastolic BP (mmHg)	83.45 ± 9.18	80.43 ± 11.26	1.818	0.231
Hypertension	317 (14.55%)	285 (15.81%)	1.205	0.272
Body mass index (kg/m ²)	23.87 ± 4.25	22.65 ± 5.15	1.048	0.301
Blood lipid				
Total cholesterol (mmol/L)	4.75 ± 0.66	5.21 ± 1.03	6.712	< 0.001 [▽]
Triglyceride (mmol/L)	1.88 ± 1.24	1.80 ± 1.16	1.619	0.108
HDL-C (mmol/L)	1.16 ± 0.23	1.33 ± 0.28	12.886	< 0.001 [▽]
LDL-C (mmol/L)	2.75 ± 0.72	2.91 ± 0.89	3.391	0.001 [▽]
Blood glucose (mmol/L)	5.04 ± 1.15	5.12 ± 1.29	1.029	0.302
Diabetes	99 (4.55%)	133 (7.38%)	14.407	< 0.001 [▽]
Kidney examination				
Urea nitrogen (mmol/L)	5.76 ± 1.54	5.09 ± 1.36	8.912	< 0.001 [▽]
Serum creatinine (μmol/L)	73.86 ± 14.49	57.50 ± 10.66	20.653	< 0.001 [▽]
Serum uric acid (μmol/L)	368.87 ± 82.18	299.30 ± 65.42	16.612	< 0.001 [▽]

[▽]p < 0.05; [▽]p < 0.01 compared to the female.

Indicators of kidney damage and prevalence of CKD among patients with MS

Prevalence of albuminuria, hematuria and decreased eGFR among patients with MS

A total of 565 patients met the MS diagnostic criteria of

IDF. Participants with MS had a higher prevalence of albuminuria and decreased eGFR compared to those without MS (6.90% vs. 4.65%, $\chi^2 = 5.184$, p < 0.01 and 5.00% vs. 0.97%, $\chi^2 = 51.148$, p < 0.01, respectively; Table 2).

Table 2. Prevalence of albuminuria, hematuria and decreased eGFR among participants with and without MS

Participants	n	Albuminuria	Hematuria	Decreased eGFR
With MS	565	39 (6.90%) [▽]	35 (6.19%)	28 (5.00%) [▽]
Without MS	3416	159 (4.65%)	217 (6.35%)	33 (0.97%)
Total	3981	198 (4.97%)	252 (6.33%)	61 (1.53%)

[▽]p < 0.05; [▽]p < 0.01 compared to participant without MS.

Table 3. Prevalence of albuminuria and decreased eGFR among participants with MS components

	Albuminuria		Statistic	p-value	eGFR		
	Case	Prevalence			Prevalence	χ^2 value	p-value
BP ≥ 140/90 mmHg							
Yes	602	42 (6.98%)	$\chi^2 = 6.021$	p = 0.014 [▽]	16 (2.66%)	$\chi^2 = 5.955$	p = 0.015 [▽]
No	3379	156 (4.62%)			45 (1.33%)		
HDL-C (< 0.9 mmol/L in men, < 1.1 mmol/L in women)							
Yes	766	30 (3.92%)	$\chi^2 = 2.243$	p = 0.134	17 (2.22%)	$\chi^2 = 2.967$	p = 0.085
No	3215	168 (5.23%)			44 (1.37%)		
Triglyceride ≥ 1.70 mmol/L							
Yes	862	51 (5.92%)	$\chi^2 = 2.069$	p = 0.150	20 (2.32%)	$\chi^2 = 4.527$	p = 0.033 [▽]
No	3119	147 (4.71%)			41 (1.31%)		
Fasting blood glucose ≥ 5.6 mmol/L							
Yes	232	35 (15.09%)	$\chi^2 = 53.305$	p < 0.001 [▽]	6 (2.59%)	$\chi^2 = 1.814$	p = 0.178
No	3749	163 (4.35%)			55 (1.47%)		
Waist circumference (≥ 90 cm in men, ≥ 80 cm in women)							
Yes	956	56 (5.86%)	$\chi^2 = 2.081$	p = 0.149	18 (1.88%)	$\chi^2 = 1.025$	p = 0.311
No	3025	142 (4.69%)			43 (1.42%)		

[▽]p < 0.05; [▽]p < 0.01 compared between participants with and without MS components.

Prevalence of albuminuria and decreased eGFR among participants with MS components

Among participants with MS components (i.e. hypertension, low HDL-C, high TG, fasting blood glucose ≥ 5.6 mmol/L, and abnormally large waist circumference), the prevalence of albuminuria and decreased eGFR was higher in those with abnormally high BP compared to those with normal BP (6.98% vs.

4.62%, $\chi^2 = 6.021$, p = 0.014 and 2.66% vs. 1.33%, $\chi^2 = 5.955$, p = 0.015, respectively). There was no statistical difference in the prevalence of albuminuria or decreased eGFR between those with abnormally low HDL-C or waist circumference and those without. Participants with abnormally high TG had higher prevalence of decreased eGFR than those with normal TG (2.32% vs. 1.31%, $\chi^2 = 4.527$, p = 0.033). There

was no statistical difference in the prevalence of albuminuria between those with and without normal TG. Participants with abnormal carbohydrate metabolism had higher prevalence of albuminuria than those without (15.09% vs. 4.35%, $\chi^2 = 53.305$, $p < 0.001$), while there was no statistical difference in decreased eGFR between these two groups, as shown in Table 3 and Figure 1A and 1B.

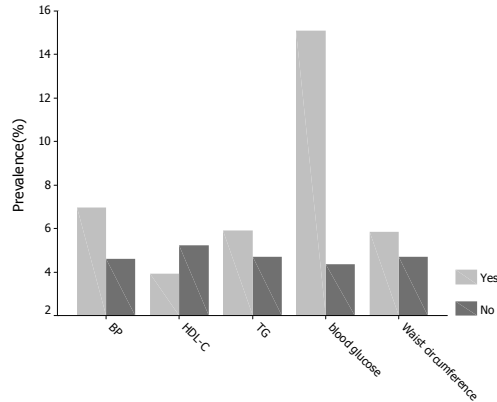


Figure 1-A. Prevalence of albuminuria among participants with MS components

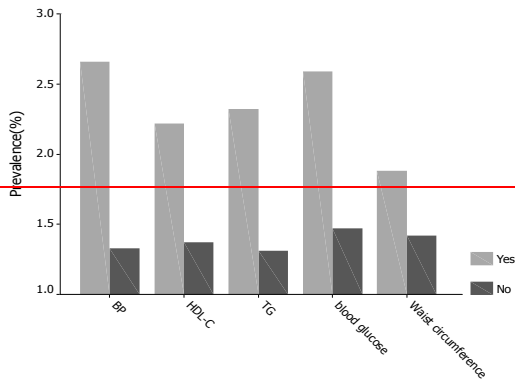


Figure 1-B. Prevalence of decreased eGFR among participants with MS components
Prevalence of CKD among participants with MS components

Of those subjects who exhibited signs of hypertension, low HDL-C, high TG, fasting blood glucose ≥ 5.6 mmol/L, and abnormally large waist circumference, the prevalence of CKD was 18.27%, 11.49%, 15.89%, 31.03% and 12.24%, respectively. In addition, participants with hypertension, high TG, or fasting blood glucose ≥ 5.6 mmol/L had a higher CKD prevalence than those without (18.27% vs. 9.20%, $\chi^2 = 44.434$, $p < 0.001$; 15.89% vs. 9.11%, $\chi^2 = 32.903$, $p < 0.001$; and 31.03% vs. 9.31%, $\chi^2 = 109.043$, $p < 0.001$, respectively), as shown in Table 4 and Figure 2.

Number of MS components and prevalence of CKD Of 1824 participants with MS components, 35.20% (642/1824), 22.15% (404/1824), 30.97% (565/1824), 7.62% (139/1824) and 4.06% (42/966) had

1, 2, 3, 4, and 5 MS components.

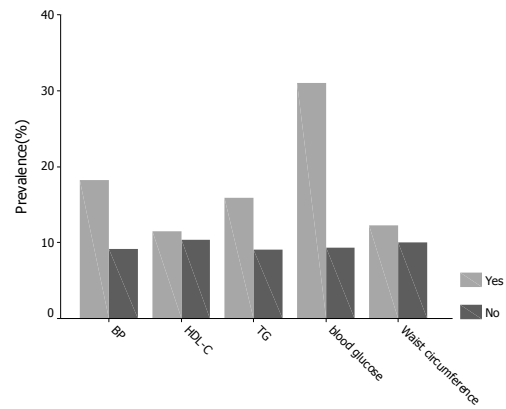


Figure 2. The prevalence of CKD among participants with MS components

The prevalence of CKD among participants who had more than one MS component was higher compared to those without MS components ($p < 0.01$). According to the chi-square for trend test, the prevalence of CKD increased as the number of MS components increased ($\chi^2 = 211.638$, $p < 0.01$), as shown in Table 5 and Figure 3.

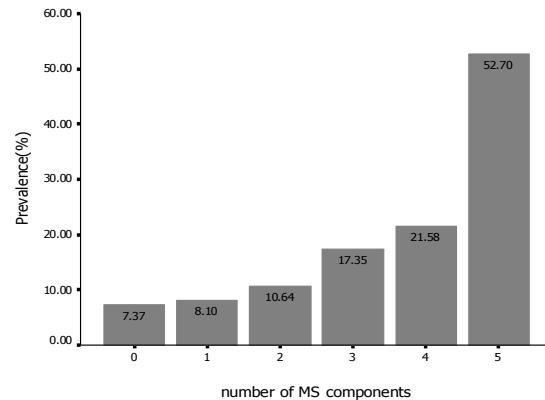


Figure 3. The prevalence of CKD among participants with varying number of MS components

4. Discussion

MS, also known as insulin resistance metabolic syndrome (IRMS), often includes obesity, hypertension, high blood glucose and dyslipidemia. Obesity, hypertension and high BP are often treated as independent risk factors for CKD (Kramer et al., 2009; Snyder et al., 2009; Muntner et al., 2010). In China, epidemiological data showed that since the 1950s, the prevalence of hypertension had increased from 5.11% to 18.8% (Zhai et al., 2010). An investigation by WHO in the year 2000 showed that the prevalence of diabetes mellitus was 5.2% among men and 5.3% among women in China (Yang et al., 2010).

Table 4. The prevalence of CKD among participants with MS components

	CKD		χ^2 value	p value
	Case	Prevalence		
BP \geq 140/90 mmHg				
Yes	602	110 (18.27%)	$\chi^2 = 44.434$	$p < 0.001^\nabla$
No	3379	311 (9.20%)		
HDL-C (< 0.9 mmol/L in men, < 1.1 mmol/L in women)				
Yes	766	88 (11.49%)	$\chi^2 = 0.836$	$p = 0.361$
No	3215	333 (10.36%)		
Triglyceride \geq 1.70 mmol/L				
Yes	862	137 (15.89%)	$\chi^2 = 32.903$	$p < 0.001^\nabla$
No	3119	284 (9.11%)		
Fasting blood glucose \geq 5.6 mmol/L				
Yes	232	72 (31.03%)	$\chi^2 = 109.043$	$p < 0.001^\nabla$
No	3749	349 (9.31%)		
Waist circumference (\geq 90 cm in men, \geq 80 cm in women)				
Yes	956	117 (12.24%)	$\chi^2 = 3.680$	$p = 0.055$
No	3025	304 (10.05%)		

$^\nabla p < 0.05$; $^\nabla p < 0.01$ compared between participants with and without MS components.

Table 5. The prevalence of CKD among participants with varying number of MS components

Number of MS components	Case	CKD		χ^2 value	p value
		Prevalence			
0	2157	159 (7.37%)	—		
1	642	52 (8.10%)	$\chi^2 = 0.377$	$p = 0.539$	
2	404	43 (10.64%)	$\chi^2 = 5.015$	$p = 0.025^\nabla$	
3	565	98 (17.35%)	$\chi^2 = 52.090$	$p < 0.001^\nabla$	
4	139	30 (21.58%)	$\chi^2 = 34.913$	$p < 0.001^\nabla$	
5	74	39 (52.70%)	$\chi^2 = 181.793$	$p < 0.001^\nabla$	

$^\nabla p < 0.05$; $^\nabla p < 0.01$ compared to participants with no MS component.

Our previous epidemiological study among residents aged 40 years or older in Henan Province showed that the prevalence rates of hypertension and diabetes were 15.04% and 5.76% respectively, the crude and standardized prevalence rates of CKD were 10.58% and 10.49% respectively (Shan et al., 2010). This is comparable to the prevalence of CKD among adults in the United States, which was about 11% (Stevens et al., 2010). The increase of hypertension and diabetes prevalence in China may have effects on the spectrum of CKD disease, making the risk factors of CKD similar to that of the developed countries. In 2005, Kurella et al (2005) reported a cohort study in which the subjects were Americans with GFR \geq 60 mL/min per 1.73 m² at baseline. After 9 years of follow-up, it was found that 7% of the participants had developed CKD (GFR < 60 mL/min per 1.73m²). The odds ratio (OR) of developing CKD in participants with MS was 1.43. Compared with participants with no MS component, those with one, two, three, four, and five components had OR for developing CKD of 1.13, 1.53, 1.75, 1.84, and 2.45, respectively. The results showed that MS was a risk factor for the development of CKD, the increased number of MS components was associated with the increased risk of CKD.

In our study, we found that persons with MS disease had greater prevalence of albuminuria and decreased eGFR than persons without. The prevalence of CKD increased with the number of MS components.

Persons with hypertension, abnormal TG, or fasting blood glucose \geq 5.6 mmol/L had greater prevalence of CKD than those without ($p < 0.01$). We guessed there were diversified pathogenesis responsible for CKD. The kidney damage caused by hypertension is mainly manifested as injury of renal blood vessel and parenchyma induced by hemodynamics changes and other factors (e.g. increase of reactive oxygen species, metabolic disorder) (Griffin et al., 2006). Animal examinations and clinical research have confirmed that dyslipidemia can induce kidney damage, including glomerular fat deposition, glomerulosclerosis, damage of epithelial cells, increase of mesangial cells, accumulation of extracellular matrix and damage of renal interstitium (Muntner et al., 2000). High blood glucose induces advanced glycation end products and hence damages the kidney; increase of polyol pathway activation leads to dysfunction of kidney cells; glomerulus hemodynamics changes cause high filtration, high infusion and increase of protein kinase C activity in kidney, and eventually lead to increase and accumulation of extracellular matrix of glomerulus (Indridason et al., 2007; Moin et al., 2008). Furthermore, it was reported that obesity induced renal hemodynamics changes, hyperplasia and hypertrophy of mesangial cells, fat deposition and hyperleptinemia, and hence led to renal damage (Iseki et al., 2004); patients with uric acid nephrolithiasis were insulin resistant and prone to have low urinary ammonium and

pH, which could result in increased risk of uric acid precipitation, producing or aggravating chronic urate nephropathy (Abate et al., 2004).

Although the prevalence of CKD has a tendency to increase each year, it has not aroused many people's attention. A survey taken in the United States showed that the awareness rate of CKD among patients with GFR at 15-59 mL/min per 1.73 m² and albuminuria was only 24.3%, and the awareness rate of CKD among patients with GFR \geq 90 mL/min per 1.73 m² and no microalbuminuria was even lower (Coresh et al., 2005). Investigative data from the U.S. National Health and Nutrition Examination Survey showed that for noninstitutionalized adults with CKD stages 1-5, the awareness rates were 40.5%, 29.3%, 22.0%, 44.5% and 100%, respectively (Zhai et al., 2010). In our study, the CKD awareness and treatment rates were even lower, at 9.5% and 8.31%, respectively. Such low awareness may be because: (1) the onset of CKD is not accompanied by readily detectable symptoms, making it difficult to ascertain; (2) the public is not educated regarding CKD; (3) physicians fail to make precise diagnosis of CKD; and (4) there are insufficient medical and public health services and resources.

In conclusion, MS is a basic risk factor for the development of CKD, and the risk of acquiring CKD increases as the number of MS components increase. Clinical physicians must attach more importance to efforts to control MS components, improve diagnosis and treatment of CKD, and prevent CKD from developing into ESRD.

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The Study of Meandering Phenomenon on the Basis of Stream Power

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Abstract: On the basis of stream power, the phenomena including regime channel establishment and river meandering can be studied. So, previous researchers have suggested and developed the stream power theory and believed that stream power minimization affects these phenomena. In this paper, the stream power theory and a criterion for meandering river modeling will be studied. Then the conclusion of case study of Ghezel Ouzan River and obtained relationships in relation to non-dimensional unit stream power will be mentioned. The Study of Meandering Phenomenon on the Basis of Stream Power.

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KEYWORDS: Meandering-Stream Power-Non Dimensional Unit Stream Power- Modeling-Regime Channel-Channel Pattern

Preface

Power is the consumed energy in unit of time. Since energy is force times distance, consumption stream power in unit of length can be written as eq.1. [1].

$$P_l = (\gamma BD)SV \dots \dots \dots (1)$$

Where, P_l - stream power in unit of length, γ - volume weight of fluid (water), B- average width of stream, D- depth of stream, S- channel slope, V- velocity of stream.

In sediment transport theories, other interpretations are also used for stream power.

A) Stream power in unit of bed area, P_a :

$$P_a = \frac{P_l}{B} = \gamma DSV = \tau_0 V \dots \dots \dots (2)$$

Where, τ_0 - bed shear stress, which, for wide channels is equal to γDS .

B) Stream power in unit of length and weight of fluid (water) or unit stream power (USP), P_w :

$$P_w = \frac{P_l}{\gamma BD} = SV \dots \dots \dots (3)$$

Several researchers, based on the theory of stream power, have introduced functions for calculating the total load of sediment, which we can point to the names as: Bagnold 1966; Engelund & Hansen 1972; Ackers & White 1973; Yang 1973, 1984; velikanov, 1954. [2].

For example Bagnold's theory (1966) is based on stream power in unit of area, P_a and Yang's formulation (1973) is based on unit stream power, P_w . [1].

Results of evaluations show that sediment transport is basically depended on the rate of dissipated energy in transport process and the hypotheses based on this basis are more general and precise compared to other hypotheses which consider the sediment transport rate as a function of discharge, average velocity, slope of energy line, and shear stress. [2]. In order to explain the dissipated energy rate in the process, so far several opinions have been offered:

A) Opinions on stream power by Bagnold (1966), Engelund & Hansen (1972), and Ackers & White (1973).

B) Opinion on unit stream power by Yang (1973)

C) Theory of gravitational power by Velikanov (1954)

Theory of stream power is based on general physical concepts and has been simply derived from basic theories of fluid mechanics. Accuracy and generality of stream power hypothesis is the main reason that equations by Engelund & Hansen (1972), Ackers & White (1973), and Yang (1973, 1979, 1984) compared to other equations, give more accurate results. As another reason, all of the parameters used in these equations are non-dimensional and therefore aren't sensitive to the small dimension of experimental flumes compared with natural rivers.

More over, the theory of unit stream power not only hasn't been based on experimental Results but also has been derived from basic hydromechanical theories and turbulent flows. From theoretical point of view, bed load, suspended load or total load amount is directly proportional to unit stream power.

The unit stream power equations are more precise than the other presented relationships for the purpose of evaluating the rate of alluvial sediment transport specially considering non-adhesive sediments under different conditions in natural channels and experimental flumes. It is mainly due to generality of the assumptions used in development of unit stream power equations, non-dimensional parameters used in the equations and the extensive range to calibrate and adjust the parameters. [2].

Hydraulic Geometry changes of river channels

Adequate physical relationships must be used to determine the hydraulic geometry of river channels. Generally, four groups of equations concerned with dynamics of flow, sediment transport, bank stability, and dynamic balance can be used. Due to dynamics balance, the two phases of liquid-solid conform together and form the channel pattern. Generally, the two following conditions must be satisfied in order to a reach be stabilized (chang 1988).

- 1) Along a reach, the sediment load must be constant; otherwise in the reach erosion or sedimentation will occurred.
- 2) Considering other limitations of river, the stream power in unit of length (γQS), must be minimized.

Chang defined this assumption as following [3]: In an alluvial river, the necessary and adequate conditions for reaching equilibrium, considering other limitations, is minimization of stream power in unit of length (γQS). In other words, in an alluvial river, Q-discharge, Q_s -sediment load and type of particles in bed and walls as independent variables regulate the width, depth, and bottom slope of river so that considering other limitations, the value of γQS to be minimized. Since Q is a known parameter, minimization of γQS happens when the slope of the channel is minimized. The hypothesis of stream power has been derived from the low of virtual work and the results based on it, are accurate for various conditions from sand to gravel channels.

Meander River Modeling

To study river meandering, physical models are more informative than mathematical models [5].

In order to simulate the global variation of a meandering river, caused by a change in flow discharge, sediment supply, upstream or downstream boundary condition, etc., a so-called meandering river model has to be adapted. The meandering river model is actually a kind of loose boundary model, which is free to change both its banks and bed, subjected to the flow erosion. Conventionally the following parameters are used in mobile bed models:

$$\theta = \frac{\tau_0}{g(\rho_s - \rho)D_s} \dots\dots\dots(\text{Shields.Parameter})\dots\dots\dots(4)$$

$$Re_s = \frac{U_* D_s}{\nu} \dots\dots\dots(\text{Particle.Reynolds.Number})\dots\dots\dots(5)$$

$$\phi = \frac{q_s}{\rho_s g \sqrt{\Delta D_s^3}} \dots\dots\dots(\text{Einstein.Parameter})\dots\dots\dots(6)$$

Where τ_0 = bed shear stress;
 ρ and ρ_s = density of water and sediment respectively;

$$\Delta = (\rho_s - \rho) / \rho \text{ relative density parameter;}$$

Case study

Ghezel Ouzan River, the second longest river in Iran, is a meandering river located in northern part of the country. After field surveys, an alluvial meandering reach of the river was selected and by using of its data, 33 experiments were carried out in a 14-meter-long, 1.5-meter-width, 0.8-meter-depth flume in the hydraulic laboratory of Watershed Management Research Center of Iran. The process of establishment and development of meandering pattern was observed and investigated. On the basis of non-dimensional unit stream power (NDUSP) and shields parameter (θ), the results were analyzed and interpreted.

According to these experiments, for very fine gravel materials (on the basis of ASCE classification method) and NDUSP at least between 5.5 to 6.5 and shields parameter between 0.01 to 0.03, the meandering pattern establishment was occurred. In the other word the initial channel which contains the mentioned values of NDUSP and θ is capable to produce a meandering pattern. After regime channel establishment, stream power decreases; because, in the situation the river dissipates its excess power due to bed and walls erosion. By the experiments, it was found that if equation (...) is confirmed between (NDUSP) and (θ), meandering pattern will be formed.

$$\theta = 0.013 \times (NDUSP)^{0.58} \dots\dots\dots(.$$

With the increase os discharge, Q, the value of NDUSP increases. Also with increasing of discharge, Q, both hydraulic radius, R, and shear stress ($\tau_0 = \gamma RS$) will increase. Since

$$\theta = \frac{\tau_0}{(\gamma_s - \gamma)D_s}, \text{ the value of } \theta \text{ will also}$$

increase; i.e. both NDUSP and θ will increase [4].

In the regime situation, P_f will minimized; i.e. for any discharge, the characteristics of channel will change in a way that P_f be minimized; since Q and

λ are constants, the changes in channel will continue until S be minimum.

Results

The theory of stream power which is based on general physical concepts and has simply been derived from basic theories of fluid mechanics, can be used for quantitative and qualitative analysis of meandering phenomenon. Non-dimensional unit stream power, which shows the importance of relation of "stream power" to "side resistance", is a ruling parameter in meandering process.

For simulating a meandering river in a model with loose boundaries, the amounts of non-dimensional unit stream power in model and prototype must be the same.

It seems unlikely to be able to replace this scaling criterion with other criteria of movement threshold of particles. The criterion of non-dimensional unit stream power as well as the shields parameter are adequate to geometrical similarity of planform.

On the basis of non-dimensional unit stream power and shields parameter it can be said whether a channel with known specifications can be meandered or not. In regime situation, minimizing of stream power in unit of length is obtained by minimizing of channel slope (for constant discharge and volume weight).

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Using of Arbuscular Mycorrhizal Fungi to Reduce the Deficiency Effect of Phosphorous Fertilization on Maize Plants (*Zea mays* L.)

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Abstract: A greenhouse study was conducted to investigate the effect of three species of arbuscular mycorrhizal fungi (AMF) *Glomus mosseae*, *G. etunicatum* and *G. clarum* at two levels of phosphorus (P) fertilization (zero and 60 µg P/g) on plant growth parameters and physiological character of maize plants (*Zea mays* L.) at seedling stage. The results showed that the best values of all plant growth parameters were recorded at P level (60 µg P/g) and inoculation with *G. etunicatum* and *G. mosseae* (The increase by rate 34.5%; 32.9% and 32.5%; 32.2% respectively) comparing with untreated plants. Also the highest value of plant dry weight was recorded in the presence of inoculation with *G. clarum* by increasing rate 55.7% comparing with control. On contrast the treatment with P causing decreased in all values of root/shoot ratios comparing with the same treatment in the absent of P fertilization. The highest values of plant P uptake were recorded in the presence of P with inoculation by *G. etunicatum*, then *G. clarum* followed by *G. mosseae* comparing with untreated plants. All treatments in zero P were decreased in values of protein content comparing with P level 60 µgP/g, and increases in proline values. The highly values of plant chlorophyll content were recorded in the presence of P fertilization and inoculation by *G. clarum*, then *G. etunicatum* (8.306, 7.840 unit respectively). On the other hand AMF root colonization% was affected by phosphorus fertilization levels. The highest values of AMF root colonization% (50.5%, 80.3%) were found when plants inoculation with *G. etunicatum* at both level of P (zero, 60 µg P/g respectively) followed by *G. clarum* then *G. mosseae*. The same results were observed in number of AMF spores/100g of soil. The AMF specie *G. etunicatum* was recorded as a highest spores numbers (252, 320 spores /100g of soil) at two levels of P followed by *G. clarum* then *G. mosseae*. In generally AMF inoculation can be used as biofertilizer to reduce the deficiency effect of phosphorous fertilization on *Zea mays* L.

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1. Introduction

The availability of Phosphorus (P) is one of the most significant determinants in plant growth (Wang *et al.*, 1998). Plants depend almost exclusively on P absorbed from soil. Total P in soil is abundant, but it is largely unavailable (Liu *et al.*, 1994). As many other plants, maize is sensitive to P and faces the dilemma of "P-deficiency in heredity" (Usuda and Kousuke, 1991). It is reported that P deficiency had a detrimental effect on morphogenesis and physiological mechanism in maize, and P deficiency symptoms and biomass have been known as indicative traits of maize in response to low P stress (Hajabbasi and Schumacher, 1994; Duan *et al.*, 2002; Liu *et al.*, 2003; Ortas *et al.*, 2011). The plant nutrition has been estimated that nearly 30 million tons of P based fertilizers (in terms of P₂O₅) are applied worldwide every year. However, the use efficiency of applied P is generally very low, ranging from 10% to 30% in the year applied (McLaughlin *et al.*, 1991). Continuous application of P fertilizers also increases the risk of P loss from soil to water, causing

toxic algal blooms in water bodies (Sharpley *et al.*, 2000). Phosphate is present in the soil in the form of inorganic orthophosphate (Pi) and is readily sequestered by cations especially in acidic conditions, of which the most abundant are iron, aluminium and calcium. The mobility of sequestered phosphate is reduced (Bucher, 2007). The arbuscular mycorrhizae (AM) fungal hyphae extend beyond the host root system to promote physiological responses in the host, such as root branching and phosphatase secretion that indirectly promote phosphate uptake (Ezawa *et al.*, 2005). Following fungal uptake phosphate is transferred to the fungal vacuole where it is polymerized to form polyphosphate chains (Ezawa *et al.*, 2001). Poly-phosphate is translocated through the vacuolar compartment to the intraradical hyphae (Ohtomo and Saito, 2005). The mechanism of poly phosphate breakdown has not been characterized but is hypothesized to require the action of fungal phosphatase enzymes present in the arbuscule (Javot *et al.*, 2007). Nye (1977) found that the increase plant uptake P due to mycorrhization results mainly from

the increased soil volume exploited by the mycorrhizal root system. The external AMF hyphae extend to soil volumes beyond the depletion zone around the roots (Sanders and Tinker, 1971), and to smaller soil pores and closer to the surfaces of soil particles than do the roots and root hairs (O'Keefe and Sylvia, 1992). Besides that, effective acquisition by external hyphae is related to rapid formation of polyphosphates in the hyphae which maintain a low internal concentration of inorganic phosphates (Callow *et al.*, 1978). A greater effect caused by an increased conversion of inorganic to organic phosphate in the leaves of mycorrhizal plants (Allen *et al.*, 1981) and a greater affinity of the absorbing sites for $H_2 PO_4$ in mycorrhizal roots have also been suggested (Cress *et al.*, 1979). Some studies have also been carried out to screen and improve the tolerance to P-deficiency in maize, most of which focused on maize in hybrid lines (Gong *et al.*, 2002; Li *et al.*, 2003; Wang *et al.*, 2003). In this study, we need to investigate the effects of plant inoculation with mycorrhizal fungi to reduce the P deficiency effect on the morphological and some physiological traits of maize plants under greenhouse condition. In addition to study the effectiveness of mycorrhizae on uptake of soil P depend extraradical mycelium and mobility of the nutrients themselves in soil.

2. Material and Methods

2.1. Preparation of mycorrhizal inoculums

The arbuscular mycorrhizal fungi (AMF) isolates used in this study are *Glomus mosseae* (Nicolson and Gerdemann), *G. etunicatum* (Becker and Gerdemann), *G. clarum* (Nicolson and Schenck) supplied from microbiology Lab. Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. The three AMF species were multiplied under onion plant root in sterilized soil. After 40 days of growth, plant shoots were removed and the substrate containing hyphae, spores and root was air dried and used as the inoculum. The inoculum was calculated based on number of spores present in 10 g dry roots (500 spores/10gm)

2.2. Inoculation and experimental design

Maize seeds (*Zea Mays* L.) were surface sterilized with sodium hypochloride (1% available chlorine) for 10 minutes, rinsed three times in sterilized distilled water, and then left to germinate for 3-4 days at $29 \pm 2^\circ C$ rolled in sterilized filter paper. Germinated seedlings were planting in plastic pots (25 cm diameter and 30cm depth) each pot was filled with 1.5 Kg sterilized Peat moss soil (pH 5.4 the soil had $33.4 \pm 3.2 \text{ mg kg}^{-1}$ extractable N, $6.2 \pm 0.45 \text{ mg kg}^{-1}$ extractable P, and $44.6 \pm 5.6 \text{ mg kg}^{-1}$ extractable K). Each pot was planted with 5 seeds and then thinned to three plants after 14 days. The

individual plant was inoculated with various mycorrhizal fungi species separately. Ten grams of AMF inoculum (500 spores) were added in a deep truck around the plant stem into each pot. The treatments were distributed in randomized complete block design with three replicates. Two treatments with different phosphorus applications levels of zero and $60 \mu\text{g P/g}$ of soil added as a rock phosphate, each block including eight treatments were replicated three times as follows: 1-Plants were treated with two level of phosphorus fertilization rate. 2-Plants were inoculated by three AM fungi species individually as 500 spores/plant. 3-Plants were inoculated by AM fungi at the above mentioned rates of phosphorus fertilization. 4-Plants were left free to serve as a check. The plants were grown in greenhouse at $23-29^\circ C$ and a relative humidity of 70-85%, with a 16h day and 8h dark photoperiod. The pots were irrigated regularly to near field capacity with tap water.

2.3. Plant sampling and biomass measurement

The plants were harvested 45 days after seedling. Root systems were separated from shoots and fresh root system was weighed immediately as well as plant shoots. Half of each root sample was fixed in FAA (37% Formaldehyde- Glacial Acetic Acid -95 Ethanol, 9:0.5:0.5, V:V:V) for quantification of AM fungal colonization and vesicular numbers. The remaining half of each samples (for root and shoot) were oven dried ($80^\circ C$ for 48h) and used for measurement of P concentration.

2.4. Measurements of mycorrhizae, and P

Arbuscular mycorrhizal fungi colonization of roots was quantified using a dissection microscope (20-40 \times) after cleaning the roots in 10% KOH (w/v) and staining them in trypan-blue. A variation of the gridline intersection method, developed by Giovannetti and Mosse, (1980), was used to determine the proportion of root length in which arbuscules, vesicles or hyphae occurred.

Shoot P concentration was determined by the molybdate blue ascorbic acid method according to Murphy and Riley, (1962) after the plant material was digested by nitric acid and perchloric acid.

2.5. Determination of physiological characteristics

Soluble protein content and proline content were determined by extraction method as described by Zhang, (1990). The youngest leaf was collected from the plant sampled and samples were stored at $-4^\circ C$ prior to analysis. The following method was used for extraction. Each sample with a weight of 1g was homogenized with a chilled mortar and pestle in 10ml of 100 mg ml^{-1} trichloroacetic acid buffer (pH 8.0). Homogenates of samples were centrifuged at 4000 rpm for 10 min. Top aqueous layer was then transferred into 5ml tubes which were incubated in a boiling water bath and quickly placed in an iced-

water bath for 5 min, then centrifuged again. Two ml thiobarbituric acid reagent was added to 2 ml of extracted supernatant. The supernatant was spectrophotometrically determined by measuring the absorbances at different wavelength. The chlorophyll content in leaves were measured by Chlorophyll Content Meter model CL-01 Co. Hansatech Instruments

2.6. Data analysis

Data was analyzed using ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA). The significance of differences within treatments was separated by using Least Significant Difference test at 5%.

3. Results

3.1. Effect of P- deficiency on plant growth parameters

Data illustrated in Fig. 1 show the effect of three species of arbuscular mycorrhizal fungi (AMF) on maize plant growth parameters in the presence or absent phosphorus (P) fertilization (zero, 60 μ g P/g of soil or with P, without P respectively). The best values of all plant growth parameters were detected in the presence of P fertilization level (with P) comparing with untreated P (without P) in all deferent treatments. The highest values of plant height and plant stem length were recorded in the presence of P fertilization and inoculation with AMF species *Glomus etunicatum* and *G. mosseae* (increase by rate 34.5%; 32.9% and 32.5%; 32.2% respectively) comparing with control plants. While the values of plant roots length and plant fresh weight were increased by rate 40%; 46.3% and 37.1%; 46.3% respectively, when maize plants inoculated with *G. clarum* and *G. etunicatum* in the presence of P comparing with values of control plants (Fig. 2). Also the highest value of plant dry weight was recorded in the case of inoculation with AMF specie *G. clarum* by increasing rate 55.7% comparing with control. On contrast the treatment with P causing decreased in all values of root/shoot ratios comparing with the same treatment in the absent of P. The largest decline values at two level of P were recorded in the plant treated with AMF specie *G. mosseae*, then *G. clarum* and *G. etunicatum* followed by control treatment.

3.2. Effects of P- deficiency on some physiological traits for maize plants

Data in Table (1) showed values of P uptake, soluble protein, proline and leaves chlorophyll content for all treatments under sufficiency and deficiency of P. The P uptake values in all treatments were significantly under P sufficiency (60 μ g P) than that under P deficiency (zero μ g P). The highest values of plant P uptake were recorded in the presence of P and inoculation with AMF specie

Glomus etunicatum, then *G. clarum* followed by *G. mosseae* comparing with untreated plants. Also all treatments of maize plants in zero level of P were observed a decrease in values of protein content comparing with P fertilization level 60 μ g, while increases in proline values were found in all plants at zero level of P comparing with other level. The highly values of plant chlorophyll content (8.306, 7.840 unit) were recorded in the presence of P fertilization and inoculation with AMF species especially *G. clarum* and *G. etunicatum* respectively.

3.3. Mycorrhizae root colonization and spores numbers

No mycorrhizae were found in all the treatments without mycorrhizal inoculation. The mycorrhizae root colonization% was affected by phosphorus fertilization levels. The rate of fertilization 60.0 μ g P/g of soil was more suitable for AM colonization in plant roots than zero level of P. The highest values of AMF root colonization% (50.5%, 80.3%) were found when plants inoculation with AMF specie *G. etunicatum* at both level of P treatments (zero, 60 μ g p/pot respectively), then *G. clarum* followed by *G. mosseae*. The same results were observed in number of AMF spores/ 100g of soil. The specie of *G. etunicatum* recorded the highest value of spores (252, 320 spores /100g of soil) at two levels of P fertilization, then *G. clarum* followed by *G. mosseae* (Figs. 3, 4).

4. Discussion

In general, P starvation induced a wide array of metabolic effects that modify plant growth. The presented data provided the strong preliminary evidence for the effects of low P stress on the morphology and physiology of maize plants, which exhibited a reduction in biomass. The inoculation maize plants (*Zea mays* L.) with three arbuscular mycorrhizal fungi (AMF) species are causing significant increase in plant growth parameters, and some physiological traits of maize plants under greenhouse condition compared to control plant. The similar result has been shown by Jakobsen *et al.* (1992); Nurlaeny *et al.*, (1996) and Ortas, (2003, 2009). The large differences in crop growth due to AMF inculation under low fertility soil conditions have been shown in similar studies (Jackson *et al.*, 2002; Martin and Stutz, 2004; Ortas *et al.*, 2011). Also under P deficient condition the density of root hairs was increase in plant roots as symptoms of P starvation. These symptoms are limited in the inculation with AMF without treatment with P. This result was agreement with Baylis, (1970) who found that density of root hairs of maize inbred lines decreased with an increase in soil P. The increase in density of root hairs, contributing to P uptake as

confirmed, appeared to be a response by the plant to low P stress. P is an essential nutrient for plant and associates with many physiological processes. In plant body, such as soluble protein, proline and chlorophyll content are usually recognized as indicative factors under stress conditions. Liang *et al.*, (2005) reported that P deficiency increased the free proline and decreased in protein and chlorophyll content. On the other hand the higher AMF root colonization% was found in the higher level of P

addition than in zero level. As well as AMF spores forming in soil increased by increasing of P fertilization. These results are agreement with Asimi *et al.*, (1980), Koide and Li (1990), Koide (1991), Toro *et al.*, (1997) and Ortas *et al.*, (2011) they studied the dramatic effects of infection by mycorrhizal fungi on the host plant, which increase in phosphorus fertilization rate, which mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots.

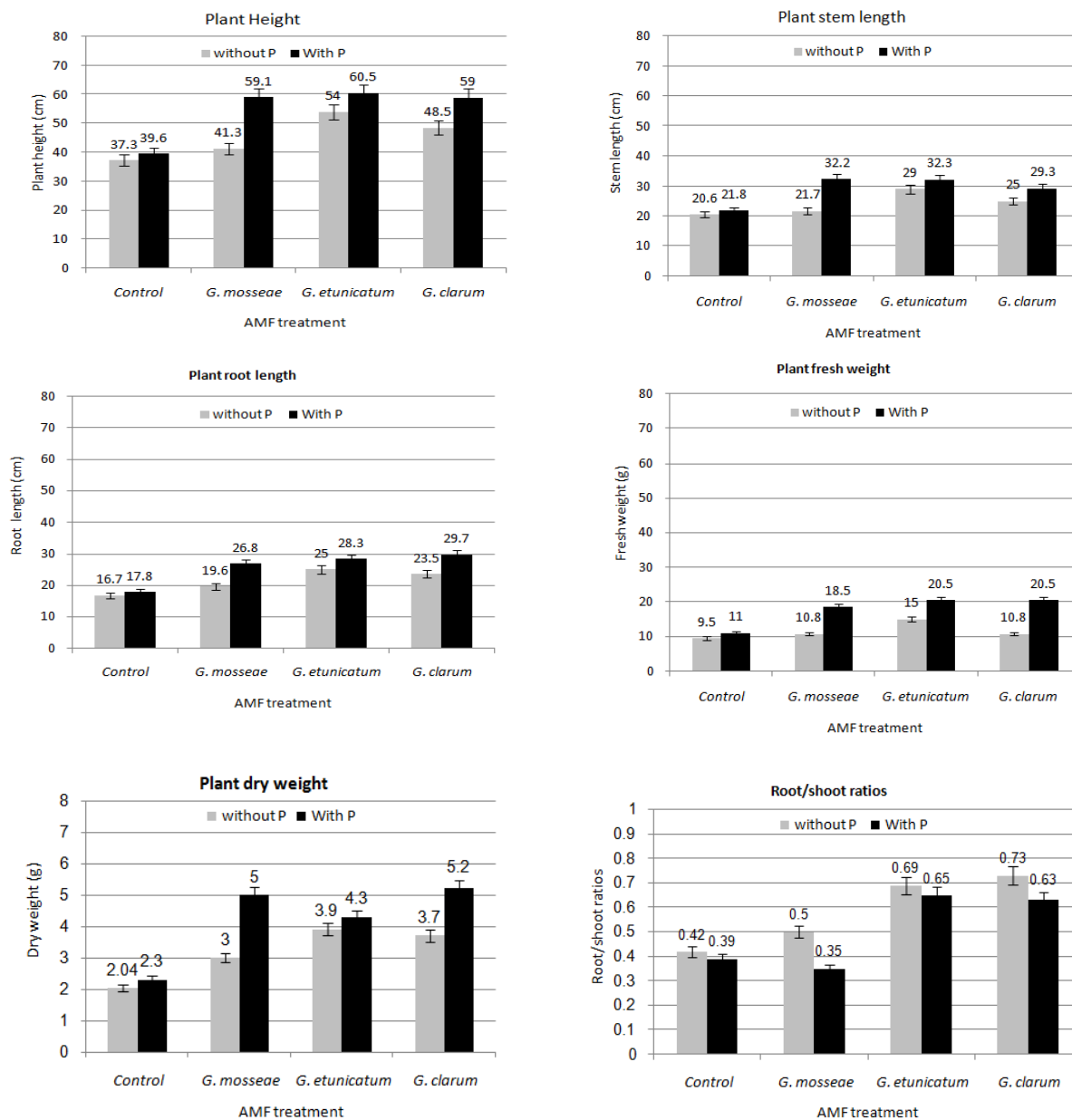


Figure 1: The effect of three AMF species on *Zea mays* L. plant growth parameters at two levels of phosphorus fertilization (without P zero μg , with P 60 μg) after 45 days from inoculation.



Figure 2: Effect of inoculation with three AM fungi species on maize plant (*Zea mays* L.) growth. A: Plants suffering deficiency of P which observing as a purple color on the back side of plant leaves and high density of root hairs (arrows). B: Plants treated with *Glomus mosseae*. C: Plants treated with *G. clarum*. D: Plants treated with *G. etunicatum*.

Table 1: Phosphorus uptake, soluble protein, proline and leaf chlorophyll of maize plant (*Zea mays* L.) at different inoculation with three AMF species in the presence two level of P

Treatment		Plant physiological parameters			
AMF species	Phosphate ($\mu\text{g g}^{-1}$)	P uptake (mg g^{-1})	Soluble protein (mg g^{-1})	Proline content ($\mu\text{g g}^{-1}$)	chlorophyll content (Unit)
<i>Glomus mosseae</i>	Zero	$0.245 \pm 0.03^*$	10.338 ± 0.731	$26.571 \pm 0.413^*$	4.726 ± 0.662
	60.0	0.698 ± 0.06	13.078 ± 0.464	19.703 ± 0.391	$6.920 \pm 0.548^*$
<i>G. etunicatum</i>	Zero	$0.203 \pm 0.05^*$	$12.609 \pm 0.461^*$	$21.304 \pm 0.562^*$	$6.110 \pm 0.806^*$
	60.0	0.774 ± 0.06	13.396 ± 0.478	19.703 ± 0.391	$7.840 \pm 0.631^*$
<i>G. clarum</i>	Zero	$0.203 \pm 0.05^*$	$11.268 \pm 0.475^*$	$25.726 \pm 0.562^*$	4.876 ± 0.856
	60.0	0.765 ± 0.04	13.632 ± 0.505	19.483 ± 0.435	$8.306 \pm 0.581^*$
Untreated	Zero	0.157 ± 0.03	10.278 ± 0.539	27.464 ± 0.496	4.733 ± 0.595
	60.0	0.665 ± 0.05	13.110 ± 0.369	20.325 ± 0.444	5.563 ± 0.190

-Mean of three replication and \pm is standard error.

- (*) significant level at 5%

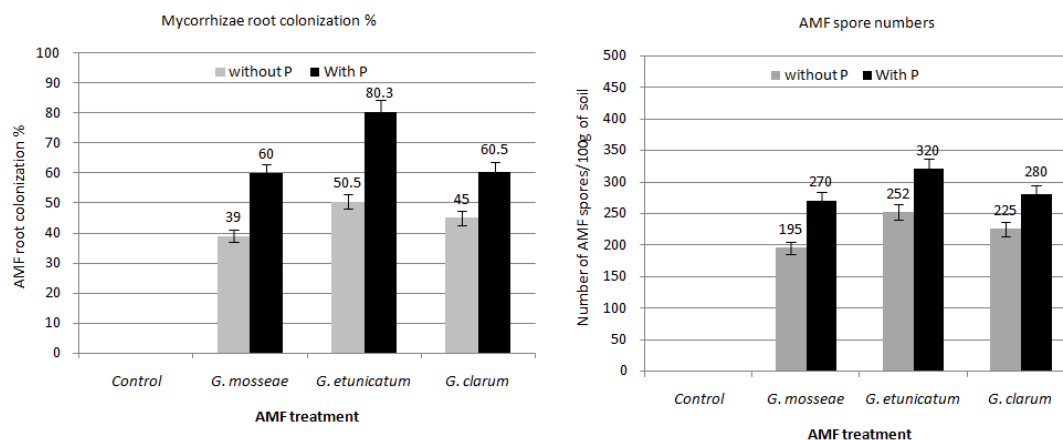


Figure 3: The AMF root colonization%, and spores numbers on maize plant roots (*Zea mays* L.) at inoculation with different AMF species in the presence two level of P

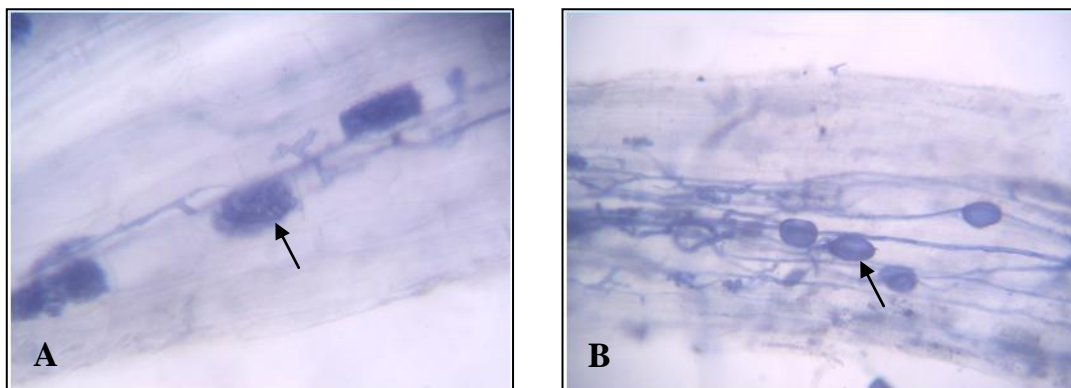


Figure 4: Photomicrographs for arbuscular mycorrhizal fungi (AMF) structures in *Zea mays* L. roots after clearing and staining (200×). The arrows showing a typical arbuscule of AMF(A) and typical vesicle was formed by AMF in the root cortex of maize plants (B).

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International Trade law and Civil Procedure Cross-Influences between Continental European

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Abstract: In order to determine whether a plaintiff in a civil case is entitled to claim, the underlying facts are often decisive. This article discusses the rules on fact-finding mechanism generally named discovery. These rules regulate how information is gathered, evidence is presented and how a decision on matters of fact is made. Romano – Canonical model and Anglo–American model have similarities and also differences mentioned in this article. But it is important to present their effective means and mechanisms for each other system to study and consider them in future legislations. The procedures that are used to resolve factual questions in civil or continental systems differs greatly from those used in American courts, we aimed to enhance our understanding of those differences and aimed to show these differences evolved throughout time. Often ,procedural rules are implemented that were tried and tested elsewhere. Comparative law may serve a useful tool to generate possible legal solutions to pressing procedural problems. In addition, experience in other jurisdictions may be of use to access possible effects of legislative change.

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1. Introduction

Continental systems (like French system as it is studied in this article), have increasingly required the parties to disclose information and have widened the possibilities for discovery. (Cadiet, 2004). Rules were introduced to prevent parties from withholding relevant information. (Kohl, 1971). Parties are required to provide complete and truthful information and they are also required to disclose in their pleadings the evidence they tend to use in support of their factual allegations. Judges have gained more powers to order the parties to produce evidence.(Levy, 1965).

The present U.S. discovery is not rooted in one, but in two distinct English procedural regimes: "common law and Equity" (Burbank, 1997). Each of these systems had their own procedure to resolve factual questions. The differences between systems are discussed in table 1 (Cannon, 2006). The fundamentals of the English rules on discovery were adopted in many North American colonies (Clark, 1935). A system based on English common law was also adopted at the federal level. Discovery in Anglo-American jurisdictions changed radically in the 18th century. (Cooter, 1995-1996) Moreover, important changes were made to the rules of evidence.(Dobie, 1938-1939).

More recently the American fact finding arrangement have changes: the 1938 Federal rules of civil procedure merged the procedures of law and equity in federal courts (Flanders, 1978-1979). The new rules on discovery aimed to prepare for trial and ensure that all relevant information was

available to both parties (Froeb, 2006).These rules enabled the parties to conduct a broad search for facts with little court intervention. (Langbein, 1995). By 1970s, Discovery was the new stage in the U.S. process of fact-finding. (Lynch, 1963).

Interrogations, depositions and requests for the discovery of documents are currently used in a large proportion of cases. (Millar, 1926).

The discovery consumes a large proportion of time and resources allotted to litigation. In addition, summary judgments became more widely available. (Millar, 1936-1937).

The developments above had led to a fourth general trend in U.S. litigation: A gradual shift in the roles of parties, lawyers, and judges in the process of discovery. (Millar, 1937-1938).

The role of court in American civil litigation was at the heart of legislative reform. Since 1983, Judges were granted more and wider discretionary powers to manage the litigation process. (74 Harv. L.Rev., 1961).

2. Research questions and general overview

What is the historical background of discovery rules and have there been cross-influences between the procedural systems and what solutions are considerable from U.S model of discovery? What are the differences between procedural systems on fact-finding?

3. Historical changes in both systems

The action to **produce and exhibit** was one in the nature of a bill of discovery which today is

called **discovery** in U.S. system and **Forced production of documents** in continental civil system. Both **Romano-canonic and Anglo-American** mechanisms to have access to the proofs in a civil procedure are **rooted in action ad exhibendum**, an action from Romano-canonical system, to enable a claimant of a proof whom the possessor refused to show it and bring it to another action.

At present, discovery is the legal process used to disclose evidence relevant to any matter at issue in a civil dispute. Each party has the right to call on others to provide discovery of relevant documents. Today we have wide discovery mechanisms in civil procedure of U.S. but in continental (civil) systems of civil procedure, there is no such means, mechanisms, and opportunities for litigants. In the U.S. system, if the discovery is called for, the formal procedure begins with opposing parties creating a list of all relevant documents which are or have been in their possession custody or power.

But in continental procedural systems, the parties can ask for that proofs before the judge, and this is the judge which verify the demand and may order the other party or third party to disclose the proof, but there is no discovery period, and there is not enough sanctions for refusal, while in U.S. model of discovery we have empowered judge jury and litigants who make a list of all relevant documents with details and the other party must comply by producing these documents in the action.

For example in French legislation, la production force des pieces is the solution when the proof which can prove the claim or defend of one party is in the possession of the other party ,if it is shown to the court that there is such a proof, and it is really in the possession of that person, the judge based on ask of that party, could order to be produced and if there is refusal of production, it could be order to pay a sum, for each day of refusal, or affirmative conclusion against refusal party. But in U.S. Federal rules of civil procedure, the role of judge is different.

There is a period before the trial is started, this time is for gathering evidences and have access for parties to all relevant documents. Discovery in U.S. approach is not asking for some proof only, it is a procedure, aimed to gather all related information for Parties of a civil case.

In this article we present American discovery to civil and continental system, although in U.S.system in some cases the existing process of discovery have caused delay and expense ,however, the disclosure is essential to achieve a just result in litigation.

4. Discussion

It is apparent that procedural rules have frequently been transplanted from one jurisdiction into another (Daigre, 1979), those that draft procedural legislation generally adopt rules and principles that were used, tried and tested elsewhere. (92 Yale L.J., 1982-1983).

There have been many examples of crossinfluence contributed to a gradual approximation of procedural systems . The pleading rules introduced by the 1848 New York Field code were similar to those on the continent. (Olivier, 2000) At the same the introduction of very liberal party driven discovery rules in the U.S. provides the clearest example. (Rosenberg, 1969 and 1988)

American model of discovery is supposed to provide the parties with relevant documents before trial. It can assist parties in preparing their cases or determining whether to settle before trial. It also should save the court time and expense through: Narrowing the issues in dispute preventing parties being taken by surprise at trial and enabling a dispute to be settled or determined at trial on its merits and not tactics.

In American model, the judge has no role of digging for facts and parties are required to have such a role, although the judge takes a more **active role** in **case management** .Thus it is strongly recommended to civil systems to make their **own** model of discovery and take the positive aspects of American model.

In American model of discovery any party may serve on any other party a request to produce and permit the party making the request, to inspect and copy any designed documents including writings, drawing, graphs, charts, photographs, phone- records electronically stored information and other data compilations from which information can be obtained, translated if necessary by the respondent or inspect and copy test or sample any tangible things which constitute or contain matters within the scope of Rule 26(b) and which are in possession ,custody or control of the party upon whom the request is served .The request may without leave of court be served upon the plaintiff after commencement of action and upon any other party with or after service of summons and complaint upon that arty. The request shall ecify a reasonable time, place and manner of making the inspection and performing related acts. The request mayspecify the form in which electronically stored formation is to be produced. The party upon whom the equest is served shall serve a written response within 30 days after the service of the request.

5. Comparing legal systems

As it was mentioned how legal systems accepted and created rules of discovery in civil procedure, it must be said that the differences in Continental legal systems and U.S. Federal rules of discovery in civil cases are numerous. (Schwarzer, 1989) In addition, there are many differences in the way in which the laws are applied. In French system (Gabdeil, 1903), the process of access to written proof and presentation of evidence may take place at a number of dispersed court sessions. In the United States there may be many procedural steps in pretrial stage of litigation. At the same time, evidence will be presented to the Trier of fact during a single uninterrupted hearing (Sofare, 1982-1983). In discussing the differences between legal systems, it is important to distinguish them.

Often the written rules in one system differ greatly from those in other systems. Out of court depositions are important within the U.S. System of discovery, unavailable in French system, for example. (Surbin, 1997-1998)

In all jurisdictions, the laws allow to have full access to all related information about the civil case, but with different features and means. (Sunderland, 1932-1933).

Most of rules of European legal system (continental) were initially influenced by the Romano – canonical model. In more recent times, and sometimes contrasted with Anglo-American systems.

Main differences are **mentioned in table 1**. These **differences** flow from two fundamental differences: different roles of judges in the civil procedure. (Sunderland, 1938-1939)

It is commonly believed that the pursuit of truth is the primary end of the process of discovery. (Fleming, 1928)

However, the pursuit of truth is not an end in itself, but a means directed towards a more remote end. (Grossen, 1960)

Technical or pure epistemological perspective does not suffice to understand the discovery arrangements of legal systems. (Keeton, 1954)

The pursuit of truth is believed to be importance to promote settlement, reach a correct decision, and level the playing field and to make the court's decision acceptable in the eyes of the litigants and the public. (Louisell, 1957) Thus in every legal system, it is relevant to identify the final ends of the litigation process. Different jurisdictions emphasize different ends of the process of discovery. Rules of discovery were designed to establish the facts "correctly". The pursuit of truth was hence of great importance in U.S. legal system. (Macclister, 1950) Thus all the means are available to achieve the facts of

civil case in civil procedure. Two distinctive features are most prominent in U.S. model of discovery in civil procedure: the civil jury and the adversarial system. (Speck, 1951) In U.S. civil procedure, the "adversarial system" provides both parties with sufficient opportunities to voice their opinion. Thus, in this system, the "role of parties" is more (Warren, 1890). The parties have the possibility of ask for production of all related documents which are "in the possession of the other party". (Weinstein, 1957).

But on the other hand, The U.S. Federal system seems to be also concerned with the "resolution of disputes" (Wigmore, 1940). We may conclude that the federal U.S. rules primarily aim for the fair and legitimate resolution of disputes. (Interim Report, 2010) Use of direct sanctions against parties in reliance of presentation of evidence in civil process is a distinctive feature of U.S. legal system. (Adams, 1998)

6. Conclusion

The procedural differences between the common law and continental systems have been thoroughly examined. Despite different features, the ultimate goal of both systems is essentially identical: to achieve the just, efficient, and speedy resolution of disputes. (Julien, 2003) Perhaps the most interesting phenomenon is that neither system is satisfied with its own performance in achieving this ultimate goal, (Kohl, 2004) and both systems are trying to seek inspiration from each other to reform their procedural arrangements. (Vincent, 2001)

The notion of active judicial management and supervision is sweeping both the United States and England and has dominated as the theme of their reform movements for the past twenty years (Heron 2002). The focus of judicial attention is shifting from trial to the pretrial stage. (Lebars, 1997) The opposite directions of these reform movements are clearly bringing the two systems into convergence. (Braas, 1945) Despite this convergent trend, the attitudes of the two systems toward civil discovery remain far apart. (Gabdeil, 1903) In the common law system, parties are equipped with discovery rights to gather information and evidence in preparing their cases. (Lewald, 1937)

DISCOVERY enables them to compel disclosure of information from their opponents and even third parties. (Jodlowski, 1967) In the continental systems, no such rights are recognized. (Nouveau Code, 2003)

The civil judges exclusively enjoy investigative power (Dunand, 1940). Almost all commentators find the answer to be rooted in different procedural arrangements and concepts of procedural justice between the two systems. (Linsmeau, 1999) I

turn to the board subject of discovery in civil actions. Viewed comparatively, in this particular realm of procedure, the civilians' mindset-still hostile to disclosure as well as discovery on the grounds of party privacy and autonomy- starkly differs from the common-law mindset. (Mougenot, 1990) However, some movement in the civil law has recently occurred, and the future should see more. Boudreau, 2006) Two points should be made perfectly clear at the outset. First, my proposal of **introducing discovery is made for the sole purpose of curing the problems arising from the continental system's lack of efficient discovery.** (Guinchard, 1999). It is not an attempt to harmonize the two systems' conflict on this issue or to build a set of universally rules. The most important lesson I find in the study of comparative civil procedure is that procedural law should be socially constructed and defined with an eye on the need and culture of a particular society. (Tarzia, 1996) Second, I would like to emphasize that while I propose to introduce discovery into the continental system, I do not propose to transplant the whole common law discovery Scheme. It would be silly to suggest such a complete transplant. (Couchez, 1998)

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Effect of *Allium ampeloprasum* on ileum function: Involvement of beta-adrenergic receptors and voltage dependent calcium channels

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Abstract: *Allium ampeloprasum* known as wild leek is a wild nutritious plant that belongs to Lilaceae. In this research, the hypoglycemic effects of the plant's leaves hydro-alcoholic extract on Wistar rat ileum contractions and its possible mechanism have been reviewed. Extraction was done through the maceration of *Allium ampeloprasum* powder with 70% alcohol. In this intervention research, 48 Wistar rats weighing between 150 and 200 grams were divided into 6 random groups of eight. The groups include: control group, the group receiving *Allium ampeloprasum* extract cumulative concentrations, the group receiving Propranolol, the group receiving Narcan, the group receiving L-name, and the group receiving cumulative concentrations of calcium chloride. On the experiment day, Wistar rats ileum contractions under 1g initial tension were separately recorded through adding potassium chloride 60(mM) in isotonic method in an organ bath containing Tyrode solution (37 °C, PH 7.4). To examine the mechanism of the extract effect, the tissue was incubated with Propranolol, Narcan or L-name, and the percentage of contraction changes were calculated and recorded. In order to determine the role of calcium channels in the tissue motor activity, ileum affected by calcium chloride cumulative concentrations was used. *Allium ampeloprasum* cumulative extracts (100, 200, and 400 mg/kg), in a dose-dependent manner, reduced ileum contractions (P<0.0001) by potassium chloride (60 mM). The intervention of beta adrenergic receptor antagonist (Propranolol, 1 µM), opioid receptors (Narcan, 1 µM), nitric oxide synthase inhibitor (L-name, 100µ M) in ileum showed that Propranolol decreases the inhibitory effects of the extract on the contractions caused by potassium chloride significantly (P<0.0001). However, L-name and Narcan did not decrease the inhibitory effect of the extract on ileum. Calcium also caused the contraction of tissue depolarized by potassium chloride. This contractive effect was significantly decreased by cumulative concentrations of the extract (P<0.0001). It can be concluded that *Allium ampeloprasum* leaf hydro-alcoholic extract could affect rat ileum motor activity by affecting beta adrenergic receptors and voltage dependent calcium channels. According to the results of the aforementioned effect, it might be used to treat digestive problems.

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Key Words: *Allium ampeloprasum* extract, Ileum, Rat

Introduction:

In recent years, the application of medical plants has increased significantly. Although some of them have toxicities (1-3), most of these plants significantly contribute to therapy of diseases (3,4). One of such plants is *Allium ampeloprasum* (wild leek) for which many medicinal properties have been reported in traditional medicine (1). Wild leek, with the scientific name of *Allium ampeloprasum*, is a wild nutritious plant that belongs to Lilaceae (1,5). The effects of wild leek are similar to garlic but milder (5). Wild leek could be found in Hamedan, Shiraz, Sanandaj, Kamyaran, Qom, and Arak provinces. The leaves and stems of young wild leeks are used as spice or medicine (3).

Wild leek contains lots of cysteine sulphoxides, saponins, tanins, and disulphide compositions (5). Its effectual constituents could protect against induced damages by damaging factors, decreases blood serum cholesterol rate, balances bodily functions, and widens blood vessels (vasodilation) (5,6). Wild leek is anti-asthma, anti-septic, Diuresic, vasodilator, expectorant, tonic, and stimulant (7). It could be considered as an anti-diabetes factor (8-10). It also has positive effects on blood serum lipid and glucose levels.

It is revealed that compounds containing sulphur in disulphides category which are amply found in allium genus plants like *Allium ampeloprasum* (wild leek) could decrease glucose levels in diabetes experimental model

through increasing peripheral glucose uptake, glucose gastrointestinal absorption inhibition, and increasing insulin secretion from remaining beta cells in Islets of Langerhans (10,11). Considering the role of oxidative stress and enzymal changes is important in the emergence of some undesirable biochemical and tissue changes in diabetes type 1 (12). The antioxidant properties of *Allium ampeloprasum* could relate to cysteine sulphoxides compounds. The antioxidant properties of such compounds is attained through increasing the level of antioxidant system enzymes including super-oxide dismutase (13,14). *Allium ampeloprasum* has pain killing properties (13). *Allium ampeloprasum* effectual constituents are similar to Garlic and Mosir having positive effects on blood serum lipids and glucose levels (15,16). No research has been done on its positive effects on intestines.

Numerous factors, affecting the cellular mechanism of muscle, could change motor activities of smooth muscle. The factors that cause smooth muscle contraction are: significant increase of extracellular potassium concentration and membrane depolarization, opening of sodium calcium slow channels and calcium entering cell, dephosphorylation of myosin phosphatase and calcium pumped into reticulum sarcoplasmic, and finally increasing the level of cytosolic calcium. Also, the factors through which cytosolic calcium decreases and myosin phosphatase activity increases could have an inhibition effect on the motor activity of smooth muscle (17,18).

Since in the previous researches, the vasodilative effect of *Allium ampeloprasum* (6) and the effects of allium from Lilaceae on aorta contractive activity with the effect of contractive response decrease in rat isolated arterial system (19) was reviewed, in this research, we studied the effect of *Allium ampeloprasum* hydro-alcoholic extract on the contractive activity of ileum, and the probable mechanism of the aforementioned effect through voltage dependent calcium channels, beta adrenergic and opioid receptors, and the role of the plant in the synthesis of nitric oxide synthase.

Materials and Methods:

Extraction Method:

In this research we used maceration method to get *Allium ampeloprasum* extract. After dehydration and powdering the plant leaves, we macerated 100 grams of the powder with 70% ethanol, and left it in the lab temperature for 72 hours. Then we filtered the solution with Buchner funnel and the solvent was distilled with a rotary evaporator in a temperature of 35 °C. The condensed solution was put in an incubator with a temperature of at most 40c so that the alcohol within the solution was completely evaporated. The resulting powder was kept in the refrigerator for later use (20). 25 grams of powder was finally resulted from 500 grams of *Allium ampeloprasum* powder.

Animals:

48 Wistar rats weighing between 150 and 200 grams provided by the Research and Laboratory Animals Multiplication Center of Shahrekord University of Medical Sciences were kept in a temperature between 20

to 24 °C and under 12 hours light/12 hours dark condition. Rats had free access to water and food, but they were deprived of food the night before the experiment to ease the job and for their tissues to be cleared (21-23).

Materials Used:

Proparanolol and L-name were prepared from Sigma Co. (USA), Narcan from Tolid Darou Co. (Iran), and all the salts from Merck Co. (Germany).

Ileum Preparation and Methodology:

Following moral principles on the day of experiment, a rat was exposed to chloroform and made unconscious, then from the end of its ileum, excluding 2 centimeters from the end, a 2-centimeter piece was cut and inside it was gently washed with Tyrode solution; then it was put between two stainless steel hooks vertically in an organ bath (50 ml), where the solution temperature and pH were 37 °C and 7.4, respectively. The initial tension on the tissue was 1 gram and the Tyrode solution in the bath was composed of the following (in millimolar):

NaCl (136), KCl (5), CaCl₂ (2), NaHCO₃ (11.9), MgCl₂ (0.98), NaH₂PO₄ (0.36), glucose (5.55).

Tissue compatibility and stability period was 60 minutes where air bubbles flowed constantly in the organ bath and every 15 minutes the solution in the bath was replaced with a new one. After compatibility, ileum was contracted by potassium chloride (60 mM) and when the contraction reaches plate state (21) the cumulative concentrations of the extract (100, 200, 400 mg/kg) (10) were added to the organ bath. Isotonic lever transducer (Harvard, UK) transferred tissue motor activity to the recording device—Universal Harvard Oscillograph—and the respective effect was recorded on paper. Then the percentage of changes in the contractile force was calculated in comparison to plate state. In order to study the mechanism of the extract effect on the tissue, it was incubated with Proparanolol with a concentration of 1µM (30 minutes), Narcan with the same concentration (1µM) (24), and L-name with a concentration of 100µM for 20 minutes. Then its effect on opioid and beta adrenergic receptors and the role of nitric oxide was studied (25). In order to study the role of extracellular calcium in the function of the extract, the tissue was first put in calcium free Tyrode solution with a high concentration of potassium chloride (60 mM). Then in a cumulative manner (2 to 8 mM), potassium chloride was added to the organ bath (21). Ileum contraction in response to cumulative concentrations of calcium chloride was recorded. After 5 minutes of incubation in the presence of the extract with cumulative concentrations, all the stages were recorded.

Statistical Methods:

Information gained through data was first saved in a computer and SPSS software and then analyzed statistically. The changes of contractile force caused by the extract and antagonists compared to the extract itself was calculated and specified in the form of SEM ± mean. Also, ANOVA statistical test and student t-test were used to compare the different concentrations of the extract and

the two groups, respectively. $P < 0.05$ was considered as the meaningful difference.

Results:

In all the stages of the experiment, adding potassium chloride to organ bath led to contraction caused by potassium chloride effect on ileum, and after a short time, the contraction reached plate state, where the percentage of ileum contraction was calculated. After the tissue reached plate state and saline added, the effect of extract cumulative concentrations, beta adrenergic and opioid receptors antagonist, the role of nitric oxide and the intervention of voltage dependent calcium channels on the

average of tissue contraction changes was calculated and recorded.

Cumulative concentrations of *Allium ampeloprasum* hydro alcoholic extract compared to contractions caused by potassium chloride in rat ileum

Table 1 shows that cumulative concentrations of *Allium ampeloprasum* hydro alcoholic extract (100, 200, 400 mg/kg) has decreased rat ileum contraction caused by potassium chloride (60 mM) in comparison with the saline group, and indicates a meaningful difference ($P < 0.0001$, $n=8$). The inhibition effect of the extract on ileum depends on dose and indicates a meaningful difference between them, too ($P < 0.05$, $n=8$).

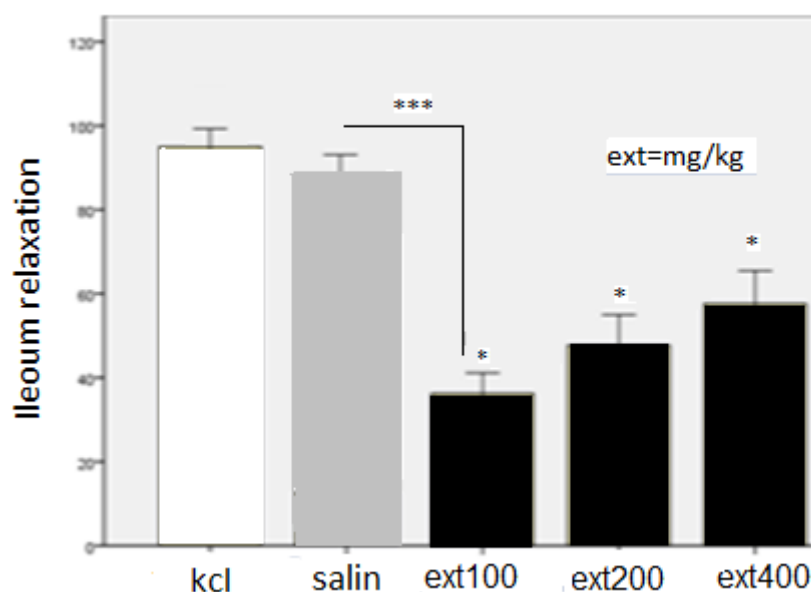


Table 1: The effect of cumulative concentrations of *Allium ampeloprasum* hydro alcoholic extract (100, 200, 400 mg/kg) on ileum contraction caused by potassium chloride (60 mM) and saline

The cumulative concentrations of the extract (100, 200, 400 mg/kg) decreased the contraction of ileum caused by potassium chloride (60 mM) in comparison with saline group (ANOVA, *** $P < 0.0001$, $n=8$). The inhibition effect is caused by the dose-dependent extract and indicates a meaningful difference between each of extract concentrations (* $P < 0.05$, $n=8$).

The comparison of the effects of beta adrenergic receptors (Propranolol) presence on the inhibitive function of the extract

Stimulating beta adrenergic receptors causes the relaxation of small intestine. It is possible that the extract has caused inhibitive function through stimulating the above-mentioned receptors. Therefore, the effects of the extract on the receptors once in the absence of Propranolol and once in its presence for 30 minutes with an interval of 15 minutes during which the tissue was washed, are compared together. The results show that the extract has caused inhibition of contraction by potassium chloride ($P < 0.0001$, $n=8$). Propranolol also caused a meaningful decrease in the inhibition effect of contraction caused by the extract ($P < 0.001$, $n=8$) (Table 2).

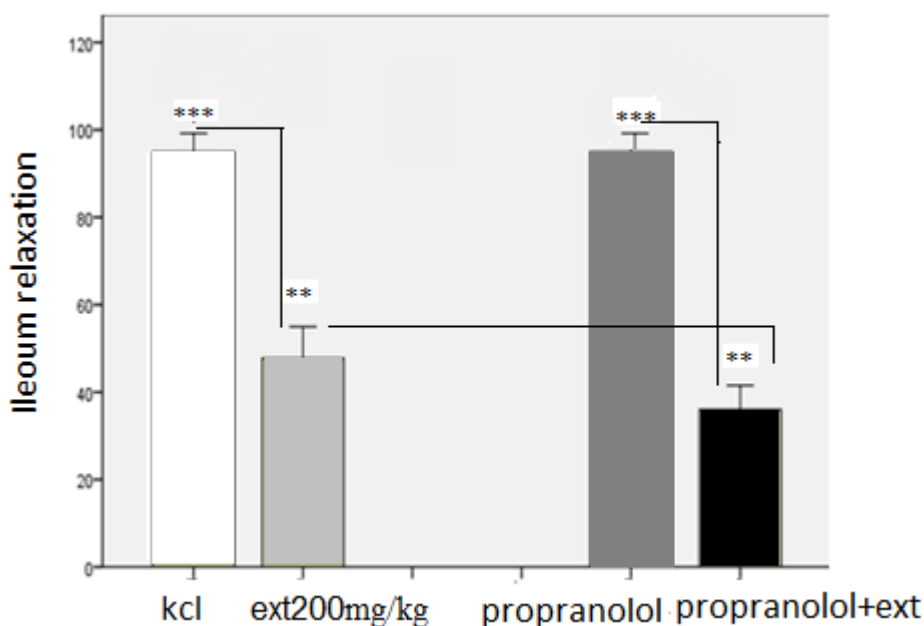


Table 2: Comparison between contractile effect of potassium chloride, inhibition effect of the extract with a concentration of 200 mg/kg, and Propranolol (1 μM) on beta adrenergic receptors in ileum (n=8). **P<0.001, ***P<0.0001

The comparison of the effects of opioid receptors antagonist presence (Narcan) on the inhibitive function of the extract

According to the fact that stimulation of opioid receptors decreases intestinal movements, there is a probability that the effectual constituents of the extract affect receptors and cause muscle relaxation. Therefore, the inhibition effect of the extract on the receptors once in the absence of Narcan (1 μM) and once in its presence for 30 minutes with an interval of 15 minutes during which the tissue was

washed, are compared together. The results show that the extract has decreased the contractile effect of potassium chloride meaningfully, but there was no meaningful difference between the contractile effect of the extract in the absence and presence of Narcan.

The extract caused the inhibition of contraction by potassium chloride (P<0.0001, n=8), but there was no meaningful difference between the contractile effect of the extract in the absence or presence of Narcan (Table 3).

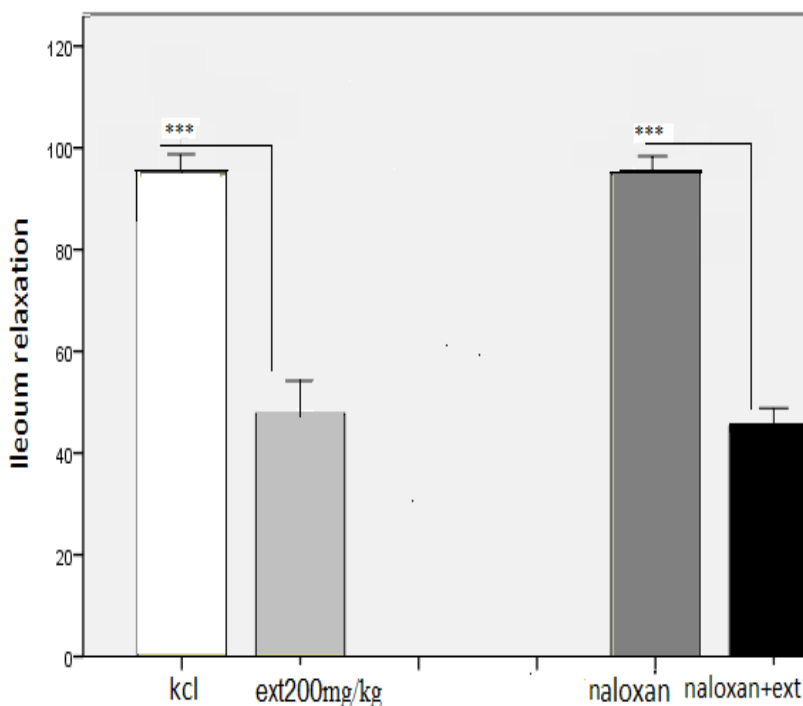


Table 3: Comparison between potassium chloride contractile effect, inhibition effect of the extract with a concentration of 200 mg/kg, and Narcan (1 μM) on opioid receptors in ileum (n=8). (***)P<0.0001

The comparison of nitric oxide synthase antagonist (L-name) on inhibitive function of the extract

It is probable that stimulating NO synthase has decreased the contractive function of the extract; L-name is also an inhibitor of nitric oxide synthase enzyme. Therefore, the effect of the extract on the receptors once in the absence

of L-name and once in its presence for 20 minutes with an interval of 15 minutes during which the tissue was washed, are compared together ($P < 0.0001$, $n=8$), but there is no meaningful difference between the inhibition effect of the extract in the absence or presence of L-name (Table 4).

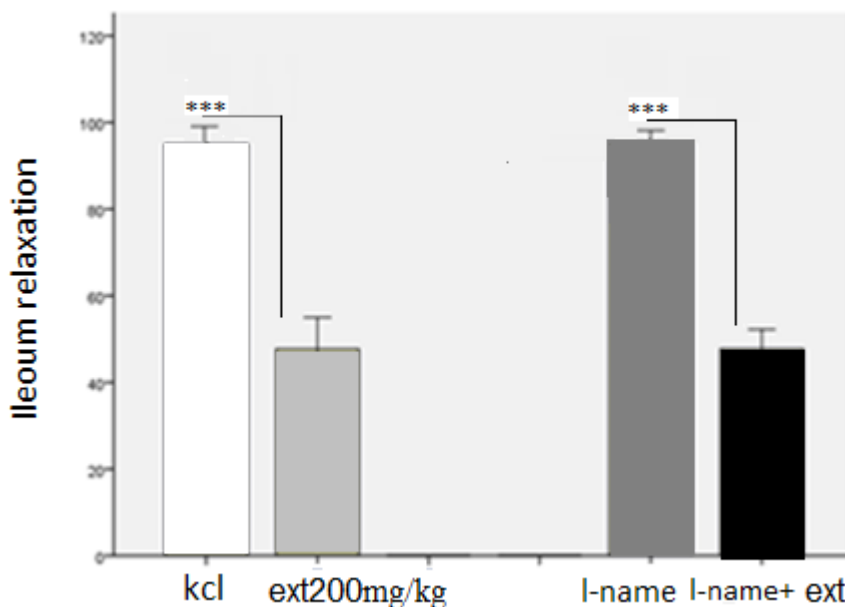


Table 4: Comparison between the contractile effect of potassium chloride, inhibition effect of the extract with a concentration of 200 mg/kg, and L-name ($100\mu\text{M}$), inhibitor of nitric oxide synthase enzyme in ileum ($n=8$). ($***P < 0.0001$)

The effect of *Allium ampeloprasum* leaf hydro alcoholic extract on contraction caused by calcium chloride in ileum depolarized by potassium chloride

Ileum contraction caused by calcium chloride cumulative concentrations (2 to $8\mu\text{M}$) in depolarized tissue by potassium chloride (60 mM) depends on the concentration of calcium chloride ($***P < 0.0001$), and the contractive responses in the presence of cumulative concentrations of *Allium ampeloprasum* extract decrease ($**P < 0.001$).

Table 5 shows that depolarized ileum of rat in a calcium free Tyrode solution with a high concentration of

potassium chloride (60 mM) contract in the presence of cumulative concentrations of calcium chloride, dependent on concentration (2,4,8 mM) ($P < 0.0001$, $n=8$). After washing the tissue with a calcium free Tyrode solution and leaving it for 15 minutes, repeating the same aforementioned stages in the presence of different concentrations of the extract (100, 200, 400 mg/kg) for 3 minutes decreases the contractile effect caused by calcium chloride in ileum, and the contractile effects of calcium in the absence or presence of the extract have a meaningful difference with each other (t-test, $P < 0.001$, $n=8$).

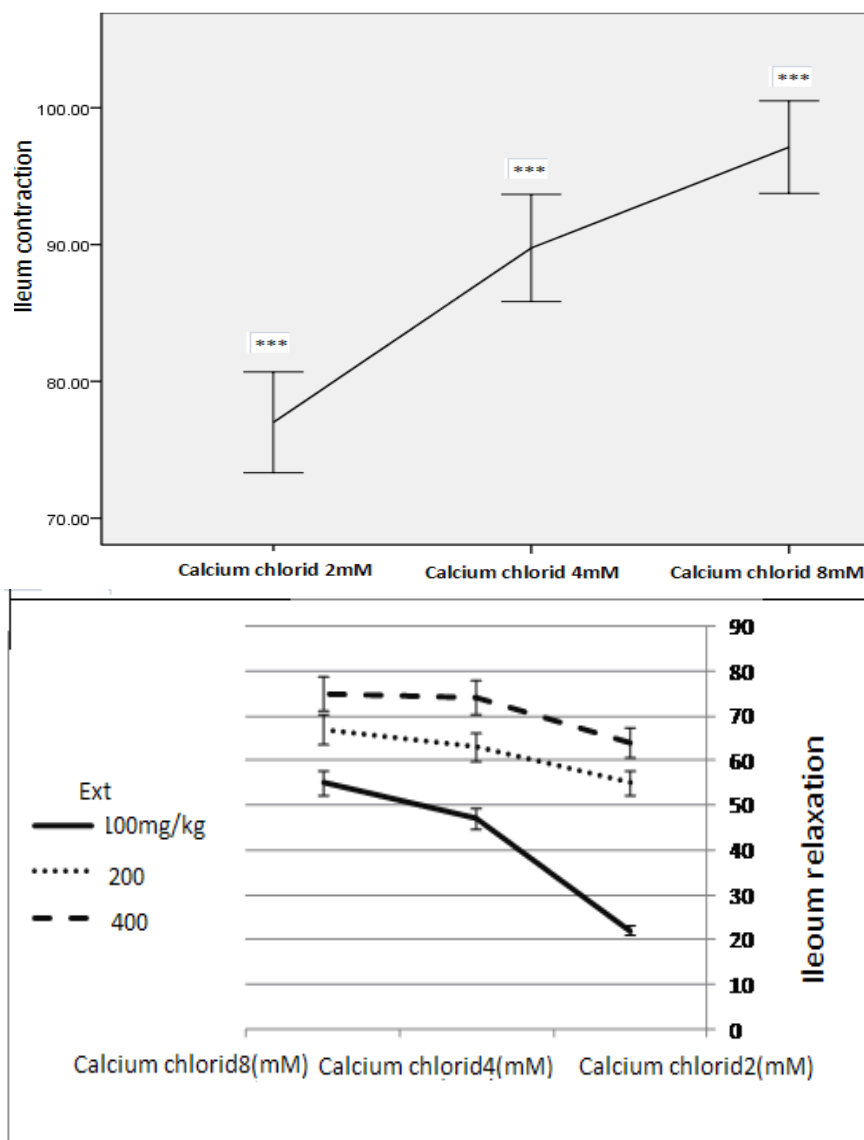


Table 5: Comparison between the contractile effect of calcium chloride cumulative concentrations (2 to 8 mM), and the inhibition effect of the extract in its cumulative concentrations (100, 200, 400 mg/kg).

Discussion:

In this research, *Allium ampeloprasum* extract could decrease contractions caused by potassium chloride for 25 minutes; however, before adding the extract, the tissue stayed in contraction during the experiment, while after adding the extract, it relaxed. This is caused by the effect of the extract on tissue, not muscular fatigue (26).

Since the major factor of smooth muscle contraction is the presence of calcium ions; these ions could enter cells through activated calcium channels and cause smooth muscle contraction. Opening of these channels doesn't change resting membrane potential much, because an enough number of potassium ions move out of the cell simultaneously to keep a natural membrane potential. Contraction continues until calcium channels are open (27). Since there are voltage- dependent calcium channels in ileum like type L channels, contraction of ileum smooth muscle caused by potassium chloride could be because of these channels (28). It is probable that by affecting ileum smooth muscle cells, *Allium*

ampeloprasum effectual constituents have prevented the increase of calcium in cells causing the muscle to relax.

About the probable mechanism of muscle relaxation, it should be noted that the activation of opioid receptors causes ileum to relax, but here, blocking the receptors with Narcan shows Narcan incapability in decreasing the inhibitive function of the extract, and confirms no intervention from receptors (29). The activation of beta adrenergic receptors causes inhibition of ileum contractive activity (30). By activating cAMP dependent protein kinases and the active transfer of calcium into sarcoplasmic reticulum, beta adrenergic receptors cause inhibition of ileum contractive activity. Incubation of ileum piece with beta adrenergic receptors antagonist by Propranolol decreases relaxing function of the extract on ileum contraction caused by potassium chloride. This could indicate that a constituent or constituents of the extract have the ability to activate beta adrenergic receptors and decrease the effect of the extract.

Meanwhile, nitric oxide is one of the most important released factors from endothelium(31-34). NO is released from L-arginine by nitric oxide synthase enzyme (35). Increase of NO synthase through the increase of cGMP causes ileum relaxation (36), but the incapability of L-name in decreasing the inhibition function of the extract confirms that nitric oxide synthase has no intervention or part in the inhibition function of the extract.

When adding calcium chloride cumulative concentrations to the tissue in a calcium free Tyrode solution with a high concentration of potassium, the tissue just gets depolarized and no contraction is observed (37). However, after adding calcium chloride to the tissue, it contracts, and then in the presence of the extract, there will be inhibition effect on contraction (38). This shows that the extract affects calcium channels and the inhibition function has come to effect.

Plants belonging to the genus of *Allium* have a strong inhibitor of aldose reductase enzyme called isoliquiritigenin that could prevent the aorta from decreasing the formation of 12 prostaglandins that have vasodilation effects, under diabetes condition. It could be explained that also the presence of the same compound (isoliquiritigenin) is why contractile effects of thoracic aorta decrease (39).

It is probable that the inhibitive function of the extract also comes from compounding with isoliquiritigenin or from a stable bond between the effectual constituent or constituents of the extract like flavonoids or saponins (40) with calcium channels meaning that the major effect of the extract comes from deactivating calcium channels and part of it probably from the effect of the extract compounds themselves with the intervention of beta adrenergic receptors. Studying each of these compounds and their effect on the above-mentioned channels demands separate researches.

Conclusion:

In general, it could be concluded that *Allium ampeloprasum* leaf hydro-alcoholic extract could affect the motor activity of rat ileum through affecting beta adrenergic receptors and voltage-dependent calcium channels, and considering its results, it could be used in treating digestive problems.

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10/28/2012

Evaluation and comparing the behavior of concrete horizontal diaphragms in linear behavior of concrete by numerical method

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Abstract: One of the most important assumptions which is being used in analysis and design of buildings against lateral forces is the rigid-floor assumption. Lateral rigidity of diaphragms depends on several factors such as: type of the structure, dimensions of structure, rigidity and location of lateral load bearing elements, stiffness of frames, type and thickness of floors, number of stories and etc. so, we should give more and more importance to this assumption. In this study, in order to investigate how concrete slabs behave, a lot of models in two cases of rigid-floor and flexible-floor in linear limitations are analyzed and compared.

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Keywords: Rigidity, flexibility, diaphragm, concrete slab

1. Introduction

Structures with flexible floor systems behave differently under dynamic lateral loading than structures with rigid diaphragms. The rigid floor assumption distributes forces between lateral resistant elements according to the proportion of elements rigidity. In addition, this assumption decreases the degrees of freedom and makes the analysis simpler. Several codes, for instance Iranian code of practice for seismic resistance of buildings (Standard 2800) present some criterions for the diaphragm. According to the mentioned standard, diaphragm is to be considered flexible when the diaphragm deflection exceeds twice the story drift. However, flexible diaphragm systems are still analyzed with criteria and recommendation developed for structure with rigid diaphragms. Variables such as structural system can affect diaphragm behavior and causes rigid diaphragm treatment was not accurate. In this study

analysis was performed in a linear mode and for each structures, modeling was performed considering both real rigidity and rigid diaphragm assumption.

2. Model description

A basic plan according to the below figure is provide in order to set up the modeling procedure, having 3m height, 5m width, 6 and 10 m spans. The diaphragm is assumed to be concrete slab.

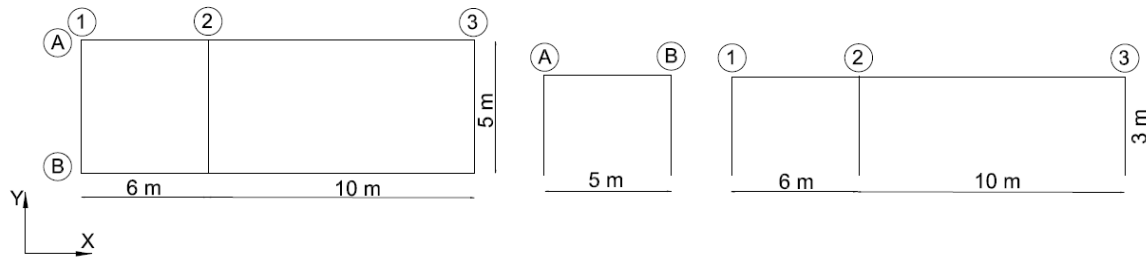


Figure.1. Model description

Loading is performed based on Iranian earthquake code and the analysis type is static. The selected structural systems are concrete structure with shear wall, concrete moment frame, steel braced frame and steel moment frame. The braces and shear walls are

in 1, 2, 3 directions and earthquake load is applied in x direction. The numerical modeling is made in SAP2000 for both rigid and flexible cases of diaphragms.

Table.1. Shear wall (SH) modeling characteristics

Mass load (flex) kg(Mass load (rigid) kg(Column cm× cm	Beam cm× cm	Shear Wall Thickness)cm(Diaphragm Thickness (cm)	Variation	Model No
93.3	9514	35×35 8Ø32	25×40	25	5	Slab thickness	SH ₁
107.4	10954				10		SH ₂
121.5	12394				15		SH ₃
149.7	15274				25		SH ₄
178	18154				35		SH ₅
133.9	13654	35×35 8Ø32	25×40	5	25	Shear wall thickness	SH ₆
139.3	14209			10			SH ₇
145.8	14869			20			SH ₈
153.7	15679			30			SH ₉
161.7	16489			40			SH ₁₀
144.8	14766	35×35 8Ø32	25×35	25	25	Beam dimensions	SH ₁₁
165.1	16839		35×55				SH ₁₂
195.4	19927		50×75				SH ₁₃

Table.2. Concrete moment (MC) modeling characteristics

Mass load (flex) kg(Mass load (rigid) kg(Column cm× cm	Beam cm× cm	Diaphragm Thickness (cm)	Variation	Model No
69.7	7109	40×40 8Ø30	30×45	5	Slab thickness	MC ₁
81.9	8357			10		MC ₂
94.2	9605			15		MC ₃
118.6	12101			25		MC ₄
143.1	14597			35		MC ₅
113.6	11588	40×40 8Ø30	25×40	25	Beam dimensions	MC ₆
133.7	13641		40×60			MC ₇
166.4	16977		55×85			MC ₈
116.7	11904	30×30 8Ø24	30×45	25	Column dimensions	MC ₉
121.1	12354	50×50 8Ø32				MC ₁₀
127.7	13028	70×70 12Ø32				MC ₁₁

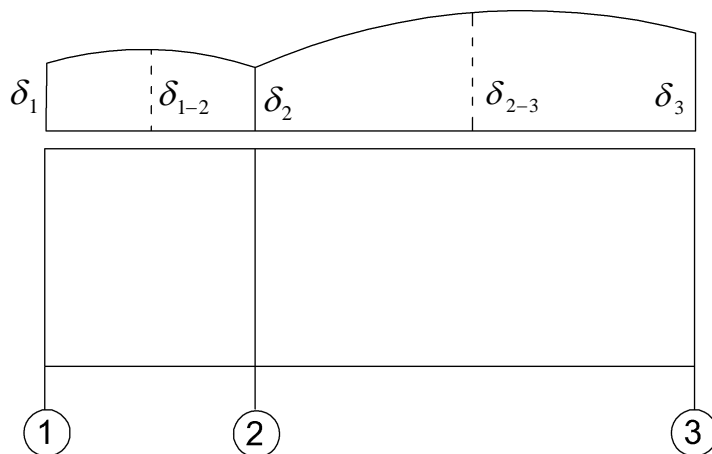
3. Comparison criterions

Here, some criterions are defined in order to make it possible to compare the results between rigid and flexible modeling results. The parameter δ is chosen as displacement symbol. In fact $\delta_3, \delta_2, \delta_1$ are the

displacement for the three direction in the below figure.

$$\delta_{\Delta} = (\delta'_2 - \delta_2) / \delta_2,$$

$$\delta'_2 = (2\delta_1 + \delta_3) / 3$$



There are also parameters such as Δ_1 , Δ_2 and finally Δ that must be defined here:

$$\Delta_1 = (\delta_{1-2} - (\delta_1 + \delta_2) / 2) / ((\delta_1 + \delta_2) / 2),$$

$$\Delta_2 = (\delta_{2-3} - (\delta_2 + \delta_3) / 2) / ((\delta_2 + \delta_3) / 2),$$

$$\Delta = \text{Max}\{\Delta_1, \Delta_2\}$$

4. Illustrative graphs

To completely understand the results for the structures modeling, in this part different graphs according to variation of some variables are plotted.

Table.3. Graphs for SH models

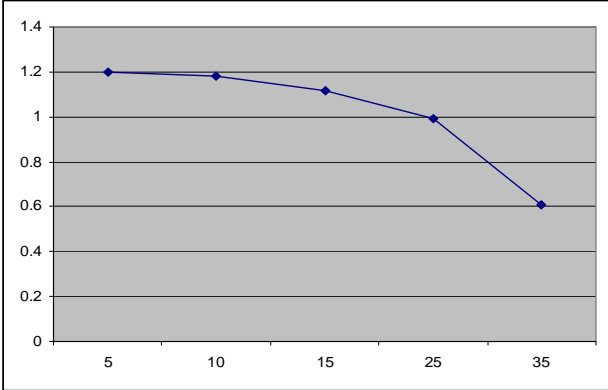
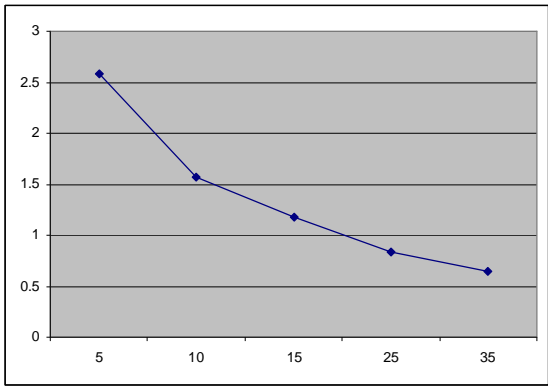
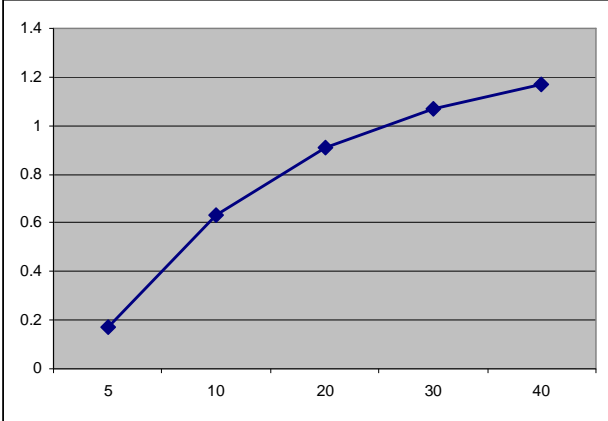
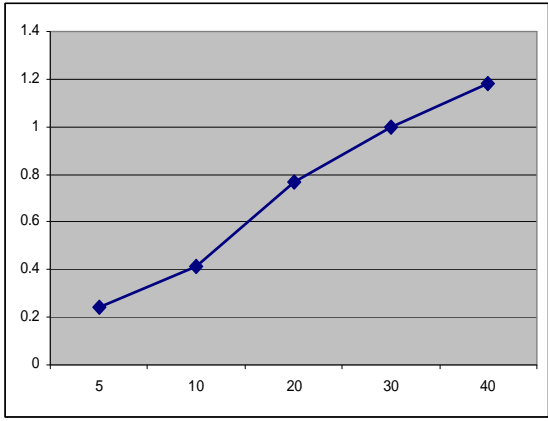
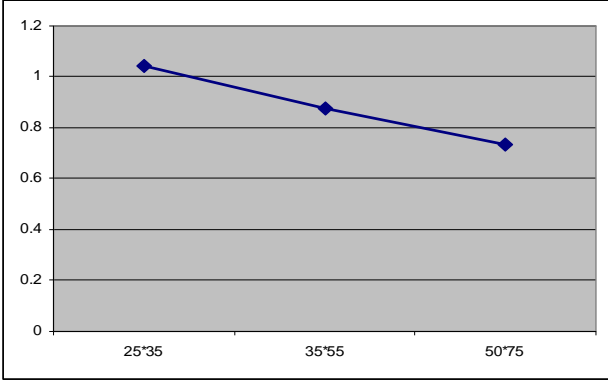
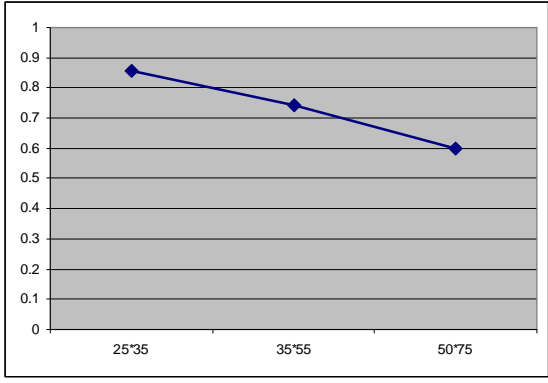
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5. Results

Based on the previous graphs for the 4 mentioned structural systems, the results are concluded here.

SH_n (n=1-13) models

According to the results for this part, table.1, $0.24 < \Delta < 2.58$ and $0.16 < \delta_{\Delta} < 1.19$. The extreme amount for this parameter shows that in this part, the diaphragms are mostly flexible. This could be because of rigid behavior of shear walls in this system.

MC_n (n=1-11) models

Based to the results for this part, table.2, $0.005 < \Delta < 0.064$ and $0.017 < \delta_{\Delta} < 0.098$. The models for concrete frames demonstrate more rigid diaphragms rather than shear wall models. The reason lies on the fact that slab rigidity is more than columns stiffness.

6. Conclusion

In this paper the effect of structural lateral load bearing systems on rigidity of concrete slabs is investigated. The results show that for a shear wall resisted structure the assumption of a rigid diaphragm is not valid. So designers should consider it as a flexible diaphragm in their designations.

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11/10/2012

Investigation of Relationship between Personality Characteristics with Dependence on Chat among StudentsBehnoush Molavi¹, Leila Pashaei² (Corresponding author)¹ MSc of personality psychology, Islamic Azad University, Science and Research Branch of Tehran, Iran.² MSc of General Psychology, Islamic Azad University, Science and Research Branch of Tehran, Iran.

Abstract: Purpose of this study was investigation of relationship between personality characteristics with dependence on chat among high school female students. Based on research project correlation form, 270 high school senior female students in the field of Mathematics – physics in the 6th district of Tehran were selected by multi-stage random sampling. Tools for implementation of this research were: a) Neo personality inventory research; b) Young inventory. In order to analysis of data, description statistics (average and standard deviation) and inferential statistics (correlation and regression) were used. Results show that: (1) Personality features are significant in explaining dependence to the chat ($p < 0.001$). (2) Among personality features, temperamental neurotic had a significant positive relation with dependence with chat ($p < 0.005$). (3) The effect of personality consistency, openness and extraversion on dependence to the chat was positive but with low impact and also was not significant ($p > 0.005$). (4) Although there was negative relationship between personality characteristics of responsibility and chat dependency, but it was not statically significant ($p > 0.005$).

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Keywords: personality characteristics, chat, dependency to chat.

Introduction:

Human beings had used different methods to contact with their fellow human beings at different ages. Using smoke, symbols (verbal, written), telephone, telegraph and also computer and Internet in recent century, all suggest the need to establish a relationship with fellow human beings (Farhangi, 2006) [4].

Internet, the emerging phenomenon of the last century, today is the obsession of many teenagers and young people. From 1992 which this technology was entered to Iran to today, tremendous growth from center to the far distance region is obvious. Coffee nets and Internet institutes grow rapidly which in every few steps, we can see one of them and it shows unique welcome of Iranian people to this technology (Welis, 2007) [5].

Although the statistics presented in the case of Internet use in Iran is far from the global statistics, But the Internet, computer games and chat are the main interests of many of Iran's youth. Countless parents ask this question that should they be concern with their children due to use Internet and chat? They concerned with some issues of this technology. Positive and negative reflections on the use of this technology have given them, (Effects that dependency to chat may have on personality features of students), put them in a conflict that finally allowed their child to sit behind the screen some times of day and stay away from the real world or not?

Nowadays, chat increased among youth people which psychologists have suggested the term addiction to chat (Farhangi, 2006) [4]. Young (2001) represented following symptoms for chat addicts based on extensive researches [13]:

1. Those who are connected to the network every day for several hours.
2. After connection to the network, lose time control.
3. They go out home very few and spend most of their time behind computer.
4. They spent little time for personal work and even eat their food in front of the monitor.
5. They deny that spend much their time in the network.
6. They check their e-mail several times a day.
7. They believe that have the best and most popular personal page.
8. They feel relieved and relaxed when communicate with their online friends.
9. They lose their jobs and other social responsibilities.
10. They do not attention to their health and physical appearance.
11. They feel bad when they are off-line (Young, 2001) [13].

It seems that people, who addict to chat, involve in pathological behavioral disorder which is same to compulsive behavior disorder. Results show that addiction to the chat is similar to the pathological gambling and had the same features and consequences. Addiction disorder to the chat is a new

clinical disorder which could be a new concern about mental health (Young, 2007) [12].

Chat with online friends in chat rooms would stimulate teenagers and young people to unlimited use of Internet. Also, Young (2007) believes that Internet vulnerabilities are just bored, depressed, introverted and without self-esteem, which may also have a history of addiction to other drugs [12]. Problems of each individual life and character and mentality of each person is effective on how to use the Internet and time which they spent (Yang, 2007) [12].

A new growing concern is optional network obsession that is include compulsive gambling in Internet, compulsive use of sites or sexy chat rooms, online auctions and extreme chat. These behaviors are highly addictive and person who have these compulsive behaviors is known as an addicted person to the Internet and chat (Mckena, 2002) [6].

Personality is a basic concept in psychology. Since personality involves all aspects of physical and emotional-mental dimensions, it can be said that behavior science efforts are in line with it. Overall, a true define of general features of personality in routine life refers to stable characteristics over time which do not change from a position to the other position and refers to person's essence. In general, behavior is influenced by the characteristics and cognitive and emotional abilities. To predict the behavior of these features, they should be closely examined (Haghshenas, 2009) [1]. Trait is feature or quality of distinguishing of personality. In our daily life, whenever we describe the personality of person that we know him/her, most time we follow trait approach and we tend to select features or prominent factors, in order to use it to summarize whole person's features in some words. This widespread define means that traits applied by three main approaches: it's possible to use them for summarize, prediction and explain of person's behavior (schultz, 1998) [3].

Some research which carried out in field of dependence to chat, suggest that chat has relationship with personality characteristics.

Jackson et al (2003) were examined the relationships between personality, cognitive style and use of the Internet in a longitudinal study [4]. Results indicated that extraversion and neurosis were associated with Internet use, but only during the first 3 months of home Internet access.

In research on Internet addiction, Tonioni et al. (2011) were discussed disadvantage of the use of Internet with long hour's online and avoiding interpersonal relationships in the real world as important criteria for the diagnosis of Internet addiction [10]. Lost communication with real people with trauma symptoms such as anxiety and

depression lead to the identification of users addicted to the Internet (Tonioni, 2011) [10].

In other research, Rayan and Xenos (2011) were examined personal impact of use or non use of social network Facebook. The results showed that Facebook users are more eccentric and narcissistic and less conscientious and also they feel less social isolation. Another finding was that Facebook users in terms of personality traits such as neurosis, loneliness, shyness and self-infatuation are different [8].

Gulliver and Ghinea (2009) examined the relationship between cognitive styles, user character and quality of multimedia perception (video, projector, TV, computers), began. Results showed that type of personality and user cognitive style affects on students' information uptake levels, their achievement in perception and also on confidence level [2].

Ranjbar (2009) examined the relationship between chat dependency and mental health of high school female students in Tehran. The research findings show that the people who spent more time to chat, their mental health and social communication would reduce [2].

Rahmani and Lavasani (2011) had investigated the Internet dependence prediction and seeking the sensation feel (subscale extroversion) in the five major personality traits and gender. Results show that there is positive significant relation between Internet dependence and seeking sensation feel and also there is negative significant relation between Internet dependence with satisfaction and loyalty [7].

Landers and Lounsbury (2004) studied the relationship between the uses of Internet and chatting with three features of 5 main characters. Results showed that using the Internet has a negative relation with three features of 5 large characters (pleasant, duty, extraversion) [3].

Saade, Kira, Nebebe and Otrakji (2006) showed that informational behavior of Internet users could be related to the experience of five major characteristics. This means that high score users openness of character test had a significant relation with the internet users [9].

According to abovementioned research, this study examined the relationship between personalities characteristics with dependency to chat was formed among students. The research hypotheses are:

1. There is relation between neuroticism (N) and dependency to chat.
2. There is relation between Extraversion (E) and dependency to chat.

3. There is relation between openness to experience (O) and dependency to chat.
4. There is relation between Appealing (A) and dependency to chat.
5. There is relation between Conscientiousness (C) and dependency to chat.

Based on our hypotheses, question is:

Do personal features have any role in dependency to chat or not?

Research Methods

Community, sample and sampling method

This research is descriptive and correlation type. Statistical society was formed from all high school senior female students in the field of Mathematics – physics in the 6th district of Tehran which were been studying 2011-2012. 270 students were selected by multi-stage random sampling.

Research Tools

1. NEO personality trait test

To evaluate the different type of personality which is personality trait, the short form of 60 questions in NEO BIG FIVE questionnaire and 60 questions type (short form) were used which 12 questions for each factor is considered and some of these questions had positive relation and some of them had negative relation with considered factor. Scoring was based on Likert method which for each trait, range from 1 to 5 was applied and 1= completely disagree, 2= disagree, 3= neutral, 4= agree, 5= completely agree. Thus, for each factor, the fewer score is 12 and the most score is 60. It means that if someone gives score 1 to all 12 questions related to each factor, 12 scores would obtain and if someone gives score 5 to all 12 questions related to each factor, 60 scores would obtain.

The reliability of the short form of questionnaire was 0.75-0.83 which determined by Mc cary and costa (1983) and its long questionnaire in the scales of neuroticism, extraversion and openness to experience was 0.68-0.83 and two factor of appealing and deontology (consciousness) was 0.79 and 0.63 respectively [5]. In this research, reliability of questionnaire calculated by Cronbach's alpha in was 0.78.

2. Young dependency to chat test (1996)

This questionnaire contains 8 questions and each question has two options (yes and no). If student mark "yes" to 5 or more than 5 questions, this student is addicted to the internet. In this research, Young questionnaire was used to evaluate the dependency to Internet and chat. Validation of questionnaire was 0.85, based on Cronbach's alpha.

Findings:

a) Data Description

Results of table 1 show that among personality features of subscales, extraversion has the highest average which was 41.79 and conscientiousness has the lowest average which was 29.97. This means that among all people in statistical sampling, amount of extraversion is more than other subscales.

Table 1: statistical characteristics of personality subscale features

Variable	Subscale	Average	S.D.
Personality Features	Neuroticism	29.97	4.88
	Extraversion	41.79	4.30
	Openness to experience	37.75	4.13
	Appealing	38.93	4.71
	Conscientiousness	40.93	3.93

Dependency to chat

Statistical characteristics of dependency to chat are highest, lowest, average and standard deviation of statistical sample in Table 2.

Table 2: statistical characteristics of dependency to chat

Variable	Lowest	highest	Average	S.D.
Dependency to chat	0	8	1.5	1.9

Results of Table 2 show that total average of dependency to chat is 1.5 and its standard deviation is 1.9. Based on cut-off point, people were divided in two groups (persons who dependent or non-dependent to chat) and results of this division are represents in Table 3.

Table 3- frequency distribution and percent of statistical sample (persons who dependent or non-dependent to chat)

Dependency to chat	Frequency	Percent
No	194	71.9
Yes	76	28.1
Total	270	100

Table 3 shows that 71.9 percent of students had not any dependency to chat and 28.1 percent had significant dependency to chat.

b) Data analysis

Table 4 showed that amount of correlation coefficient (R) between personality characteristics and dependency to chat is 0.281 and explains 7.9 percent of variable variance of dependency to chat.

Table 4: indexes and regression analysis statistics between personality features and dependency to chat

Correlation coefficient (R)	Explanation coefficient (R Square)	Adjusted coefficient	Error of standard estimation
0.281	0.079	0.062	1.87

In table 5, F is 4.54 and *df* is 5 and also personality features to define dependency to chat is significant ($p < 0.001$).

Table 5: summary of regression analysis of dependency to chat through personality features

Index source	Sum of squares	Degrees of freedom (<i>df</i>)	Mean square	F	Sig.
Regression effect	79.825	5	15.973	4.54	0.001
Residual effect	928.909	264	3.519		
Total	1008.774	269	---		

Results of analysis and β amount of Table 6 show that among personality features, temperamental neurotic had a positive and significant relation with chat ($p < 0.005$).

Also, according to standardized coefficient regression division (Beta) in Table 6, it can be concluded that the effect of personality consistency, openness and extraversion on dependency of people to chat is positive, but its effect was small and not statistically significant ($p > 0.005$).

However, there is negative relation between personality features of responsibility and dependency to chat, but it's not significant statistically ($p > 0.005$).

Table 6: variables which entered to regression equation

Index parameter	β coefficient		Standardized β coefficient Beta	t ratio	Significant t level
	B	Standard error			
Constant amount	-2.188	1.931	---	-1.133	0.258
Responsibility	-0.026	0.032	-0.053	-0.81	0.419
Compatibility	0.04	0.025	0.098	1.582	0.115
Openness	0.014	0.029	0.031	0.495	0.621
Extraversion	0.002	0.029	0.006	0.087	0.931
Temperamental neurotic	0.087	0.026	0.218	3.294	0.001

Explanation of research hypotheses

Results of this research showed that: 1. personality features are significant in explanation of dependency to chat ($p < 0.001$). 2. Among all personality features, temperamental neurotic had a positive and significant relation with dependency to chat ($p < 0.005$). 3. Impact of personality features such as compatibility, openness and extraversion on chat is

positive, but has a low impact and is not significant statistically ($p > 0.005$). 4. However, there is negative relation between personality features and dependency to chat, but it's not significant statistically ($p > 0.005$).

Findings of this research are accordance with findings of Jackson et al. (2003), Tonioni et al. (2011), Ryan and Xenos (2011), Ranjbar (2009), Rahmani and Lavasani (2011), Landers and Lounsbury (2004), Saade, r. g., Kira, d., Nebebe, f., & Otrakji, c. (2006).

Chat is a media tool which helps to have extensive communication. Extensive communication in virtual world, would help to people to familiar with factors associated with communication with other people. This kind of learning would be generalized with real world. Many students put away shyness by chat and think to have more communication. Excessive use of chat would lead to kind of separation from real world for students and spent more time in virtual world and more be secluded and do not attend in community (Ranjbar, 2009).

One of the main effects of chat is chat addiction which we investigate it based on pathological dimension. Symptoms such as obsessive thoughts about the Internet, to reduce the incentive to control appetite, inability to stop the use Chat and their sinking (withdrawal), as non-healthy features of the chat is quoted (Young, 2007).

Probably dependent people to chat would express anxiety, anger and impulses through their free speech in virtual environments such as chat rooms. In addition to the mentioned negative feelings, lack of compliance with the surroundings is probably more in addicted people to chat. Maybe temperamental neurotic people do not like to have extensive social relation or do not have ability to do it, so they selected chat as a way to have a limit and controllable relation with other people. A new published study in ABC site suggested that 80 percent of users which have significant dependency to chat, spent much of their time in chat rooms to escape from negative emotions, accelerating to reach legal adulthood, intimate relationships and express their feelings without embarrassment (Ranjbar, 2009).

If people were more dependent on outward-oriented chat, had a chance to chat as well, more social ties and establish more relationships because eliminating the need for their interaction, the rate for unattached people to chat reduce the compactness. On the other hand, people are less outward-oriented and some are inclined toward introspection, they more enjoy from individual activities and working with computers. Participants in this study showed less intention to introversion because of over use of chat rooms.

Since many students in this research are students, their curiosity and enjoyment of the unusual things can also be specific to this age group, as dependency to chat can not have a significant effect on personality difference.

It seems that students, who are dependent to chat, select the chat rooms as an unpleasant environment for expressing emotions. They express pleasant and unpleasant sensations in the virtual space comfortably and the comfort of their compatibility with the surrounding environment would be helpful. On the other hand, the dependence on chat shows positive approach to activities in virtual spaces. Persons, who receive a lower score in the pleasant, would be cynical and skeptical and they seem to compete with other people. It's possible that addicted people to chat show contrary traits due to obtain high score in pleasant scale. Chat like other technological phenomena, is a neutral technology and has positive and negative effects on the target user.

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Real-time Quantitative PCR Monitoring of Antioxidant Enzyme Gene Expression in Wheat Radicles Treated With Cu^{2+} and Cd^{2+}

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Abstract: Real-time quantitative PCR was used to study the differential expression of three antioxidant enzyme genes copper/zinc-superoxide dismutase (Cu/Zn-SOD), peroxidase (POD) and Glutathione S-transferase (GST) – in winter wheat (*Triticum aestivum L.*) radicles following treatment with two heavy metals (Cu and Cd). The effects of varying the concentration of the heavy metals and the duration of exposure were investigated. It was found that Cd stress has a more profound effect than Cu on antioxidant gene expression for all tested mass concentrations and that heavy metal exposure induces GST expression more strongly than that of POD or Cu/Zn-SOD, with POD being expressed more strongly than Cu/Zn-SOD.

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Key words: antioxidant enzyme genes; heavy metals stress; real-time quantitative PCR.

Abbreviations:

AOS	active oxygen species
Cu/Zn-SOD	copper/zinc-superoxide dismutase
GSH	glutathione
GST	glutathione S-transferase
H_2O_2	hydrogen peroxide
$\cdot\text{OH}$	hydroxyl radicals
POD	peroxidase
ROS	reactive oxygen species

1. Introduction

During growth and development, a plant has to cope with a range of different internal and external stresses, so its ability to adapt to metabolic and environmental changes is essential for survival. Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($\text{O}_2^{\cdot-}$) and the more toxic hydroxyl radicals ($\text{OH}\cdot$) and singlet oxygen ($^1\text{O}_2$), are produced continuously during plant growth and development, but their abundance increases when plants are exposed to various biotic and abiotic stresses (Elstner, 1982; Asada, 1994; Dat et al., 2000). These toxic ROS oxidize proteins, unsaturated fatty acids and DNA, causing cellular damage and cell death.

Plants have a number of different defense mechanisms by which they respond to oxidative stress. These include producing non-enzymatic antioxidants such as ascorbate and glutathione, and enzymatic antioxidants such as catalase, superoxide dismutase and ascorbate peroxidase. If these defenses fail to protect the plant from the ROS, cell death will result. Several heavy metals have become widely distributed in the environment due to human activities such as mining and the disposal of garbage and sewage sludge in field sites. In terms of their

environmental impact, the most important of these heavy metals are cadmium, zinc, copper, and lead. However, the accumulation of large amounts of any heavy metal in the organism as a whole or in specific organs can cause significant damage (Clemens and Krämer 2003).

Heavy metal stress is one of the major abiotic stresses affecting germination, crop growth and productivity. In nature, plants encounter a number of biotic and abiotic stress factors simultaneously, including drought, heat, shock and heavy metals. Copper is an essential trace mineral that is present in almost all living organisms; it is a cofactor that is required to maintain the structural and catalytic properties of various enzymes. Cadmium has a wide range of toxic effects, which are exacerbated by its long biological half-life and low excretion rate (Tully et al. 2000). Its acute toxicity in mammals has been attributed in part to its ability to induce oxidative stress. In plants, Cd affects photosynthesis, respiration and nitrogen metabolism, and causes oxidative damage similar to that observed in mammals (Dixit et al. 2001).

Under optimal conditions, cellular homeostasis is achieved by the coordinated action of several biochemical pathways. Stress factors can

have different effects on these pathways and their coordination; in plants, this can change the flow of metabolites through the various homeostatic pathways. Metabolic activity results in the continuous formation of reactive oxygen species (ROS), and their abundance increases under stress conditions. This increase is accompanied by the activation of defense genes whose products have a variety of functions that may include ROS scavenging. ROS such as the superoxide radical (O_2^-) cause oxidative damage to various cellular components such as membrane lipids and oxidation-sensitive enzymes, affect vital processes such as the synthesis and denaturation of proteins, and can induce mutations in DNA. Cells therefore produce a number of protective enzymes that are activated in response to oxidative stress. These include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and Glutathione S-transferase (GST). Glutathione-dependent enzymes and metabolites are useful target species for biomonitoring of oxidative damage and the effects of ROS because glutathione is involved in phytochelatin biosynthesis (Rausser 1990) and glutathione S-transferases (GST, Habig et al. 1974; Schröder and Berkau 1993) are potent detoxification enzymes, catalyzing the nucleophilic attack of reduced glutathione on electrophilic pollutant molecules and products of oxidative stress.

This paper describes an investigation into the activity of antioxidant enzymes under various conditions and the expression of the corresponding genes in the wheat radicle. The results obtained provide increased understanding of plant responses to Cu^{2+} and Cd^{2+} stresses and to stressful environmental conditions in general, and will be useful in future crop engineering programs aimed at adapting crops to challenging environments and increasing their agronomic value.

2. Materials and Methods

Sowing and heavy metal treatments

The crop examined in this work was the "Aikang 58" variety of winter wheat (*Triticum aestivum*). Seeds of uniform size were selected, washed with distilled water and treated with 0.1% mercuric chloride (w/v) for 5 min. The seeds were then thoroughly washed with deionized water and sown in petri dishes (100 seeds per dish) lined with filter paper and precultured for 72h. Sets of Cu and Cd solutions containing 5 mg/L, 30 mg/L, and 60 mg/L of the metal salt were prepared by dissolving copper sulfate ($CuSO_4 \cdot H_2O$) or $CdCl_2$ in tap water; control experiments were conducted using tap water alone. The precultured seeds were then soaked in tap water or the appropriate metal ion solution and left to grow for 24h, 48h, 72h, or 96h before subsequent

experimentation.

Total RNA extraction and cDNA synthesis

0.1g of wheat radicles were ground in liquid nitrogen. Total RNA was extracted using the RNAiso Plus kit (TaKaRa/Invitrogen, Japan) according to the manufacturer's instructions. All preparation and handling steps involving RNA were performed in a laminar flow hood, under RNase-free conditions. RNA was used to generate single-stranded cDNA by reverse transcription using an oligo-dT primer and the ABI PCR System (Applied Biosystems, Foster City, CA, USA) in conjunction with the PrimeScript™ RT-PCR Kit (TaKaRa, Japan). Reverse transcription was performed according to the kit manufacturer's instructions.

Design and identification of qRT-PCR primers

Real-time PCR primers for the amplification of Cu/Zn superoxide dismutase (SOD), peroxidase (POD) and Glutathione S-transferase (GST) were designed based on the wheat gene sequences in GenBank using the Primer Primer 5.0 software (Premier Biosoft International, Palo Alto, CA, USA), along with a primer for the 18S rRNA as a control. The primers used to quantify the mRNA levels of the genes of interests were given in Table 1. The mRNA sequences of *Cu/Zn-SOD*, *POD*, *GST* and *18S rRNA* were obtained from the following GenBank accession numbers: TAU69536, X56011, AJ441055 and AJ272181, respectively. The extracted RNA samples were subjected to DNA-free treatment to avoid genomic DNA contamination, and amplified PCR products of all four genes were sequenced and blasted to ensure that the correct mRNA sequences were quantified.

Quantitative real-time RT-PCR (qRT-PCR)

QRT-PCR was performed using the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) in conjunction with the SYBR Premix Ex Taq™ kit (TaKaRa, Japan), using the procedure specified by the kit manufacturer. Each sample was split into three separate sub-samples and three reactions were performed in parallel. A standard two-step amplification procedure was carried out as follows: 95°C for 30s, then 95°C for 5s and 60°C for 34s go-around 35 cycles. Following amplification, the samples were subjected to the dissociation protocol. Each sample was done in triplicate.

Statistical analysis

The expression data were analyzed using the SPSS 11.5 statistical package for Windows (SPSS Inc., USA). Analyses of variation were computed for statistically significant differences determined using the appropriate F-tests. Results are presented as the means \pm SD of at least three independent replicates. Mean differences were compared utilizing Tukey's

test at $P < 0.05$.

3. Results

Quantitative analysis of *Cu/Zn-SOD* mRNA expression following Cu and Cd treatment

To examine the ability of different heavy metals to induce transcription of *Cu/Zn-SOD* gene, wheat radicles were exposed to aqueous solutions of Cu or Cd salts containing 0, 5 mg/L, 30 mg/L, or 60 mg/L of the metal salt. Quantitative RT-PCR analysis indicated that Cd stress induced significantly stronger expression of the *Cu/Zn-SOD* gene than did Cu stress at all tested mass concentrations, as shown in Figure 1. For both metal salts, the level of *Cu/Zn-SOD* expression decreased over time following exposure, with the lowest levels being observed after 96h. For all treatments, the level of *Cu/Zn-SOD* expression was significantly lower than that in the control and was reduced by exposure to higher mass concentrations of the metal salt.

Quantitative analysis of *POD* mRNA expression following Cu and Cd treatment

POD is important because it prevents the accumulation of H_2O_2 , which oxidizes membrane lipids to malondialdehyde (MDA). It thus plays a vital role in maintaining the integrity of the cell membrane. As shown in Figure 2, quantitative RT-PCR analysis revealed that Cu stress reduced the expression of the *POD* gene significantly more than did Cd stress. Treatment with either of the two heavy metals caused a rapid decrease in *POD* expression compared to the control, with levels beginning to increase towards the end of the treatment. Treatment with low mass concentrations of Cu caused *POD* gene expression to remain consistently low throughout the experiment whereas treatment with higher mass concentrations caused *POD* expression to increase towards the end of the treatment; the lowest level of *POD* expression observed occurred with the 30 mg/L treatment, 72 hours after exposure. Cd stress caused *POD* expression to decrease initially but in most cases, it then began to increase later in

the experiment – typically, after 48 hours. The lowest level of *POD* expression was observed after treatment with the 5mg/L Cd solution.

Quantitative analysis of *GST* mRNA expression following Cu and Cd treatment

As shown in Figure 3, *GST* gene expression increased in response to treatment with either Cu or Cd. The abundance of *GST* mRNA in treated wheat radicles increased significantly between 24h and 48h after treatment for all concentrations of Cd (by a factor of 1.5-2 at 24h and 1-3 at 48h). Cu exposure caused rapid increases in the abundance of *GST* mRNA, especially following treatment with the 60mg/L and 30mg/L solutions. In general, *GST* gene expression was initially reduced by treatment with the metal salts but began to recover as the experiment proceeded.

4. Discussion

In this work, wheat radicles were treated with solutions of Cu and Cd salts in order to investigate the expression of genes encoding antioxidant enzymes following heavy metal stress. In general, copper salts had more pronounced effects on the expression of the *Cu/Zn SOD*, *POD*, and *GST* genes than did cadmium salts. In addition, *GST* was expressed more strongly than *POD*, which in turn was expressed more strongly than *Cu/Zn-SOD*.

There seem to be species-specific differences in the induction of anti-oxidant gene expression following heavy metal stress: in some plants, primary defense genes such as *SOD*, catalase, and *POD* are expressed more strongly whereas in others, the main response involves glutathione-dependent enzymes. In the case of wheat, it seems that some of its detoxification capacity – specifically, that originating from glutathione-based enzymes – is initially employed to deal with heavy metal stress but is quickly reallocated for other purposes. It has previously been reported that glutathione S-transferases generally respond strongly to heavy metal exposure (Noctor et al. 2002).

Table 1. Genes targeted for expression profile analysis and primer sequences for their cDNA

Gene	Acc.no	Primer sequence	Product (bp)
18SrRNA	AJ272181	F-TTAGTTGGTGGAGCGATT R-TGTTATTGCCTCAAACCTCC	145
Cu/Zn-SOD	TAU69536	F-CGATAGCCAGATTCCTTTG R- AACCAGCGACCTACAACG	176
POD	X56011	F-CAGCGACCTGCCAGGCTTTA R-GTTGGCCCGGAGAGATGTGG	196bp
GST	AJ441055	F-GGAGCACAAGAGCCCCGAGC R-CGGGTTGTAGGTGTGCGCGT	217bp

Note: PCR primers were designed for three antioxidant genes (*Cu/Zn-SOD*, *POD* and *GST*) and one housekeeping genes (18S rRNA).

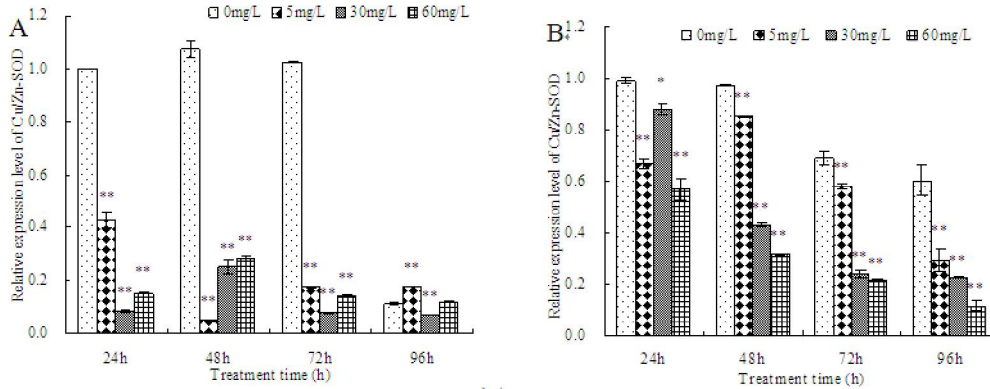


Figure 1. Quantitative real-time PCR data on the number of mRNA transcripts of the *Cu/Zn-SOD* gene in wheat radicles treated with 0 (control), 5, 30, or 60mg/L of Cu (A) or Cd (B) for 24h, 48h, 72h, 96h. The values reported are means \pm SD (n=3). Single asterisks indicate significant deviation from the control and double asterisks indicate extremely significant deviance form the control (P<0.05) as determined by ANOVA followed by a multiple range test (LSD) with respect to duration of exposure.

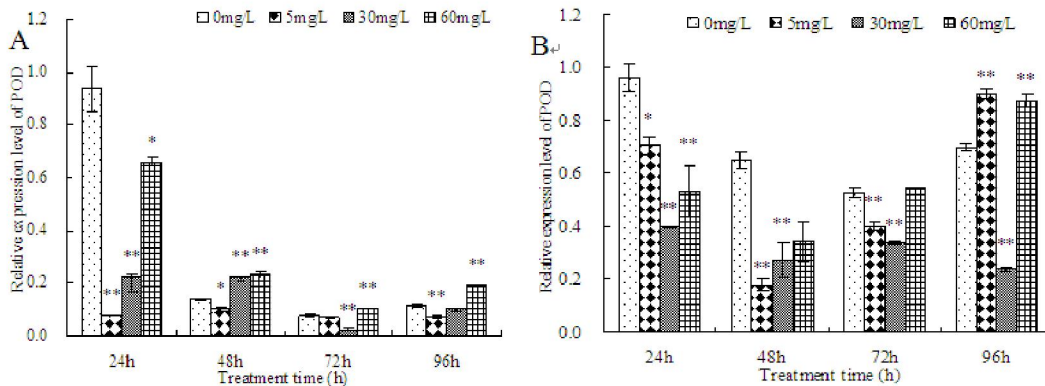


Figure 2. Quantitative real-time PCR data on the number of mRNA transcripts of the POD gene in wheat radicles treated with 0 (control), 5, 30, or 60mg/L of Cu (A) or Cd (B) for 24h, 48h, 96h. The values reported are means \pm SD (n=3). Single asterisks indicate significant deviation from the control and double asterisks indicate extremely significant deviance form the control (P<0.05) as determined by ANOVA followed by a multiple range test (LSD) with respect to duration of exposure.

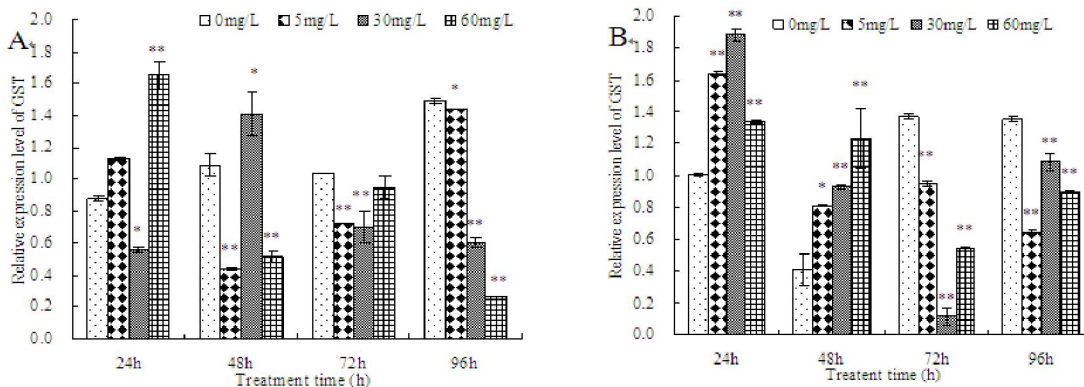


Figure 3. Quantitative real-time PCR data on the number of mRNA transcripts of the GST gene in wheat radicles treated with 0 (control), 5, 30, or 60mg/L of Cu (A) or Cd (B) for 24h, 48h, 72h, 96h. The values reported are means \pm SD (n=3). Single asterisks indicate significant deviation from the control and double asterisks indicate extremely significant deviance form the control (P<0.05) as determined by ANOVA followed by a multiple range test (LSD) with respect to duration of exposure.

Long-term exposure to heavy metals can affect plant physiological processes and reduce cellular control over the formation and destruction of ROS (Srivalli et al., 2003). Plants have well-organized antioxidative defense systems comprising enzymatic and non-enzymatic antioxidants to scavenge ROS. The cooperative functioning of antioxidants such as SOD, POD and GST plays an important role in scavenging ROS and maintaining the organism's physiological redox balance (Wise, 1995; Foyer and Nector, 2000; Cho and Seo, 2005). Under normal growing conditions, oxidative damage to cellular components is minimized by efficient elimination of ROS. However, if ROS production exceeds the capacity of the cell's antioxidant systems, damage will start to accumulate.

The superoxide dismutases (SODs) are a family of metalloenzymes with a range of different isoforms, including Cu-Zn-SOD, Mn-SOD and Fe-SOD. They are present in almost all cells that are exposed to oxygen and are the primary scavengers of O_2^- radicals (Alscher et al., 2002). To determine how SOD expression is affected by heavy metal stress, we monitored the abundance of Cu/Zn-SOD mRNA in wheat radicles that had been treated with Cu or Cd. Our results indicate that there were significant differences in Cu/Zn-SOD expression between groups and that heavy metal exposure reduced the abundance of Cu/Zn-SOD mRNA relative to the control group (Figure 1). This demonstrates that heavy metals can affect SOD levels in wheat plants at both the molecular and the cellular levels.

Peroxidases (PODs) are widely distributed in the plant kingdom and play a major role in eliminating active oxygen species (AOS). They catalyze the oxidation of a sacrificial substrate by H_2O_2 . A previous study investigated changes in the expression profiles of 10 POD genes in sweet pea following exposure to air pollutants and UV radiation (Kim et al., 2007). It was found that certain POD genes played specific roles in defending against oxidative stress. In another study, it was demonstrated that treating plants with three heavy metals – Cd, Cu and Zn – affected POD expression and that the magnitude of the effect varied depending on the duration of exposure (Kim et al., 2010). The results obtained in the current study suggest that POD enzymes are important in protecting the wheat radical from oxidative stress induced by heavy metal treatment (Fig. 2). Significant changes in POD expression were observed irrespective of the type of metal applied; while the response to Cu stress was more pronounced than that induced by Cd, both metals resulted in substantial changes compared to the control. POD destroys H_2O_2 by oxidizing various hydrogen donor molecules and thus protects plant

tissues that have been exposed to direct oxidants or heavy metals (Wang and Yang, 2005; Song et al., 2007; Xue et al., 2008). The abundance of POD mRNA may therefore be a useful indicator of damage to plant tissues caused by heavy metal exposure.

The glutathione S-transferases (GSTs) are dimeric and multifunctional enzymes that are ubiquitous in aerobic organisms (Edwards et al., 2000). GSTs play a crucial role in detoxifying xenobiotic compounds in cells by catalyzing the nucleophilic attack of the thiol group (SH) of reduced glutathione (GSH) on diverse electrophilic molecules, including herbicides, insecticides, carcinogens and other xenobiotics (Pascal et al., 1998; Edwards et al., 2000; Yin et al., 2008). They play a central role in the antioxidant defense mechanisms of both eukaryotes and prokaryotes. Exposure to environmental stress (acidic pH) has been shown to induce GST expression in shrimp. Moreover, it has been reported that cadmium exposure affects the expression of a number of GST isoforms in the river puffer fish *T. obscurus* (Kim et al., 2010). Our results suggest that GST is one of the main antioxidant components in the responses to Cu and Cd exposure in wheat, since its expression increased significantly following treatment. For all metal treatments examined in this work, the level of GST expression was substantially higher than in the control in the 48 hours following the initiation of the treatment and then began to gradually decrease. This suggests that GST plays an important role in detoxifying heavy metals in wheat radicles.

The results obtained in this work demonstrate that antioxidant gene expression following exposure to Cu and Cd varies over time and depends on the concentration of the metal salt. The origins of the differences between the two metals in terms of gene expression are unclear and there are several factors that should be considered when searching for explanations. First, we observed differences in the responses of antioxidant genes to different superoxide-generating compounds, although these were relatively minor; in general, the responses to the two metals were similar for all three genes considered. Second, antioxidant gene expression is controlled by a wide range of different promoters and control elements. The number, order, and types of protein binding sequences present in promoters play a major role in determining gene expression patterns. Third, cadmium is very toxic towards a wide variety of species, affecting the behavior, growth, and physiology of many plants (Liu et al., 2007). Cd has multiple effects on cells and can interfere with cell cycle progression, proliferation, differentiation, DNA replication and repair, and apoptotic pathways (Bertin et al., 2006). It also induces oxidative stress

by increasing the concentration of the superoxide anion and hydrogen peroxide in cells (Szuster-Ciesielska et al., 2000). Cadmium ions (Cd^{2+}) can bind to free thiol (SH) groups in proteins, cysteine, and glutathione, and inhibit the functions of these biomolecules. It seems that Cd can both induce and inhibit GST activity, depending on its concentration (Yano et al., 2005). Moreover, our results indicate that the duration of Cd exposure has a significant effect on the expression of three antioxidant genes (Cu/Zn-SOD, POD, and GST). The expression of all three genes considered in this work increased following Cd exposure.

Copper is an essential trace mineral that is present in almost all organisms. It functions as a cofactor and is required for the structural and catalytic properties of various enzymes. Cells must balance their need for small quantities of copper against its toxic effects when present in excessive quantities. Cu deficiency decreases the activities of enzymes involved in oxidation defense systems and also alters the cellular abundance of ROS scavengers, such as metallothioneins and glutathione (Uriu-Adams et al., 2005). Excessive Cu levels cause ROS formation by promoting hydroxyl radical formation. These are the most strongly oxidizing ROS and are highly toxic (Gaetke et al., 2003). Our results indicate that Cu/Zn-SOD and POD expression are less strongly induced by Cu exposure than is that of GST, which suggests that ROS generated by Cu exposure are primarily detoxified by glutathione peroxidase.

The findings obtained in this work constitute an important contribution to our understanding of plants' responses to stress factors and provide some baseline information on the cascades or networks of events that are triggered by heavy metal stress. In the long run, unraveling the stress response mechanisms of plants will be extremely useful because it will expand our understanding of gene regulation in all eukaryotes and may eventually allow us to design or adjust mechanisms that regulate gene expression to create crops that are better adapted to challenging environments and have increased agronomic value.

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