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# Study of bis{2-(naphtha [3,4]imidazol-2-yl) quinolinato} Magnesium

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**Abstract:** The study of bis{2-(naphtha[3,4]imidazol-2-yl) quinolinato} Magnesium (MgNIQ) is presented in this report. It was observed the decomposition temperature is high to 577℃ but no melting transition ($T_m$) of MgNIQ up to 450℃. By using of MgNIQ as emitted layer exhibits a broad maximum spectrum peak at 615 nm. The color of the emitted light is in the orange-red region in the CIE coordinate of x = 0.36 y = 0.53.
[Yu-Feng Lin, Pin-Wen Cheng, Shih-Hsuan Chiu, Chen-Hao Wang, Shung-Jim Yang, Anchi Yeh. **Study of bis{2-(naphtha [3,4]imidazol-2-yl) quinolinato} Magnesium.** Life Science Journal, 2012;9(2):307-310] (ISSN:1097-8135). http://www.lifesciencesite.com. 48.

**Keywords** Electroluminescence; red light; device; bis{2-(naphtha [3,4]imidazol-2-yl) quinolinato}

## 1. Introduction

Luminescent organic/organometallic compounds have attracted much attention recently because of their potential applications in electroluminescnt (EL) displays [1-5]. Organic and polymer devices provide advantages over their inorganic counterparts, such as high luminous efficiency and fine-pixel formation. Luminescent chelate complexes have been shown to be particularly useful in electroluminescnt (EL) displays because of their relatively high stability and volatility. The most well-known example of such chelate compounds is $A1q_3$, not only a good emitter but also a highly efficient electron-transporting material, where q is the 8-hydroxyquinolinato ligand [6, 7]. Via the modification of the ligand of metal chelate compound, the emission color of a metal chelate compound may be tuned. Other properties, such as thermal stability and carrier mobility, may also be improved upon. In the present work, we report the synthesis and electroluminescent (EL) property of bis{2-(naphtha[3,4]imidazol-2-yl) quinolinato} Magnesium (MgNIQ). The attachment of the naphtha[3,4]imidazol group at 2-position would allow the ligand to form stable complexes with metal ions similarly to 8-hydroxyquinoline. Therefore, the thermal stability, an important character for the practical application in the electronic fields, of this metal complex is investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The organic emitting device using MgNIQ as emitting layer has been fabricated to study the electroluminescent property of this metal complex.

## 2. Experimental

The synthesis of the title compound was accomplished by following processes, as shown in Scheme 1. The dibutylmagnesium solution (0.5M in heptane, 0.6927mL, 10mmol) was slowly added to 100 ml of THF solution containing 2-(naphtha[3,4]imidazol-2-yl) quinoline [8] (2.95g, 10mmol) at 0℃ under $N_2$. After the resulting mixture was stirred at room temperature for 6 hours, 5 ml isopropyl alcohol was added to quench the reaction. The solvents were removed under vacuum condition at $5\times10^{-3}$ Torr, and the residual solid was sublimed to purify the final product. Orange powder of MgNIQ was obtained in 85% yield. The formula of this compound has been determined by $^1$H NMR and elemental analysis. The organic light emitting device, Fig. 1, using MgNIQ as the emitting layer were fabricated on the transparent conductive indium-tin oxide (ITO) glass substrate. The organic layers and the cathode were sequentially deposited by conventional vacuum vapor deposition in the same chamber without breaking the vacuum under $3\times10^{-7}$ Torr. In the present work, the N,N'-bis- (1-naphthyl)-N,N'-diphenyl-1,1'-biphenyl-4,4'-diamine (NPB) was used as the hole-transport material (HTM), and tris (8-quinnolinolato) aluminum ($Alq_3$) was employed as the electron-transporting material (ETM). The EL spectrum and the Commission International de l'Eclairage (CIE) co-ordinates were measured by Pro-650 Spectroscanner (step size is 1.0 nm and bandpass is 4nm), the current-voltage (I-V) characteristic was measured by Keithley 2400 Source meter.

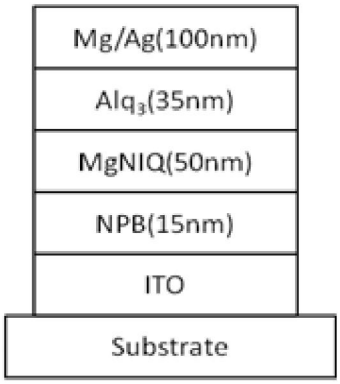
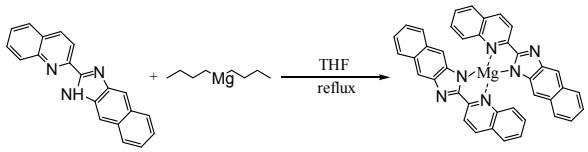



**Fig. 1.** Device structure of organic light emitting device (OLED) fabricated in this work

**Scheme 1.**




Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer thermogravimeter (Pyris 1) under a dry nitrogen gas flow at the heating rate of 20℃/min. Glass transition temperature ($T_g$) and melting point ($T_m$) of materials were determined by differential scanning calorimetry of the Perkin-Elmer differential scanning calorimeter (DSC-7).

## 3. Results and discussion

Fig. 2 shows the TGA of MgNIQ that possesses a maximum rate of weight loss occurring at 577℃ and no weight loss was observed at the temperature lower than 465℃. Above 600℃, there is about 13 wt % of residue composed of Magnesium ash. This Magnesium complex is reasonably stable upon exposure to air and exhibited a very high thermal stability in nitrogen, which is attributed to the fact that the Mg-N (imidazole) bond is highly polarized [9, 10]. The melting temperature ($T_m$) of MgNIQ was not observed up to 450℃ with DSC curve. The DSC and TGA results indicate that the MgNIQ possesses a very high thermal stability, which may serve as an advantage for the fabrication of organic light emitting device because the use of the materials with high thermal stability as the active emissive layer or carrier transporting layer may provide the device with greater longevity [11, 12].

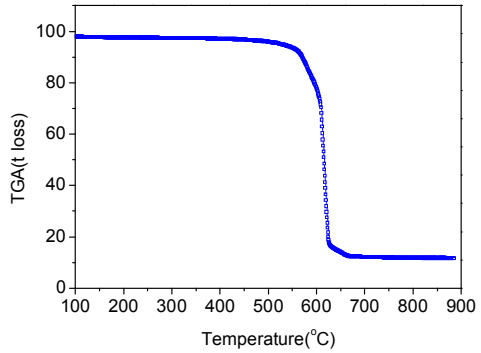



**Fig. 2.** TGA curve of MgNIQ.

The Photoluminescent (PL) spectra of the MgNIQ solutions and neat film, excited with 350 nm laser line, were illustrated in Figure 3. At low concentration, $1\times10^{-5}$ M in DMF, only one emission band is observed with maximum at 489nm, corresponding to the relaxation of MgNIQ from the excited state of a single molecule into ground state. Besides the 489 nm band, a new emission band appeared while the concentration of MgNIQ increased from $1\times10^{-5}$ to $1\times10^{-3}$ M. This new emission band having a maximum at 565nm is observed in the spectrum of the MgNIQ neat film. We have assigned this new emission band to the excimer and higher aggregates emission [13, 14] resulting from the relaxation of collision complex into the lower energy state. The EL spectrum of organic light emitting device at the bias voltage of 13 V, Fig. 4, shows the broad emission band in the 500-700nm region with the maximum at 615nm. The emission is almost fixed in the orange-red region in the CIE coordinate of x = 0.36 y = 0.53, Fig. 5. For the small molecular organic materials, to develop the new type of material with red emission is very important because this kind of material is very seldom prepared so far, and it is very important for the fabrication of full color display panels. The change of the spectral wavelength may be achieved also by general conception of search and design of modified materials for wide band emission consists in substitution of the backside groups by electron acceptors like halogens etc. and different kind of donors [15, 16]. At the same time important role here may play electron-vibration interactions determining the spectral broadening of the emission lines. So the future strategy of the materials design may be in this way also.

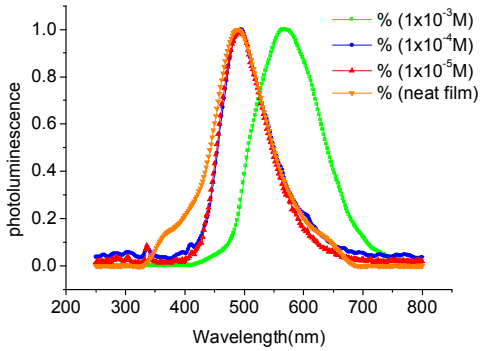



**Fig. 3.** Photoluminescent spectra of the MgNIQ in solutions and neat film

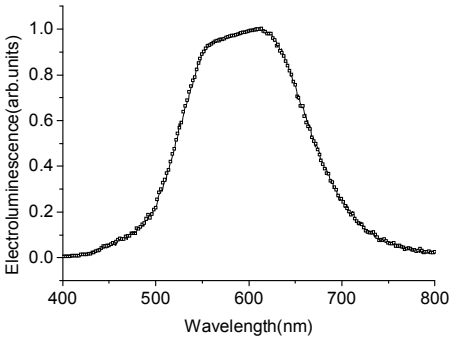



**Fig. 4.** EL spectrum of OLED fabricated in this work.

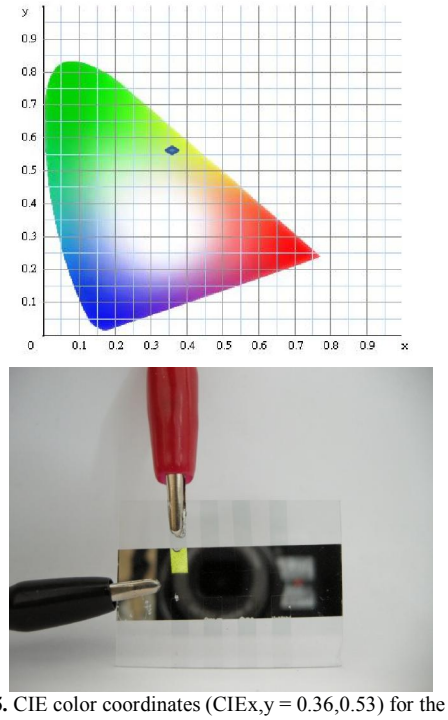


**Fig. 5.** CIE color coordinates (CIEx,y = 0.36,0.53) for the light emission produced by the OLED devices.

Figure 6 shows the energy level diagram of the HOMO and LUMO of the different organic materials and the work function of cathode and anode. The LUMO energy of MgNIQ is 2.6eV determined form the HOMO energy (5.8eV) obtained from the cyclic voltammetry (CV) method and the optical band gap estimated from the absorption onset. Comparing the energy level of MgNIQ with NPB, it is clear that the MgNIQ has the much higher hole injection barrier than that of NPB; in fact, it is impossible for the hole injection from ITO into MgNIQ without the assistance of NPB or some other kind of HTLs. This diagram also pointed out that the $Alq_3$ has the lower electron injection barrier than that of MgNIQ, so the electron injection from the MgAg into MgNIQ will be enhanced and confines the recombination zone at the interface between NPB and MgNIQ. Fig. 7 shows the current-voltage and luminance- voltage characteristics of this device having a low turn on voltage of about 6.0V for current and luminance. This device shows a brightness of 2414 $cdm^{-2}$ at the driving voltage of 13V with current density of 334 $mA/cm^2$, decaying to 25 $cdm^{-2}$ in 120 hours.

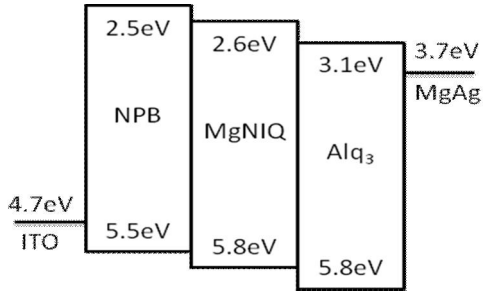



**Fig. 6.** Energy level diagram of OLED materials, ITO, and Mg-Ag alloy

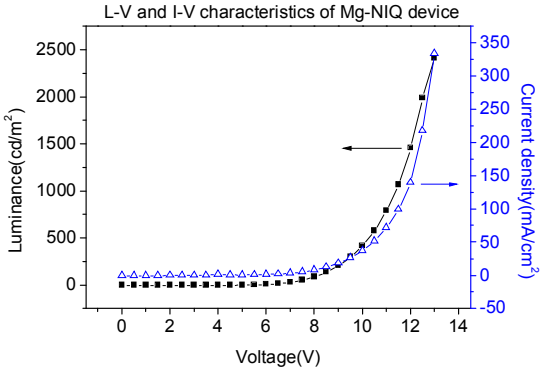



**Fig.7.** Current-voltage and luminance- voltage characteristics of OLED fabricated in this work.

## 4. Conclusion

A novel metal complex, bis{2-(naphtha[3,4] imidazol-2-yl) quinolinato} magnesium (MgNIQ), was successfully prepared by the reaction of 2-(naphtha[3,4]imidazol-2-yl) quinoline and dibutyl-

magnesium. The investigation demonstrated that this compound possess charge transfer and film-forming properties and has high thermal stability..

The excimer emission resulting from the collision complex was observed. The devices composed of MgNIQ as the emitting layer can tune the emitting color via the controlling of carrier recombination region. Because of its high thermal stability and excellent electrical characteristics, MgNIQ and its related compound suggest a possible application for the use of the organic light emitting devices.

**Acknowledgements**

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# Preparation of P(MMA/EGDMA/GMA)/Ni functional composite particles by dispersion polymerization and electroless plating

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**Abstract-**In this study, copolymer beads were prepared from glycidylmethacrylate (GMA) and methylmethacrylate (MMA) in the presence of a cross-linker (i.e., ethyleneglycol dimethacrylate,EGDMA) via dispension polymerization. Preparation of P(MMA/EGDMA/GMA)/Ni functional composite particles that the synthesis particles having various size from 0.5 to 5μm by dispersion polymerization which the particle size was controled with initiator concentration, and polymerization temperature. In this study,Poly(MMA/EGDMA /GMA )/Ni beads were synthesized by dispersion polymerization and electroless nickel. The core-shell structure of polymer--nickel composites and the structure of polymer spheres were characterized by TGA, FESEM,EPMA and FTIR. The results indicate that the nickel was coated on the surface of microspheres. The inner diameter of the microspheres with nickel shell was about 0.6~1.6μm. A possible formation mechanism of the core-shell structure of P(MMA/EGDMA /GMA)/Ni spheres was proposed.
[Xin-Liang Chen, Yen-Chung Chen, Chang-Pin Chang, Ming-Der Ger, Shung-Jim Yang. **Preparation of P(MMA/EGDMA/GMA)/Ni functional composite particles by dispersion polymerization and electroless plating.** Life Sci J 2012;9(2):311-315] (ISSN:1097-8135). http://www.lifesciencesite.com. 49.

**Keywords:** electroless plating, copolymer beads, dispension polymerization, glycidylmethacrylate (GMA), methylmethacrylate (MMA), ethyleneglycol dimethacrylate (EGDMA)

## 1. Introduction

Anisotropic conductive adhesive (film) provides both electrical and mechanical interconnections between electronic components andthe supporting substrate. These anisotropic conductive films (ACFs) offer numerous advantages in surface mount assembly, including flexible andsimple process at low temperature, fluxless bonding which eliminates the need for cleaning, lead-free formulations and low cost [1,2]. The anisotropic nature of these materials makes them excellent candidates for very fine pitch component such as flip chip. These flip chip applications on flexible substrates such as smart cards, disk drives and driver chips for LCDs have attracted much interests andwid espreaduse [3]. However, in flip chip technology, ACF dose not selfalign [4] that would allow misplaced chips to be pulled into correct position corresponding to substrate electrodes by surface tension forces of molten solder.

Composite particles that contain an inner core covered by a shell (core-shell particles) exhibit significant different properties from those of the core itself. The surface properties are governed by the characteristics of the shell coating. The surface engineering of particles is to produce core-shell particles, where the core consists of a solid coated by a number of methods. We have introduced the metallic nickel component into co-polymer cores using electroless plating to form composite particles with response to external electric fields.

Poly(methyl methacrylate)-(glycidyl methacrylate)-(ethyl glycol dimethacylate), poly(MMA-GMA-EGDMA) particles were prepared by dispersion polymerization. Conventional, poly(GMA-MMA-EGDMA) beads were prepared via suspension polymerization. This work examines the feasibility of adopting a novel dispersion polymerization method [5-11], as seldom done previously, to polymerization the copolymer microsphere. The epoxy group containing poly(glycidyl methacrylate-co methylmethacrylate) beads were prepared by dispersion polymerization and the beads were cross-linked with ethyl glycol dimethacylate (EGDMA). The functional epoxy groups of the beads were converted into amino groups. The size and structure of the beads were characterized using SEM and FT-IR spectroscopy, respectively.

Recently, The epoxy groups of the beads were converted into amino groups has been used as a polymeric cross linking agent of latices with epoxy groups, to reduce the release of harmful components into the environment, and to enhance the properties of water and solvent resistance and the mechanical strength of the films formed from the latices.[12] However, highly hydrophilic polymers are not easily prepared by conventional polymerization. Dispersion polymerization might give monodisperse

microspheres but the polymerization is apt to leave free stabilizers in the medium, which would be a harmful contaminant in their applications.[13,14]

In this paper, the Ni-coated Poly(MMA/EGDMA /GMA ) microspheres were fabricated by electroless plating. The surface activating treatment and electroless nickel plating process were optimized in order to obtain the uniform and continuous coatings. And the electrical properties of Ni-coated Poly(MMA/EGDMA /GMA ) microspheres were also investigated in order to develop the novel conductive microparticles.

## 2. Experimental Setup

### 2.1. Materials

Methyl methacrylate (MMA, ACROS Chemicals). glycidyl methacrylate (GMA, ACROS Chemicals). ethyl glycol dimethacylate (EGDMA; Kishida Chemical Industries, Tokyo, Japan) were of commercial grade. They were each distilled under reduced pressure and stored in a refrigerator before use. 2,2_-Azobisisobutyronitrile (AIBN; ACROS Chemicals) was used as a hydrophobic initiator for the suspension polymerization and dispersion polymerization.

### 2.2. Polymerization for dispersion polymerization

Dispersion polymerization was carried out in a 250 mL round flask with a mechanical stirring at 200 rpm under nitrogen atmosphere at 70 °C. Pre-weighed ethanol and aqueous PVP solution were charged in the reactor and followed the addition of MMA, GMA and EGDMA. Then, the AIBN dissolved in ethanol was added and the polymerization was initiated. During the polymerization, aliquots of the reaction mixture were withdrawn from the reactor to examine the conversion upon reaction time and the characteristics of the particles. The withdrawn polymerization products were rinsed off with DDI water and ethanol, centrifuged repeatedly to remove the nonreacted materials and dried in vacuum oven at 60 °C for 2 days, then used for characterization. The detailed polymerization method and the optimum conditions for the preparation of monodisperse polymer particles are described in the elsewhere.

### 2.3. Nickel nanoparticles coating on PMMA-GMA-EGDMA polymer spheres

Nickel was coated onto PMMA-GMA-EGDMA copolymer beads by electroless plating. The known amount of parent PMMA-GMA-EGDMA particles were first sensitized using an acid $SnCl_2$ solution (0.1M $SnCl_2$/0.2M HCl) ultrasonically for 40 min. Then the mixture was washed with deionized water. This step resulted in creating nanoscopic metallic Pd particles as catalytic sites on the PMMA-GMA-EGDMA particles surfaces. The Pd-modified particles were rinsed again using de-ionized water and introduced into electroles splating solution bath. The Pd catalytic sites created on the polymer sphere surfaces allow the second surface redox reaction to begin. These beads were rinsed repeatedly with de-ionized water. The beads were then immersed in an electroless Ni plating solution (ICP Nicoro GIB, Okuno Chemical Co. Ltd.,Japan) for 8 min at 80℃ to produce an Ni layer under constant stirring. Finally, the product was separated by washed twice with de-ionized water.

### 2.4. Characterization

After the polymerization, the final monomer conversion was measured gravimetrically. The size and size distribution of MMA-GMA-EDGMA copolymers were observed by scanning electron microscopy (SEM, S-3500N; HITACHI, Japan) for the dispersion polymerization. Thermal gravimetric analysis (TGA) of PMMA-GMA-EGDMA was carried out on a TA-STDQ600 (TA Instruments, New Castle, DE). The thermograms were acquired between 25 and 500℃ at a heating rate of 10℃/min. Nitrogen was used as the purge gas at a flow rate of 20 mL/min. Fourier transform infrared (FT-IR) spectra of the copolymer microsphere were recorded with a Nicolet (Madison, WI) 170SX FT-IR spectrometer in the attenuated total reflection mode, wavelength range 4000–650 $cm^{-1}$.

## 3. Results and Discussions

Dispersion polymerization is actually a precipitation polymerization in which the medium is miscible with the monomer but not the polymer. When the polymerization starts, free radicals formed by initiator decomposition grow in the continuous phase until their size reaches a critical chain length, at which point they precipitate by either a self or aggregative nucleation process[15], forming nuclei.

When sufficient mature particles are formed ehich can capture all the radicals and nuclei in the continuous phase, no more particles will be formed, and the particle formation stage is completed [16].

### 3.1. Microsphere thermal analysis

Fig.1. show the TGA thermographs of MMA-GMA-EGDMA copolymer microsphere. The TGA curves of MMA-GMA-EDGMA copolymer microsphere revealed there main weight loss regions. The first region at 80–150℃ was due to the removal of water; the second transition region at 200–400℃ was due to degradation of the polymer films; and the peak of the third stage, at 435℃, was due to cleavage

of the polymeric backbone. The temperature of hot loss weight analysis is 234°C. We find hot loss weight analysis result, PMMA-GMA-EDGMA copolymer micro sphere than one PMMA polymerization micro sphere of hot loss weight analysis. The reason has adding micro EDGMA that the structure inner take shape cross linking. The temperature increases of hot loss weight analysis.

### 3.2. Temperature

As illustrated in Fig.2 The copolymer prepared at temperatures of 70, 75 and 80°C, had narrow size distributions with the particle diameter increasing from 0.8 um at 70°C to 0.9 um at 75°C to 1um at 80°C

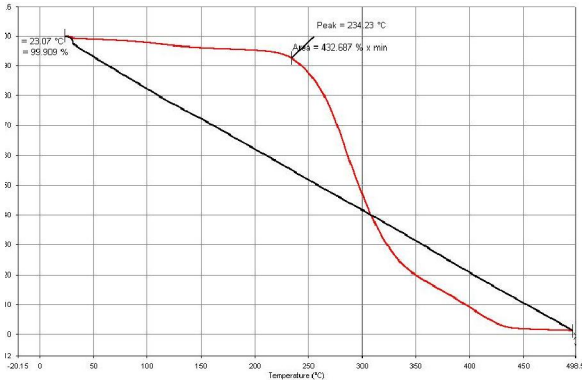


**Fig.1.** The PMMA-GMA-EDGMA copolymer microsphere of hot loss weight analysis.

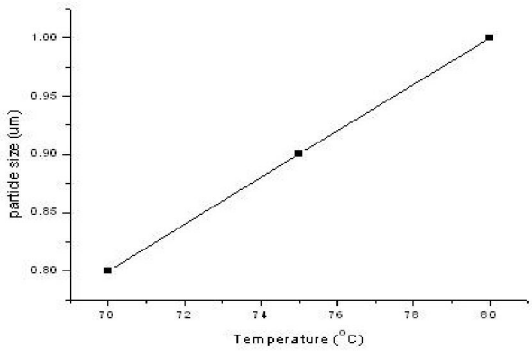


**Fig.2.** Effect of temperature on copolymer particle size

### 3.3. Initiator concentration

Narrow particle size distributions were obtained for copolymer particles prepared with AIBN initiator concentrations ranging from 0.1 to 0.5 wt% at a constant concentration PVP of 5%. The particle size increased from 0.5 to 1um as initiator concentration was increased over this range.Fig.3. shows effect on initiator (AIBN) concentration on copolymer size.

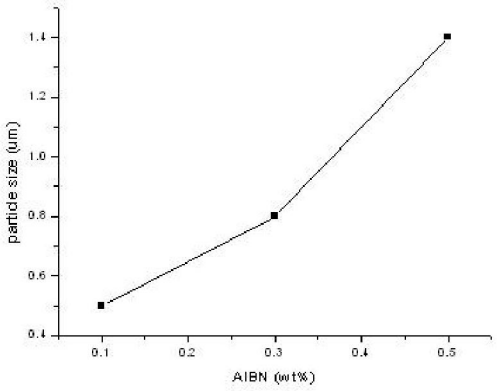


**Fig.3.** Effect of initiator (AIBN wt%) on copolymer particle size

### 3.4. FT-IR analysis

The FT-IR spectrum of PMMA-GMA-EGDMA (shown in fig.4.) indicates the details of functional groups present in the synthesized polymer beads. A sharp intense peak at 1721 $cm^{-1}$ appeared due to the presence of ester carbonyl group stretching vibration. The broad peak ranging form 1260-1000$cm^{-1}$ can be explained owing to the C-O (ester bond) stretching vibration. The broad band from 950-650 $cm^{-1}$ is due to the bending of C-H. The broad peak ranging form 3100-2900 $cm^{-1}$ is due to the presence of stretching vibration. Have epoxy function groups in the structure of Glycidylmethacrylate. Because other function groups are too strong to cover the signal of epoxy function group in the polymer.

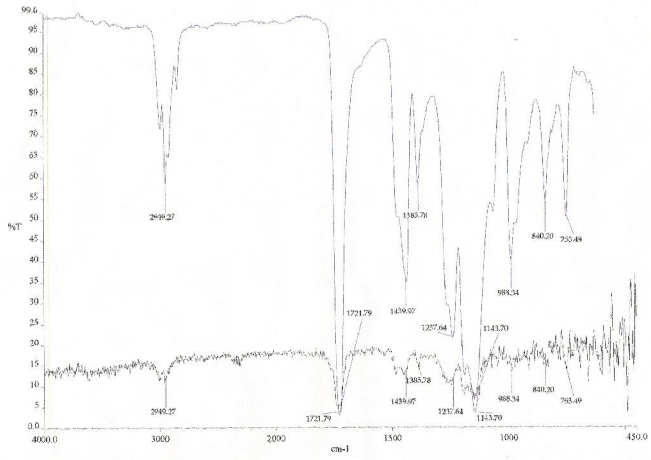


**Fig.4.** FT-IR spectra of MMA-GMA-EDGMA copolymer, MMA polymer

### 3.5. Microsphere morphology

The technique for electroless metal plating is based on the use of a chemical reducing agent that permits the reduction of the metal from solution on the surface of the substrate. For this process, the

beads surfaces need not be electronically conducting, while the kinetics of electron transfer should be slow enough to avoid the reduction of the metal ions and nucleation in solution. The surface acts then as a catalyst to ensure that reduction only takes place on the surface, so that the metal remains attached. The scanning electron microscopy images of PMMA-GMA-EDGMA for dispersion polymerization polymer particles, shown in Fig.5. The presence of the loaded nickel nanoparticles on the PMMA-GMA-EDGMA polymer micro spheres can be confirmed initially by the scanning electron microscopy. While the SEM image of the nickel loaded particles, PMMA-GMA-EDGMA/ Ni particles, in Fig.6. clearly become opaquely black.

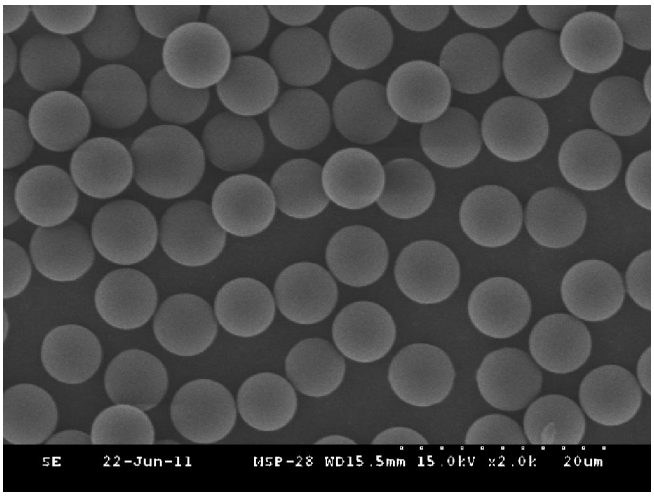


**Fig.5.** The SEM image of PMMA-GMA-EDGMA particles.

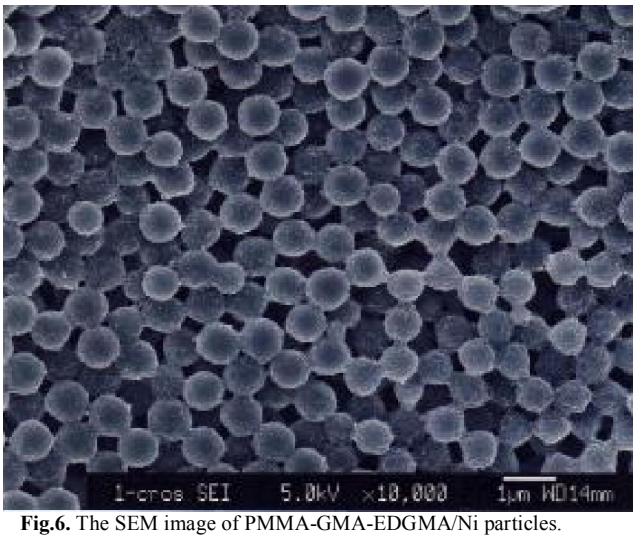


**Fig.6.** The SEM image of PMMA-GMA-EDGMA/Ni particles.

The primary importance of an Electron probe micro-analyzer (EPMA) is the ability to acquire precise, quantitative elemental analyses at very small "spot" sizes about 1~2 microns, EPMA analysis of the PMMA-GMA-EGDMA/Ni particles, as shown in Fig.4, indicated that there were 89.77wt.% of nickel and 10.23 wt.% of phosphorous in the deposited nickel–phosphorous component. The phosphorous peak derived from the reducing agent, which is considered to affect the electric property of the Ni–P coating. Then,for comparison the Pd, Ni and P loading after step-by-step electroless plating was measured by ICP-MS. The loading was measured to be 0.35 wt%,35.65 wt% and 4.34 wt% after nickel plating, respectively. After the subsequent nickel deposition process, which corresponds to an average coating thicknesses of the Ni-P layers of 100~150 nm, shown in Fig.5.

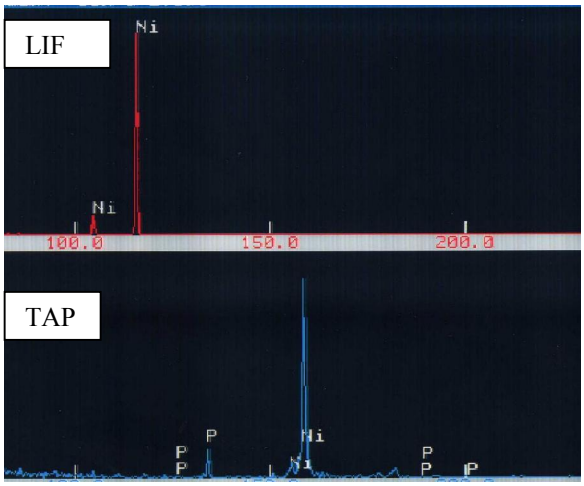


**Fig.7.** Electron probe micro-analyzer analysis of PMMA-GMA-EGDMA/Ni particles.

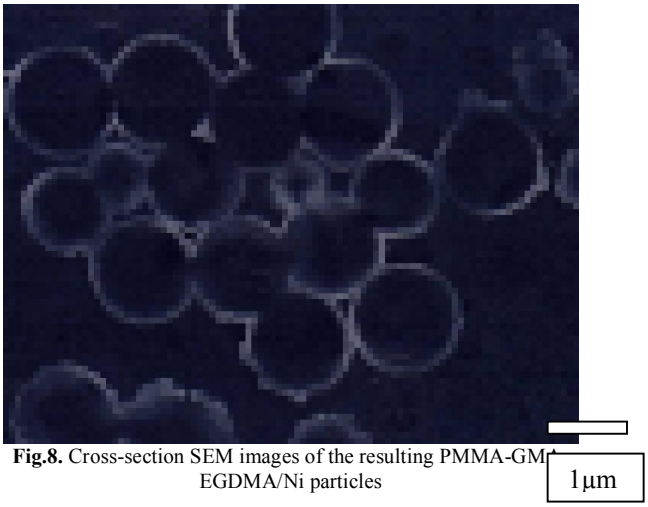


**Fig.8.** Cross-section SEM images of the resulting PMMA-GMA-EGDMA/Ni particles

## 4. Conclusions

In conclusion, the novel PMMA-GMA-EDGMA/Ni particles were prepared by nonelectroless plating technique. The resulting particles were of 0.8~1 um average diameter. This provided a new route to control and selective surface modification of colloidal particles.

4/6/2012

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# Synthesis and Electroluminescent property of bis (2-(benzimidazol-2-yl) quinolinato) Magnesium

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**Abstract:** An emission material, (2-(benzimidazol-2-yl)quinolinato)magnesium (MgBIQ) used for organic light emitting devices, has been synthesized. The melting transition ($T_m$) of MgBIQ is 436℃ and no glass transition temperature ($T_g$) was observed up to 430℃. The emission spectrum of organic emitting device using MgBIQ as emitted layer exhibits a broad maximum at 596 nm. The color of the emitted light is in the red region in the CIE coordinate of x = 0.46 y = 0.46.

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**Keywords:** Electroluminescence; red light; device, bis (2-(benzimidazol-2-yl) quinolinato)

## 1. Introduction

Since an organic light emitting diode was reported by Tang and Vanslyke [1], LEDs based on organic or polymeric materials have generated considerable interest and enabled the development of low-cost, full-color, flat-panel displays along with other emissive products [2-5]. Organic and polymer devices provide advantages over their inorganic counterparts, such as high luminous efficiency and fine-pixel formation. The best-known EL metal chelate compound is $Alq_3$, not only a good emitter but also a highly efficient electron-transporting material, where q is the 8-hydroxyquinolinato ligand [6, 7]. Via the modification of the ligand of metal chelate compound, the emission color of a metal chelate compound may be tuned. Other properties, such as thermal stability and carrier mobility, may also be improved upon. In the present work, we report the synthesis and electroluminescent (EL) property of (2-(benzimidazol- 2-yl)quinolinato)magnesium (MgBIQ). The attachment of the benzimidazol group at 2-position would allow the ligand to form stable complexes with metal ions similarly to 8-hydroxyquinoline. Therefore, the thermal stability, an important character for the practical application in the electronic fields, of this metal complex is investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The organic emitting device using MgBIQ as emitting layer has been fabricated to study the electroluminescent property of this metal complex.

## 2. Experimental Setup

The synthesis of the title compound was accomplished by following processes, as shown in Scheme 1. The dibutylmagnesium solution (0.5M in heptane, 0.6927mL, 10mmol) was slowly added to 100 ml of THF solution containing benzimidazol-2-yl-quinoline (2.45g, 10mmol) at −10℃ under $N_2$. After the resulting mixture was stirred at room temperature for 4 hours, 5 ml isopropyl alcohol was added to quench the reaction. The solvents were removed under vacuum condition, and the residual solid was sublimed to purify the final product. reddish orange powder of MgBIQ was obtained in 75% yield. The formula of this compound has been determined by 1H NMR and elemental analysis. The organic light emitting device, Fig. 1, using MgBIQ as the emitting layer were fabricated on the transparent conductive indium-tin oxide (ITO) glass substrate. The organic layers and the cathode were sequentially deposited by conventional vacuum vapor deposition in the same chamber without breaking the vacuum under $3\times10^{-7}$ Torr. In the present work, the N,N'-bis-(1-naphthyl)- N,N'-diphenyl-1,1'-biphenyl-4,4'-diamine (NPB, 1c) was used as the hole-transport material (HTM), and tris (8-quinnolinolato) aluminum ($Alq_3$, 1d) was employed as the electron-transporting material (ETM). The EL spectrum and the Commission Internationale de l'Eclairage (CIE) co-ordinates were measured by Pro-650 Spectroscanner, the current-voltage (I-V) characteristic was measured by Keithley 2400 Source meter.

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer thermogravimeter (Pyris 1) under a dry nitrogen gas flow at the heating rate of 20℃/min. Glass transition temperature ($T_g$) and melting point ($T_m$) of materials were determined by differential scanning calorimetry of the Perkin-Elmer differential scanning calorimeter (DSC-7).

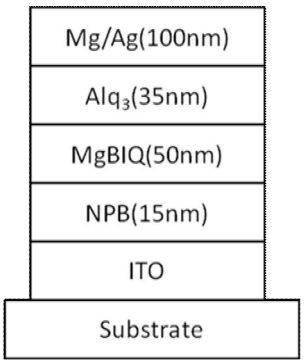
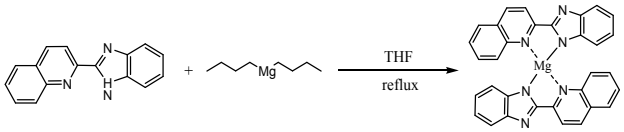



**Fig. 1.** Device structure of organic light emitting device (OLED) fabricated in this work

**Scheme 1.**




## Results and discussion

Fig. 2 shows the TGA of MgBIQ that possesses a maximum rate of weight loss occurring at 501℃ and no weight loss was observed at the temperature lower than 362℃. Above 600℃, there is about 16 wt % of residue composed of Magnesium ash. This Magnesium complex is reasonably stable upon exposure to air and exhibited a very high thermal stability in nitrogen, which is attributed to the fact that the Mg-N (imidazole) bond is highly polarized [8, 9]. The DSC curve of MgBIQ, Fig. 3, shows that the melting transition (Tm) of MgBIQ is 436℃and no glass transition temperature ($T_g$) was observed up to 430℃. The DSC results indicate that the MgBIQ possesses a very high transition temperature, which may serve as an advantage for the fabrication of organic light emitting device because the use of the materials with high transition temperature as the active emissive layer or carrier transporting layer may provide the device with greater longevity [10, 11].

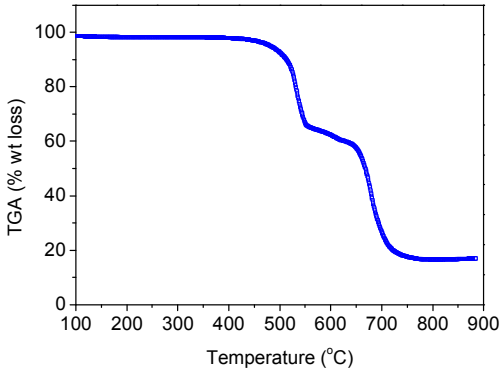



**Fig. 2.** TGA curve of MgBIQ.

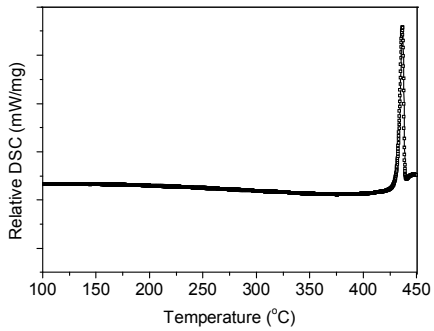



**Fig. 3.** DSC curve of MgBIQ, under nitrogen gas flow at the heating rate of 20℃/min.

The Photoluminescent (PL) spectra of the MgBIQ solutions and neat film, excited with 323 nm laser line, were illustrated in Figure 4. At low concentration, $5\times10^{-5}$ M in DMSO, only one emission band is observed with maximum at 436 nm, corresponding to the relaxation of MgBIQ from the excited state of a single molecule into ground state. Besides the 436 nm band, a new emission band appeared while the concentration of MgBIQ increased from $5\times10^{-5}$ to $1\times10^{-3}$ M. This new emission band having a maximum at 500 nm is observed in the spectrum of the MgBIQ neat film. We have assigned this new emission band to the excimer and higher aggregates emission [12,13] resulting from the relaxation of collision complex into the lower energy state.

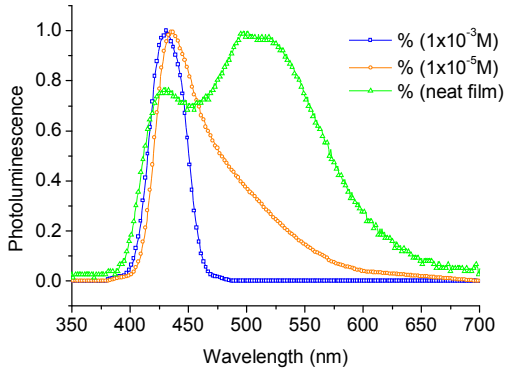



**Fig. 4.** Photoluminescent spectra of the MgBIQ in solutions and neat film

The EL spectrum of organic light emitting device at the bias voltage of 11.5 V, Fig. 5, shows the broad emission band in the 550-700 nm region with the maximum at 596 nm. The emission is almost fixed in the red region in the CIE coordinate of x = 0.46 y = 0.46, Fig. 6. For the small molecular organic materials, to develop the new type of material with red emission is very important because this kind of

material is very seldom prepared so far, and it is very important for the fabrication of full color display panels.

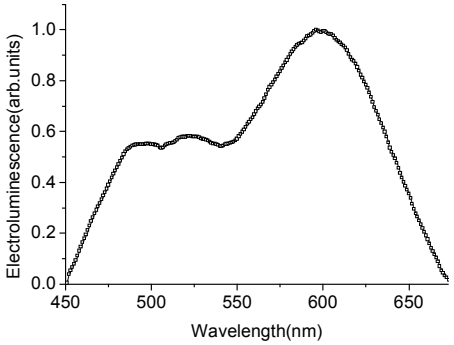



**Fig. 5.** EL spectrum of OLED fabricated in this work.

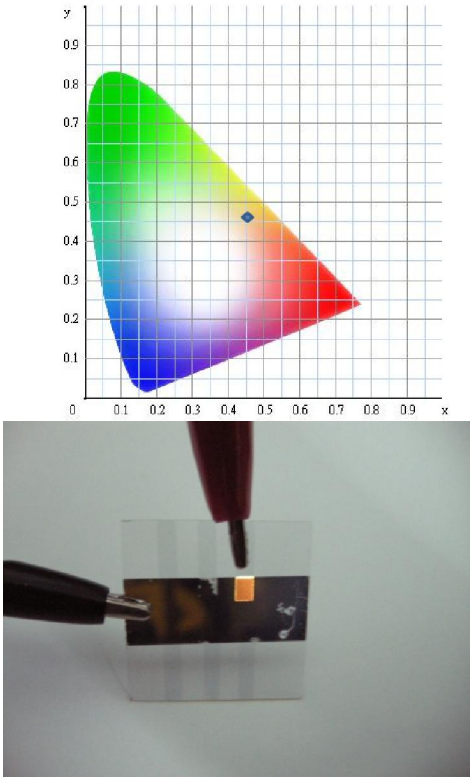



**Fig. 6.** CIE color coordinates (CIEx,y = 0.46，0.46) for the light emission produced by the OLED devices.

Figure 7 shows the energy level diagram of the HOMO and LUMO of the different organic materials and the work function of cathode and anode. The LUMO energy of MgBIQ is 2.6eV determined form the HOMO energy (5.9eV) obtained from the cyclic voltammetry (CV) method and the optical band gap estimated from the absorption onset. Comparing the energy level of MgBIQ with NPB, it is clear that the MgBIQ has the much higher hole injection barrier than that of NPB; in fact, it is impossible for the hole injection from ITO into MgBIQ without the assistance of NPB or some other kind of HTLs. This diagram also pointed out that the $Alq_3$ has the lower electron injection barrier than that of MgBIQ, so the electron injection from the MgAg into MgBIQ will be enhanced and confines the recombination zone at the interface between NPB and MgBIQ. Fig. 8 shows the current-voltage and luminance- voltage characteristics of this device having a low turn on voltage of about 5.5V for current and luminance. This device shows a brightness of 1315 $cdm^{-2}$ at the driving voltage of 11.5V with current density of 167mA/$cm^2$.

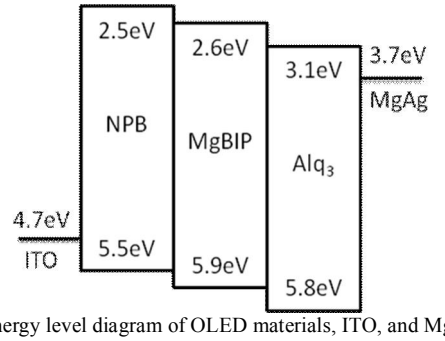



**Fig. 7.** Energy level diagram of OLED materials, ITO, and Mg-Ag alloy

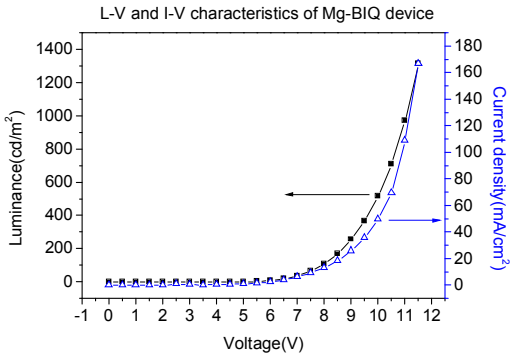



**Fig. 8.** Current-voltage and luminance- voltage characteristics of OLED fabricated in this work.

## 4. Conclusion

A novel metal complex, bis (2-(benzimidazol-2-yl) quinolinato) magnesium, was successfully prepared by the reaction of benzimidazol-2-yl-quinoline and dibutylmagnesium. Because of its high thermal stability and excellent electrical characteristics, MgBIQ and its related compound suggest a possible application for the use of the organic light emitting devices.

## Acknowledgements

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4/22/2012

# Chemical Characteristics and Antioxidant Capacity of Egyptian and Chinese Sunflower Seeds: A Case Study

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**Abstract:** In the last few years Chinese sunflower seed has invaded our Egyptian market increasingly. We took it and the Egyptian sunflower seed as a comparable case study to characterize and investigate them as a source of effective natural antioxidants, oil and protein. Chemical characteristics of the two seeds revealed that protein, oil, ash, moisture and total phenolic contents (TPC) increased significantly ($P<0.05$) after dehulling with pronounced larger amount of these parameters in the Egyptian sunflower seed compared to the Chinese one. Fatty acid analysis showed that Egyptian sunflower oil contains more than 86% and Chinese sunflower oil contains more than 80% unsaturated fatty acids which give these oils a relative advantage. Chlorogenic acid was the major phenolic compound present in TPC as measured by HPLC. Antioxidant activity (AA %) of the phenolic extracts was followed up by measuring radical scavenging activity (RSA %) of the stable DPPH• radical, the degradation rate of β-carotene-linoleic acid o/w emulsion, and the oxidation stability measured by the fully automated active oxygen method (Rancimat). Egyptian sunflower seed have more AA % than Chinese seed as revealed by the higher RSA%, less degradation rate of β-carotene-linoleic acid color and longer induction period measured by Rancimat. Results also demonstrated the suitability of Egyptian and Chinese sunflower seed to be an effective source of protein, with some good functional properties such as solubility, dispersibility water absorption capacity, and emulsifying capacity. Contrary, it showed poor foaming and gelling abilities. Egyptian sunflower oil can also be used as an effective source of unsaturated fats and natural antioxidants. Hence, can be supplemented in many foods and can replace the synthetic antioxidant with their remarkable hazards.
[S.F. Hamed, Suzanne M. Wagdy, and M.G. Megahed. **Chemical Characteristics and Antioxidant Capacity of Egyptian and Chinese Sunflower Seeds: A Case Study.** Life Sci J 2012;9(2):320-328]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 51.

**Key words**: sunflower seed, chlorogenic acid, total phenolic content, antioxidant activity.

## 1. Introduction

Sunflower is one of the major oilseed crops ranking fourth with a worldwide production of about 10.6 million metric tons in 2006 [(1)]. Sunflower is an annual plant native to the Americas belonging to the family Asteraceae. Per 100 g the seed enclose protein up to 20.78 g, total lipid (fat) up to 51.46 g, ash up to 3.02 g, fiber up to 8.6 g with total energy of 2445 kj. The oil accounts for 80% of the value of the sunflower crop, as contrasted with soybean which derives most of its value from the meal. Sunflower oil is generally considered a premium oil because of its light color, high level of unsaturated fatty acids and lack of linolenic acid, bland flavor and high smoke points. The primary fatty acids in the oil are oleic and linoleic (typically 90% unsaturated fatty acids), with the remainder consisting of palmitic and stearic saturated fatty acids. The primary use is as a salad and cooking oil or in margarine. In the USA, sunflower oils account for 8% or less of the market, but in many sunflower-producing countries, sunflower is the preferred and the most commonly used oil [(2)].

Egypt's production of edible vegetable oils suffers several problems nowadays. During the early sixties, Egypt used to be self-sufficient in edible vegetable oils, where self-sufficiency ratio reached 95%. Such ratio followed a declining trend until reaching as low as 31.6% in 2007, which led to increasing volume of oil imports that reached 5.6 thousand tons worth L.E 1.992 billion in 2007 [(3)]. The problem is further complicated by the reliance of the edible oils industry in Egypt on imported raw materials, where private sector's dependency ratio is estimated at 85% [(3)] and according to Egyptian-British Chamber of Commerce Egypt imported 92% of edible oil consumed in 2010 [(4)]. As a result of such a gap between consumption and production Chinese sunflower seed has increasingly invaded the Egyptian market during the last few years.

Lipid oxidation is well known to cause deterioration of fats or fat-containing foods. Also, reactive oxygen species (ROS) produced during natural biological activity in the human body tends to accumulate therein. Antioxidants are needed to retard or delay lipid oxidation and to scavenge ROS. Antioxidants can act by the following mechanisms in lipid peroxidation: (1) decreasing localized oxygen concentrations, (2) preventing chain initiation by scavenging initiating radicals, (3) binding catalysts, such as metal ions, to prevent initiating radical generation, (4) decomposing peroxides so they

cannot be reconverted to initiating radicals, and (5) chain-breaking, to prevent continued hydrogen abstraction by active radicals [5]. Commonly utilized chain-breaking antioxidants include butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tert- butylhydroquinone (TBHQ), propyl gallate (PG) and the naturally occurring tocopherols. There are some serious problems concerning the safety and toxicity of BHA, BHT and TBHQ related to their metabolism and possible absorption and accumulation in body organs and tissues [6]. Therefore, the search for natural antioxidants is highly desirable. The phenolic components and tocopherols are the most important antioxidants for storage stability, as well as, nutritional quality of food made from sunflower seeds. Cells contain a complex system of antioxidant defenses to protect against the harmful consequences of activated oxygen species. When such a complex mechanism inside the cell fail to get rid of ROS it may cause many dangerous diseases such as inflammation, cardiovascular-diseases, cancer and aging [7, 8]. Numerous scientific articles refer to several natural phenols delaying the in vitro oxidation of simple or complex lipid matrices [9].

So, the aim of this work was to take Egyptian and Chinese sunflower seeds as a comparable case study for investigating their suitability as effective sources for natural antioxidants, oil and protein.

## 2. Materials and Methods

### 2.1. Materials

Two samples of sunflower seed, an Egyptian and a Chinese, were purchased from the local market, Dokki district, Cairo, Egypt. Chlorogenic acid (CGA) and TBHQ were purchased from Sigma. All reagents were BDH or of analytical grade.

### Methods

### 2.2. Proximate composition of sunflower seeds

Sunflower seeds were divided into two parts. Half of the seeds were used as such with hull and designated whole seed (WS) while the rest half was dehulled manually and designated (DS). Hulls were separated by aspiration. WS and DS were ground and sieved to pass an 80 mesh screen and then analyzed for their proximate composition that is moisture, crude protein, oil content, crude ash, and crude fiber as recommended by A.O.A.C. [10]. Oil content was measured using a soxthlet extractor and n-hexane as a solvent. Hexane was evaporated using a rotary evaporator (Buchi Rotavapor Switzerland) at 40 ºC. The oil was dried over anhydrous sodium sulphate then placed in a vacuum oven until constant weight. The oil was kept at -20 ºC until analyses.

The defatted meal resulting from WS and DS was spread to dry at room temperature and designated defatted meal of whole seeds (DMWS) and defatted meal of dehulled seeds (DMDS). The meal was kept in closed containers at -20 ºC until further work.

### 2.3. Phenolic extract preparation

Preparation of phenolic extracts was carried out following scientific literature regarding this subject [11-13]. Briefly, ground samples of whole (WS) or dehulled (DS) sunflower seeds (20 g) were extracted with 200 ml of solvent consisting of methanol, 0.16 M hydrochloric acid and water, mixed in proportion 8:1:1, respectively, for 2 h. The above mentioned procedure was repeated on the residue and extracts were combined and washed three times with 15 ml hexane to remove escaped oil using separatory funnel. The combined methanol layer was dried using rotary evaporator at 40 ºC and stored in darkness at -20 ºC.

### 2.4. Total phenolic content (TPC)

Total phenolics were determined colorimetrically using Folin-Ciocalteau reagent according to Hung et al. [14]. The absorbance was measured at 725 nm using a UV – 1601 PC UV-visible spectrophotometer (Shimadzu, Japan). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of chlorogenic acid and the results were expressed as milligrams chlorogenic acid equivalent (CAE) per 100 g extract.

### 2.5. Chemical characteristics and fatty acid composition

The chemical characteristics of the oils used for the experiment have been determined according to A.O.A.C. [10]

For determination of fatty acid composition sunflower oil methyl esters were prepared according to A.O.A.C. method [10]. Determination of fatty acids composition was performed using a Hewlett Packard HP 6890 gas chromatograph, operated under the following conditions: Detector, flame ionisation (FID); column, capillary, 30.0 m X 530 µm, 1.0 µm thickness, polyethylene glycol phase (INNO Wax); N2 with flow rate, 15 ml/ min with average velocity 89 cm/s (8.2 psi); H2 flow rate, 30 ml/min; air flow rate, 300 ml/min; split ratio, 8:1, split flow, 120 ml/min; gas saver, 20 ml/min. Detector temperature, 280 ºC; column temperature, 240 ºC; injection temperature, 280 ºC. Programmed temperature starting from 100 ºC to reach a maximum of 240 ºC was used for eluting the fatty acid methyl esters. The identification of the peaks was made as compared with chromatograms of standard fatty acids methyl esters (Sigma, USA).

**2.6. Antioxidant Activity (AA%)**

Antioxidant activity was determined by three methods: Radical scavenging activity [15], by the β-carotene/ linoleic acid method described by Al-Shaikhan et al. [16] and the fully automated active oxygen (i.e. Rancimat) method. The later method was carried out using the Rancimat 679® (Metrohm AG, Herisau, Switzerland) instrument at 110 °C with the air flow rate of 20 L/hr [17]. The oxidative stability was expressed as induction time (hr).

All antioxidant activity experiments were performed using 500 ppm of phenolic extracts in purified (stripped**)** sunflower oil**.**

**2.7. Sunflower oil stripping (purification)**

Sunflower oils were stripped from antioxidants and from trace metals and other prooxidants according to Fuster et al., 1998 [18] via adsorption chromatography to yield purified sunflower triacylglycerols fraction. A glass column (40 × 2.5 cm i.d.), plugged with glass wool, was packed with 250 g of alumina (activated at 100°C for 8 h and then at 200°C for 12 h) suspended in n-hexane, capped with sea sand, and conditioned by prewashing with 200 mL of n-hexane. The oil (100 mL) was dissolved in an equal volume of hexane and passed through the column, which was then washed with 200 mL of n-hexane. The chromatographic column was wrapped with aluminium foil to prevent light-induced oxidations during the purification process, and triacylglycerols were collected in an aluminum foil wrapped flask. Analysis of the purified oils by thin-layer chromatography (Merck precoated silica gel 60 thin-layer chromatographic plates, 0.25 mm layer thickness and chloroform diethyl ether; 90:10, vol/vol) showed that they are composed mainly of triacylglycerols (data not shown).

**2.8. Functional properties of sunflower defatted meals**

Nitrogen solubility index (NSI), protein dispersibility index (PDI) were determined as described by Smith and Circle [19]. Water absorption capacity (WAC) was estimated according to Huber [20]. Oil holding capacity (OHC) according to Childs and Forte [21]. Emulsifying Capacity (EC) as indicated by Shahidi *et al*. [22]. Foam Stability (FS) as described by A.A.C.C. [23]. Gelation according to Circle et al.[24].

**2.9. HPLC Analysis**

Methanolic extracts (5 µL) were injected in An Agilent 1100 Series HPLC system with a quaternary solvent delivery system, an online degasser, an autosampler, and a DAD detector was used for the analysis. The column was a Phenomenex Luna C18 (5 µm, 250 mm X 4.6 mm) and column temperature was maintained at 30 °C. Two mobile phases, A 0.1% phosphoric acid and B acetonitrile were used in a gradient elution at a flow of 1 ml/min with the following gradient profile: 20 min from 10-22% B, 20 min with a linear rise to 40% B, 5 min reverse to 10% B, and additional 5 min equilibration time [34]. The system was controlled and data analysis was performed by Agilent Chemstation Software. All the calculations concerning the quantitative analysis were performed with external standardization by the measurement of peak areas.

**2.9. Statistical analysis**

All chemical analyses were performed in three replicates and the results were statistically analysed. Statistical analysis was performed using the GLM procedure with SAS (25) software. Duncan's multiple comparison procedure was used to compare the means. A probability to $p \leq 0.05$ was used to establish the statistical significance.

**3. Results and Discussion**

**Proximate composition of sunflower seeds**

Results in Table 1. Revealed that Moisture content in WS (9.2±0.85%) and in DS (8.9±0.24%) of Chinese seeds was higher than that of the Egyptian ones (7.02± 0.66% and 7.45±0.35 in whole and dehulled seeds, respectively. Protein content in WS (22.96±1%) and DS (28.46±0.5 %) of Egyptian sunflower were higher compared to the Chinese ones (21.22±0.99% and 26.69±1.99%, for WS and DS, respectively). The same trend was recorded for oil content where Egyptian sunflower seeds had higher oil content both in WS and DS than the Chinese seeds. The former recorded oil content of 22.11 ±1.01% and 29.09 ±0.99% while the latter recorded oil content of 16.33 ±0.96% and 20.36 ±0.89% in WS and DS, respectively. Whereas, the ash content of Chinese seeds recorded higher percentages both in whole (7.36±0.14%) and dehulled (8.3±0.33%) seeds than whole (3.95±0.22%) and dehulled (5.53±0.62%) seeds of the Egyptian type. Chinese sunflower seeds showed lower crude fiber content (31.51± 2.1%) in WS than that of Egyptian WS (34.91± 2.1%) but, higher crude fiber (18.81± 0.36%) in Chinese DS than Egyptian DS (16.36± 0.34%) were recorded. Nitrogen free extract (calculated) was higher in DS than WS, also higher in Chinese seeds than the Egyptian ones.

**Table 1.** Proximate composition of Egyptian and Chinese sunflower seeds

| Parameter | Egyptian | | Chinese | |
|---|---|---|---|---|
| | WS* | DS* | WS | DS |
| Moisture (%) | 7.02±0.66[a] | 7.45±0.35[b] | 9.2±0.85[d] | 8.9±0.24[c] |
| Protein (%) | 22.96±1.2[b] | 28.46±0.5[d] | 21.22±0.99[a] | 26.69±1.99[c] |
| Oil Content (%) | 22.11 ±1.01[c] | 29.09 ±0.99[d] | 16.33 ±0.96[a] | 20.36 ±0.89[b] |
| Ash (%) | 3.95±0.22[a] | 5.53±0.62[b] | 7.36±0.14[c] | 8.3±0.33[d] |
| Crude Fiber (%) | 34.91±2.1[d] | 16.36±0.34[a] | 31.51±2.1[c] | 18.81±0.36[b] |
| Nitrogen Free Extract | 9.05±0.86 [a] | 13.11±0.59 [b] | 14.38±0.75 [c] | 16.94±0.98 [d] |

Means followed by the same letter within the same row are not significantly different (P<0.05) *WS: Whole seed; DS: Dehulled seeds. Values are mean ± SD.

From the results we notice that after dehulling there is a significant increase in all constituents except crude fiber which is high in the seed hulls. Similar finding were also found by some other scientists [26, 27]. Bhagya and Sastry [28] also reported similar effects of dehulling on Niger seeds.

**Total phenolic content (TPC)**

According to the results (Table 2.) the total phenolic content (TPC), expressed as chlorogenic acid equivalent (mg/100g), was significantly higher (P<0.05) in dehulled seeds (DS) than in whole seeds (WS) of both Egyptian and Chinese sunflowers. The TPC was lower by about 35% in WS than DS. The highest TPC in WS (772 ±3.3 mg CAE/100 g) and in the DS (1088 ±3.95 mg CAE /100 g) was in Egyptian seeds. Chinese seeds showed less phenolics content both in WS (625 ± 2.1 mg CAE /100 g) and in the DS (886 ±3.5 mg CAE /100g). Various scientists have investigated the content of phenolic compounds in sunflower seeds [29-32]. De Leonardis and coworkers [30] reported that TPC in sunflower was in the range of 1.11 to 1.15 mg/mL chlorogenic acid equivalent which is in good agreement with our findings, while Fisk et al.[31] determined the TPC in sunflower seeds and found it to be 2700 mg/100 g. Comparison is hardly possible because of differing analytical methodologies, and differences in the sample material and origin.

**Table 2.** Total phenolic compounds (mg/100g CAE*) in Egyptian and Chinese sunflower seeds

| Parameter | Egyptian | | Chinese | |
|---|---|---|---|---|
| | WS* | DS* | WS | DS |
| Total Phenolics (mg CAE*/100g, (spectrophotometric) | 772±3.3[b] | 1088±3.95[d] | 625±2.1[a] | 886±3.5[c] |
| Chlorogenic acid (mg/100gm, HPLC) | 501.8[b] | 728.96[d] | 400.2[a] | 602.3[c] |

Means followed by the same letter within the same row are not significantly different (P<0.05), CAE *: chlorogenic acid equivalent *WS: whole seed; DS: dehulled seeds. Values are mean ± SD.

In the two (Egyptian and Chinese sunflower oils) samples, chlorogenic acid was the most abundant phenolic compound, 602.3-728.96mg/100gm for DS and 400.2- 501.8 for WS, respectively constituting ≈ 65% of total phenolics (Table2.) as measured by HPLC. Other phenolic constituents were present in too small amounts to be detected by HPLC. Results of Žilić et al.[33] were comparable to our results where they found that TPC of sunflower oil comprised principally of chlorogenic acid, while caffeic acid, ferulic acid, rosmarinic acid, myricetin, and rutin were found in very small percentages. De Leonardis et al.[30] showed that phenolic spectrum of sunflower seeds included seven components (chlorogenic acid, protocatechuic, caffeic acid, o-cinnamic acid, ferulic acid, syringic acid and an unidentified phenolic compound). These authors also reported that the chlorogenic acid was the most abundant phenolic compound (≈79.4% of total phenols). Phenolic compounds in seeds and kernels of sunflower deserve much more attention because the total phenolic content, strongly correlate (r=0.93, P<0.05) with the total antioxidant activity [30,33].

**Chemical characteristics and fatty acid composition**

The chemical characteristics of the Egyptian and Chinese sunflower oil samples are shown in Table 3. Results revealed that acid and peroxide values of the two samples were moderate and comparable to each other and were not significantly different (P< 0.05). Iodine value (IV) which represents the degree of unsaturation indicated that Egyptian sunflower oil has higher IV (129.41) compared to IV of the Chinese oil (115.18), hence Egyptian sunflower oil had significantly (P< 0.05) higher total unsaturation than the Chinese one. Regarding the ester value, saponification value, and unsaponifiable matters, results showed that the Egyptian sunflower oil had higher values than the Chinese one (P< 0.05).

**Table 3.** Chemical characteristics of Egyptian and Chinese sunflower oils

| Parameter | Egyptian | Chinese |
|---|---|---|
| Acid value (mg KOH /g oil) | 3.23±0.22[a] | 3.8±0.31[a] |
| Peroxide value (meq.$O_2$/kg oil) | 0.71±0.33[b] | 0.88±0.23[b] |
| Iodine value | 129.41±1.44[d] | 105.18±1.54[c] |
| Saponification value (mg KOH / g oil) | 189.4±2.1[b] | 187.6±2.3[a] |
| Unsaponifiable matter (%) | 1.85±0.15[b] | 1.74±0.13[a] |
| Ester value | 186.17±1.53[b] | 183.8±0.99[a] |

Means followed by the same letter within the same row are not significantly different (P<0.05), Values are mean ± SD.

Regarding fatty acid profile, Table 4 shows that palmitic acid contents ranged from 6.50 to 9.53%, palmitoleic acid contents from 1 to 1.3%, stearic acid contents from 7.25 to 9.78%, oleic acid contents from 32.95 to 27.8% and linoleic acid contents seeds ranged from 52.3 to 51.56% for Egyptian and Chinese sunflower oils, respectively. As seen from Table 4. almost 86% of Egyptian sunflower oil and 80% of Chinese sunflower oil are of good unsaturated type. Clinical studies show that higher unsaturated fat diets may be preferable even to low-fat diets because they lower total cholesterol, low density lipoprotein (LDL) or bad cholesterol and triglycerides, while maintaining beneficial high density lipoprotein (HDL) cholesterol, which is needed to carry the "bad" cholesterol away [(21)].

**Table 4.** Fatty acid composition of Egyptian and Chinese sunflower oils

| Fatty Acid | Egyptian | Chinese |
|---|---|---|
| Palmitic acid | 6.5±0.77[a] | 9.53±0.98[b] |
| Palmitoleic | 1±0.39[a] | 1.33±0.64[a] |
| Stearic | 7.25±0.44[a] | 9.78±0.86[b] |
| Oleic | 32.95±0.79[b] | 27.8±0.69[a] |
| Linoleic | 52.3±0.85[b] | 51.56±0.8[a] |
| Total Saturation (SFA) | 13.75[a] | 19.31[b] |
| Total unsaturation (UFA) | 86.25[b] | 80.69[a] |
| SAT/USAT | 0.16[a] | 0.24[b] |

Means followed by the same letter within the same row are not significantly different (P<0.05), Values are mean ± SD

**Antioxidant activity (AA%)**

The antioxidant property of sunflower seed extracts influenced by the presence of phenolic compounds was followed up by measuring the capacity of scavenging DPPH• (RSA %), the oxidation of β-carotene- linoleic acid o/w emulsion and as well as measuring oxidation stability by the automated active oxygen method (Rancimat).

**Radical scavenging activity (RSA%)**

The stable DPPH• is scavenged by accepting a hydrogen atom or an electron from the antioxidant and DPPH• transforms into its reduced form, DPPH-H [(34-36)]. DPPH• has a maximum UV–Vis absorbance at 516 nm. Decreasing the absorbance of DPPH solution indicates an increase in DPPH radical scavenging in terms of hydrogen-donating ability. The solution of the purple-colored DPPH radical changed to yellow-colored DPPH-H after reduction. The time taken for the initial DPPH• concentration to reach 50% is called $TC_{50}$. Decrease of $TC_{50}$ indicates high RSA% and vice versa. $TC_{50}$ of Egyptian and Chinese were shown in Fig. 1. As shown in Fig.1 the RSA% of dehulled seed extracts were higher ($TC_{50}$ ranged 7.5-15.33 min) than whole seed extracts ($TC_{50}$ ranged 10.5-18.5 min) for Egyptian and Chinese sunflower, respectively. Egyptian sunflower seeds revealed significantly (P<0.05) higher RSA% both in WS and DS than Chinese seeds.

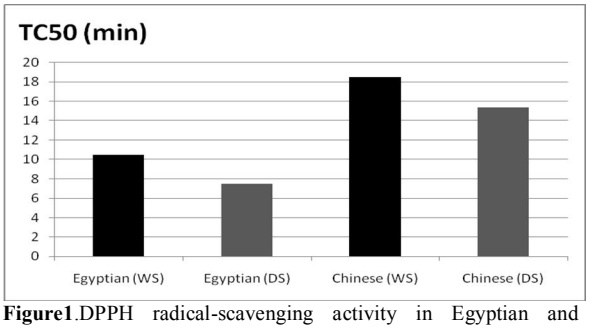


**Figure1**.DPPH radical-scavenging activity in Egyptian and Chinese sunflower seed extracts expressed as $TC_{50}$; WS: whole seed; DS: dehulled seeds.

**β-Carotene – linoleic acid assay**

The oxidation stability of WS and DS of Egyptian and Chinese sunflower TPC extracts in emulsions, was assessed by the coupled oxidation of β-carotene and linoleic acid in o/w emulsion. The test is based on the fact that β-carotene undergoes rapid discoloration in the absence of antioxidant and during oxidation an atom of hydrogen is abstracted from the active methylene group of linoleic acid located on carbon-11 between the two double bonds[37, 38] . The pentadienyl free radical so formed then attacks highly unsaturated β-carotene molecules to reacquire an hydrogen atom. As the β-carotene molecules lose their conjugation, they lose their characteristic orange color. This process can be monitored spectrophotometrically[39]. The presence of phenolic antioxidant can hinder the extent of β-carotene degradation by neutralizing the linoleate free radical and any other radicals formed within the system. The rate of β-carotene bleaching by Egyptian (WS and DS), Chinese (WS and DS) sunflower seeds, TBHQ and α-tocopherol was shown in Fig.2. Among the six tested samples the least β-carotene bleaching (i.e. highest antioxidant activity) was recorded for TBHQ while highest β-carotene bleaching (i.e. least antioxidant activity) was that of Chinese WS. The order of decreasing antioxidant as shown in Fig.2 was TBHQ>Egyptian DS> Egyptian WS > α-tocopherol >Chinese DS > Chinese WS.

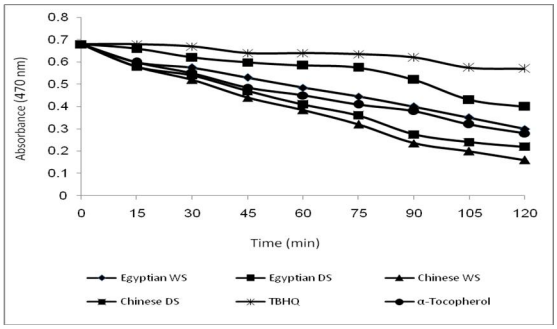


**Figure 2**. Effect of Egyptian and Chinese sunflower seed extracts on bleaching of β-carotene/linoleic acid w/o emulsion bleaching β-carotene/linoleic acid, WS: whole seed; DS: dehulled seeds.

**Active oxygen method (Rancimat)**

The measurement of fat and oil oxidation stability is commonly assessed by the fully automated version of active oxygen method available in Rancimat apparatus (Metrohm Ltd, Herisau, Switzerland) and is accepted as a standard method by American Oil Chemists' Society (AOCS Cd 12b-92) [10, 40-42].

Rancimat method determines the induction period by measuring the increase in volatile acidic by-products released from the oxidizing fat at 100-110 °C. The concentration of degradation products which are transferred into distilled water is monitored by measuring the conductivity. Longer induction periods suggest stronger activity of the added antioxidants.

It is clear that TBHQ revealed the highest protection as indicated by its longest induction period (12.3 hr) among all the tested samples, whereas the control (stripped sunflower oil without any addition) showed the least induction period (3.28 hr). The descending order of antioxidant capacity was TBHQ>Egyptian DS> Egyptian WS ≥ Chinese DS >Chinese WS >control.

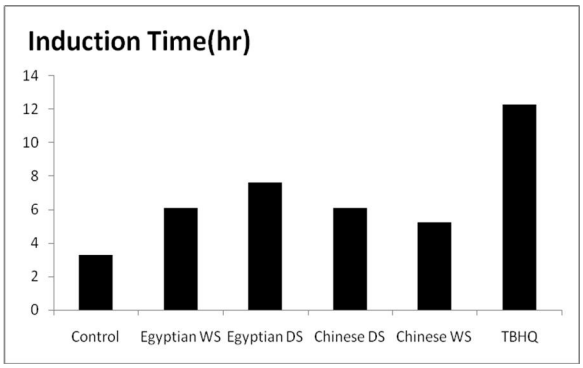


**Figure 3.** Effect of Egyptian and Chinese sunflower seed extracts on oxidation stability of stripped sunflower oil measured by Rancimat, WS: whole seed; DS: dehulled seeds.

**Functional properties of defatted sunflower meals.**

Proximate composition of the defatted meal of whole seed (DMWS) and defatted meal of dehulled seeds (DMDS) for both Egyptian and Chinese seeds are represented in Table 5.

Removal of the oil from WS meal and DS meal resulted in concentration of almost all other constituents specially protein.

Protein content was raised from 22.96 and 28.46% (Table 1) for Egyptian WS, and DS, respectively, to 45.36 and 51.43% protein for DMWS and DMDS (Table 5), respectively. While the Chinese WS and DS contained 21.22 and 26.69% protein respectively (Table 1), which increased upon defatting to 43.89 and 49.72% protein for DMWS and DMDS, respectively (Table 5). Other values in table 5 are self explanatory.

Apart from their nutritional properties, the functional properties of protein and protein products must be taken into account as stated by Finch [43]. Pour –El [44] had broadly defined functionality as any property of a food or food ingredient except its nutritional ones that affected its utilization. The range of desirable and attractive functional properties that should be looked for is almost as broad as the range of foods themselves. Some of the important functional properties were chosen and investigated for DMWS and DMDS protein, and their results are illustrated in Table 6.

**Table 5**. Proximate composition of defatted meal of whole seed (DMWS) and defatted meal of dehulled seed (DMDS) of both Egyptian and Chinese sunflower seeds

| Parameters (%) | Egyptian | | Chinese | |
|---|---|---|---|---|
| | DMWS* | DMDS* | DMWS | DMDS |
| Moisture | 8.23±0.56 | 7.69±0.66 | 7.99±0.85 | 9.01±0.23 |
| Protein | 45.36±0.62 | 51.43±0.55 | 43.89±0.98 | 49.72±0.42 |
| Oil | 0.5±0.16 | 0.4±0.35 | 0.2±0.57 | 0.4±0.62 |
| Ash | 6.7±0.52 | 8.04±0.43 | 9.01±0.72 | 10.12±0.29 |
| Crude fiber | 35.91±0.64 | 18.23±0.86 | 34.69±0.39 | 20.12±0.65 |
| Nitrogen free extract | 3.3±0.01 | 14.21±0.34 | 4.22±0.41 | 10.63±0.33 |

*DMWS= defatted meal of whole seeds; DMDS= defatted meal of dehulled seeds

**Table 6:** Functional properties of defatted meal of whole seed(DMWS) and defatted meal of dehulled seed (DMDS)proteins of both Egyptian and Chinese sunflower seeds, as well as soybean meal for comparison.

| **Functional Properties** | **Egyptian** | | **Chinese** | | **Soya bean meal*** |
|---|---|---|---|---|---|
| | DMWS | DMDS | DMWS | DMDS | |
| **NSI (%)** | 5.5±. 23 | 7.9 ±.11 | 5.6 ±.35 | 6.9 ±.27 | 15.48 ±.66 |
| **PDI (%)** | 6.0 ±.44 | 9.4 ±.45 | 4.0 ±.36 | 15.5 ±.55 | 16.25 ±.51 |
| **WAC (%)** | 480 ±.35 | 450 ±.26 | 480 ±.16 | 460 ±.36 | 300 ±.32 |
| **OHC (%)** | 5.4 ±.72 | 6.3 ±.56 | 6.3 ±.32 | 7.14 ±.14 | 1.875 ±.71 |
| **EC (%)** | 20.0 ±. | 20.8 ±.31 | 20.0 ±.55 | 20.8 ±.46 | 20.8 ±.42 |
| **GE (%)** | 1.0 ±.42 | 1.0 ±.37 | 1.0 ±.53 | 1.0 ±.12 | 3. ±.41 |
| **Foam Stability After** | | | | | |
| 40 Second | 18.3 ±.34 | 12.85 ±.51 | 19.76 ±.31 | 10.86 ±.43 | 32.5 ±.33 |
| 50 Second | 18.4 ±.25 | 15.67 ±.17 | 21.7 ±.11 | 14.13 ±.44 | 130.5 ±.54 |
| 60 Second | 20.03 ±.55 | 20.03 ±.55 | 21.35 ±.53 | 8.7 ±.62 | 160.3 ±.66 |

*Taha and Ibrahim [46]. N.S.I: Nitrogen Solubility Index E.C.: Emulsifying Capacity P.D.I.: Protein Dispersibility index
O.H.C : Oil Holding Capacity ml oil to mg sample W.A.C: Water absorption Capacity G.E: Gelation

**Nitrogen Solubility Index (NSI)**

NSI is a very important measure of the functionality of the proteins in different food systems, especially in fortifying nutritious beverages, instant foods, bakery products , salad dressings, soups and others. The American Dairy Products Institute emphasized that a high value of the NSI indicates that the product is less soluble. NSI of sunflower protein products indicate very good solubility of protein compared to soybean meal protein. NSI values for DMWS(Egyptian), DMWS (Chinese), DMDS (Chinese), DMDS (Egyptian),and soybean meal were 5.5, 5.6, 6.9, 7.9, and 15.48%, respectively.

**Protein Dispersibility Index (PDI):**

PDI is another criterion similar to NSI. It confirms the good solubility of sunflower protein. Soybean meal possessed 16.25% PDI, while DMDS (Chinese) had a close PDI 15.5% to soybean. On the other hand sunflower products, namely DMDS (Egyptian), DMWS (Egyptian), DMWS (Chinese) had 9.4, 6.0, and 4.0 % PDI, respectively, showing superiority to soybean protein.

**Water absorption capacity (WAC):**

It is the ability of a product to absorb water or swell. This property is important in the manufacture of bakery products, pastas, doughnuts and others. Sunflower protein with better WAC than soybean meal protein thus is even more suitable to fortify the above mentioned products . DMDS (Chinese), DMWS (Egyptian), DMWS (Chinese), DMDS (Egyptian) showed 480, 480, 460, and 450% WAC, respectively, compared to 300 % WAC of soybean meal.

**Oil Holding Capacity (OHC)**

OHC is the ability of a protein to bind with oil. It is an important criterion in the meat industry (sausages, hamburgers etc.) OHC % for DMDS (Egyptian), DMDS (Chinese), DMWS (Egyptian), DMWS (Chinese), and soybean meal were 6.3, 7.14, 5.4, 6.3, and 1.875, respectively.

**Emulsifying capacity (EC)**

Emulsifying and film forming ability of plant proteins is essential for those proteins to perform well in meat systems. Also a protein's ability to form emulsion is critical to their application in mayonnaise, salad dressing, milks, and frozen desserts. EC of whole seed proteins is 20ml oil/100g sample which means less than soybean meal. EC of dehulled meals is comparable to that of soybean meal (20.8ml oil/100g) sample. González –Pérez and Vereijken [46] reported that the emulsifying

properties of sunflower protein, show very interesting perspectives to enhance their usage, as they seem at least comparable to those of soy protein.

**Gelling Ability or Gelation (GE)**

It is an important criterion as a protein's EC in comminuted meat systems. It is reported as the lowest concentration of protein that remained as a stable gel after 30 min at room temperature. Soybean meal gelled at 3% protein concentration while sunflower protein products gelled at 1% protein concentration which indicates better gelling properties. On the other hand González –Pérez and Vereijken ([46]) reported that gelling properties of sunflower were not as promising as the EC.

**Foam Stability (FS)**

FS Is the capacity to form stiff, stable foam and is a requirement of proteins to be incorporated into gel cakes, whipped toppings, desserts and soufflé like products. Results in Table 6. reveal low foam stability of all sunflower protein products compared to soybean meal. Poor foaming properties of sunflower protein was in agreement with González – Pérez and Vereijken ([46]) who concluded poor foaming properties for sunflower protein.

In conclusion Egyptian and Chinese sunflower seed and meals did not show much difference between the functional properties of their meal proteins.

**4. Conclusion**

This work assessed that dehulling of sunflower seeds either Egyptian or Chinese increase significantly total proteins, total fats (of which the majority is unsaturated), and total phenolics. Egyptian seed and oil was found to be superior to the Chinese ones in most chemical characteristics and in its content of protein, fat and antioxidant activity. Although, its production is insufficient to meet the consumption of edible oils Egyptian sunflower seed can be used efficiently in supplementation of many foods due to its superior protein, unsaturated fat, and natural antioxidant contents. On the other hand, Egyptian and Chinese sunflower seeds and meals did not show much difference between the functional properties of their meal proteins.

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4/25/2012

# Adoption of Aquaculture Technology by Fish Farmers in Lagos State, Nigeria

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**Abstract:** This paper focused on the extent of adoption of aquaculture technology introduced to fish farmers in Lagos State, Nigeria. Researchers have developed technology packages and disseminated it to the fish farmers through extension agents so as to improve aquaculture in Nigeria. The package included nine practices that fish farmers were expected to adopt. Data were collected from fish farmers through structured interview scheduled. Frequency counts and percentages were used as descriptive method of analyzing the data. Results showed that fish farmers adopted mainly three aquaculture technologies introduced by extension agents. These include pond fertilization (89.8%), water quality management (81.9%) and weed control (81.9%). Reasons for non-adoption of technologies include fund (99.1%), effect of the technology (60.0%), skill/manpower (59.0%). To increase the level of adoption of aquaculture technologies in Nigeria, it is necessary to provide fish farmers with credit facilities. Also, extension agents should be provided with motorcycles to enable them visit fish farmers more frequently to provide skill and encourage them to adopt technologies introduced to them.
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**Kew words:** Adoption, Aquaculture, Technology, Extension agents, Fish farmers.

## 1. Introduction

The rapid increase in world population has resulted in a huge increase in the need for animal protein and other nutritional requirements. This is particularly crucial in developing countries like Nigeria where there is widening gap between supply and demand of fish leading to the large scale fish importation. Fish provides roughly 40% of the protein intake for nearly 2/3 of the world's human population (Oyetoro and Akinboye 2010). The use of fish as source of protein to aid growth and development of both human and livestock cannot be over emphasized. Fish complements meat since the cost of the later is beyond what most Nigerians can afford. (Adeokun et al 2006). Aquaculture is the farming of fish in confined waters (Omitoyin 2006). It also includes the husbandry, management, and multiplication or breeding of all useful aquatic organisms in manmade ponds, cages or other enclosures in lakes and coastal waters' (Ogunremi 2010). Green facts (2004) showed that aquaculture is the fastest growing animal based food production sector particularly in the developing countries mainly China and other Asian countries. In Africa, the governments of the continent under the aegis of the African Union, have identified the great potential of aquaculture and are determined to encourage private sector investment (NEPAD, 2005).

Nwachukwu and Onuegbu (2007) reported that the development of aquaculture can only be enhanced by the introduction of modern technologies. While there have been instances of successful introduction of technologies to boost production in Ghana (World fish centre 2005), the major problem has been the lack of appropriate technology. The adoption of new technology is described as innovation decision process through which an individual passes through the time of first knowledge of the innovation to a decision stage of either adoption or rejection and confirm the decision (Ekong 2002). The decision to adopt innovations involves risk on the part of the farmer. The farmer therefore has to be convinced of the superiority of recommended technology over the existing one. (Adeokun et al 2007). The main objective of the study was to identify the level of adoption of aquaculture technology by fish farmers in Lagos State, Nigeria. The specific objectives were to: describe the personal characteristics of fish farmers in the area of study; identify various types of technologies disseminated to the fish farmers via extension service and various levels of adoption and determine reasons for non-adoption of technologies.

## 2. Materials and Methods

The research was carried out in Lagos State of Nigeria. List of farmers were obtained from Lagos State Agricultural Development programme (LSADP). Eighty-eight fish farmers were randomly selected to constitute 60 percent of the total fish farmers. Structured interview schedule was used to collect information from the respondents. The fish farmers were asked to list technologies disseminated

to them by extension agents, technologies adopted and reasons for adoption of innovation. The data collected were analyzed using such statistical tools as frequency counts and percentages.

## 3. Results

Table 1 presents the personal characteristics of the respondents, while Table 2 shows the adoption of aquaculture technologies by farmers and figure 1 indicates the reasons for non- adoption of aquaculture technologies.

**Table 1.** Distribution of Fish Farmers by Demographic Characteristics

| | Variable | Frequency | Percentage |
|---|---|---|---|
| Gender | Male | 87 | 98.9 |
| | Female | 1 | 1.1 |
| | Total | 88 | 100 |
| Age (years) | 20-30 | 10 | 11.4 |
| | 31-40 | 20 | 22.7 |
| | 41-50 | 37 | 42.0 |
| | > 50 | 21 | 13.8 |
| | Total | 88 | 100 |
| Marital status | Single | 7 | 8.0 |
| | Married | 74 | 84.1 |
| | Divorced | 6 | 5.7 |
| | No response | 1 | 2.2 |
| | Total | 88 | 100 |
| Dependents | None | 16 | 18.2 |
| | 1-5 | 51 | 58.0 |
| | > 5 | 21 | 23.8 |
| | Total | 88 | 100 |
| Educational | Non formal | 2 | 2.3 |
| | Adult Literacy | 4 | 4.5 |
| | Primary | 11 | 12.5 |
| | Secondary | 31 | 35.2 |
| | Tertiary | 29 | 33.0 |
| | No response | 11 | 12.5 |
| | Total | 88 | 100 |

**Table 2.** Technologies introduced to Fish Farmers

| Technologies | Adoption of technologies | Percentages |
|---|---|---|
| Fingerlings Production technique | 53 | 25.9 |
| Pond fertilization method | 12 | 5.9 |
| Stocking method | 195 | 95.1 |
| Water quality management technique | 124 | 60.5 |
| Fish feeding technique | 195 | 94.6 |
| Weed control method | 9 | 4.4 |
| Pond draining method | 130 | 63.4 |
| Fish seed Transportation method | 50 | 24.4 |

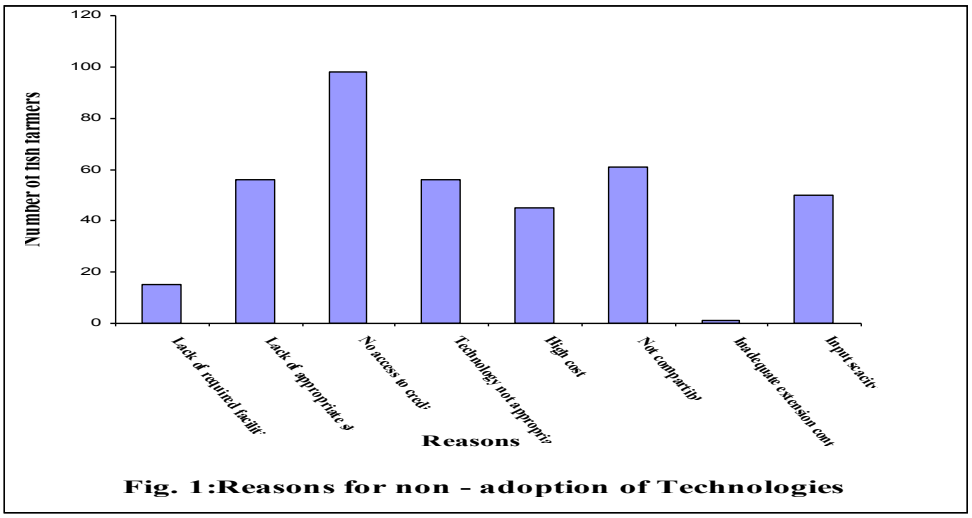


**Fig. 1.** Reasons for non-adoption of Technologies

**Discussions**

From table 1, almost all the respondents were male 98.9%, more males are involved in fish faring than females, most of the farmers are between the age of 30 - 50years (674.7%). Age is very important in adoption decision-making because it requires maturity, mental and psychological alertness. Age can affect perception, attitude and adoption of innovation (Adesiji 2004). Thus, there is need to encourage younger people to go engage in fish farming. It can be observed on the table that the highest percentage accounted for married respondents was (84.1%), only (8.0% were single (Oyetoro and Akinboye 2010, Nwachuwu and Onuegbu 2007) reported higher percentages of married among fish farmers. It can be inferred that the married have extra hands to work with on their farms hence can embark on more farming activities. Also, 85.0% have between 1 and 5 dependents, while 23.8% have more.

On the educational level of the respondents, 12.5% had minimum education of primary school, 35.2% attended secondary schools while 33.0% attended tertiary institutions. The implication of this is that, information dissemination by extension agents through leaf lets, extension guides, and other print media might make impact in improving fishing activities in the area of study since most of the fishermen could read and write it could also be seen that majority of the fish farmers in the study area (84.1%) engage in fish farming as their primary

occupation while the remaining (15.9%) engage in fish farming as their secondary occupation.

Results from Table 2 shows higher adoption level for stocking (95.1%), fish feeding (94.6%), pond draining (63.0%) and water quality management (60.5%). Pond fertilization (5.9%), weed control (4.4%) and fish breeding (25.9%) recorded low adoption. High adoption rate of fish feeding was reported by (Tejiri and Fregene 2011). The reason for the high adoption level of stocking, fish feeding and pond draining were paramount because it invariably determines the yield. Stocking signifies the number of fish to put in water, for feeding the quality and quantity of nutrients needed by fish and the timing of feed application is important. Good water quality management will prevent retarded growth and outbreak of diseases, if a pond is not well drained when harvesting, it will affect fish stocked later as those remaining will cannibalize on them thus the farmer will incur loss. Omitoyin (2002) reported that feed is a major input in aquaculture. The adoption of new technology and production practices is often the key to maintaining a profitable agricultural operation (Ogunremi 2010). Figure 1 shows reasons for non- adoption of technologies introduced to fish farmers in the study area. Technologies were generated for farmers without their input in most cases. Inputs for developed ones are either not available or avoidable; consequently, most of the technologies generated are not suitable to the farmers needs and have relatively limited acceptability (Akinbile, 2002). It has been noted that people do not just adopt a technology because it is available to them. Even when the technology is available and appropriate, some personal and socio-cultural factors bear on the decision of clienteles to adopt or not (Adesehinwa and Bolorunduro 2007). Most of the fish farmers (98%) asserted that credit was a major reason for non- adoption of technologies. As regards cost implication, Abadi *et al*; (2003) agreed that if it is perceived that an innovation is more subject to price variability. Adu (2007) submitted that farmers' needs and objectives are the primary stimuli for adoption of technology. Angba (2000) however, discovered that adoption takes place only when the constraints due to a new technology are overcome such that farmers are able to take adoption decisions. Male were mostly into fish farming in Lagos State. The factors influencing adoption of technologies in the study area were mostly fund, skill or manpower, how applicable the technology could be. When fish farmers do not see a technology as generating in come immediately, the motivation to commit resources to the venture will not be there. It is therefore recommended that:

1) Enough find should be made available to fish farmers for expansion of their farming activities.
2) The government through the extension agents should enlighten fish farmers on various ways of raising fund. (Nigerian Agricultural Cooperative and Rural Development Bank (NACRDB, loan Scheme).
3) Extension agents should also come up with programmes that will encourage fish farmers.
4) Extension agents should visit fish farmers timely so as to provide the needed skill, guide and draw up programmes that will encourage fish farmers on adoption of technologies to boost fish production in the country.
5) Fish farmers should properly organize themselves in to cooperatives so that government can channel various aids, and loan.

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# Effect of cases of loading and distribution of shear connectors on the behavior of One-Way composite pre-slabs

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**Abstract:** Many structures have been recently constructed using composite reinforced concrete elements. One of the most common types is the pre-slabs which are used extensively in the construction of both buildings and bridges. It consists of a pre-cast concrete layer serves as a form or shuttering for the cast-in-place concrete layer. Also the cast-in-place concrete layer can be used for strengthening an existing slab. One of the most governing factors in design of sections of these elements is the shear transfer along the interface which is major factor to achieve the composite action between the two layers. In this research, the behavior of one way composite pre-slabs was studied. An experimental program was carried out to test nine simply supported slabs, three of them were reference monolithic slabs and the remaining six slabs were composite pre-slabs composed of two layers with different distributions of shear connectors according to shear force distribution. All slabs were tested under different cases of loading. Finally; comparison between experimental results of tested specimens and theoretical results obtained from analysis using finite element program was made and valuable recommendations for structural designers were suggested.
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**Key Words:** Concrete, Shear transfer, Composite, Pre-slabs

## Introduction:

The composite concrete-concrete construction, as one of several techniques of prefabrication and precasting, has been more and more employed. In the composite construction, the precast concrete acts in conjunction with the cast-in-place concrete to form what are called “composite section”.

Most of the recent codes of practice permit design of composite flexural member as monolithic one provided that its composite interface has enough shear transfer capacity. The increase of composite interface roughness and the use of steel ties, shear keys or adhesive materials, improve the shear transfer capacity and thus insure the full composite action.

Abd El-Hay A.S. (1) tested nine composite continuous one way pre-slabs 2.36x0.8x0.1 m. under the action of distributed load, the results showed that the pre-slab with rough interface and concentration of dowels in the outside ¼ span gives an ultimate load as monolithic slab, also the use of epoxy painting without dowels or roughness was very poor in resisting the shear stress along the interface.

Rabie(2) tested four composite two-way simply supported pre-slabs 2x2x0.1 m. under the effect of distributed load, the result showed that the ultimate load for the composite slab with rough interface only was about 87% of that of monolithic slab, also a slightly higher values of both deflection and concrete compressive stress was measured up to the complete separation of the two layers. Also; the pre-slab with distributed dowels 1ϕ8@ 40 cm. gives ultimate load about 92% of that of monolithic slab. While the use of concentrated dowels decreases both deflection and stress in dowels till the separation of the two layers in the interior zone which led to sudden increase in both deflection and stress in dowels.

EL-Behairy S.A, and Abou El-Enin A.W.(3) carried out tests on concrete pre-slabs cast in different ages; the effect of surface condition was studied. The result showed that the specimens with roughened interface gave the best results while the pre-slabs with smooth or toweled interface with steel dowels of area less than 0.15% did not reach the monolithic stage.

El-Rakib (4) made a series of push-off specimens for the evaluation of shear transfer parameters, he concluded that the use of shear connectors had a significant effect on increasing the ultimate shear strength and decreasing both slippage and crack width. Also he recommended that the imbedded length of the shear connectors not less than 10ϕ in the old concrete layer and 20ϕ in the new concrete layer.

Dong *et al.* (5) made a test on eight concrete pre-slabs 4x1.4x0.2 m. with four different concrete strength 19, 28, 32 and 51 Mpa.

He concluded that the shear stress versus slippage behavior of unbounded-smooth interface was distinctly different from that of an unbounded-rough interface.

Ihab A. H.(6) and El-Sayed M. (7) discussed the shear transfer.

Abou El-Matty (8) and Easterling W.S., and Young C.S. (9) discussed the behavior of composite slabs.

**Experimental work:**

Experimental program was carried out on six composite pre-slabs and three monolithic slabs; all slabs were supported on two edge supports to represent the case of one way simply supported slabs.

Each composite slab consists of two concrete layers; the first layer was slab with dimensions 106 *80*5 cm. with main bottom reinforcement of 10 Φ 12 mm. and secondary reinforcement of 6 Φ 6 mm. The second layer had the same dimensions as the first layer 106 *80 *5 cm without reinforcement, as shown in figure (1).


**Figure (1):** Pre-slab before casting the second layer.

All slabs are of total thickness of 10 cm and were tested under the case of uniformly distributed loads, one line load and two line loads, but they had a different dowels distribution as follows:

**S1:** Monolithic slab tested under the effect of uniformly distributed loads.

**S2:** Monolithic slab tested under the effect of one line load acts at a distance of 20 % of the span from one edge.

**S3:** Monolithic slab tested under the effect of two line loads act at a distance of 20 % of the span from the two edges.

**S4:** Composite slab tested under the effect of uniformly distributed loads and had a uniform dowels distribution.

**S5:** Composite slab tested under the effect of uniformly distributed loads and had a concentrated dowels distribution according to the shearing force diagram.

**S6:** Composite slab tested under the effect of one line load acts at a distance of 20 % of the span from one edge and had a uniform dowels distribution.

**S7:** Composite slab tested under the effect of one line load acts at a distance of 20 % of the span from one edge and had 50 % of dowels area put in one quarter of the span under the line load as the other 50% of dowels area put uniformly in the remaining span.

**S8:** Composite slab tested under the effect of two line loads acts at a distance of 20 % of the span from the two edges and had a uniform dowels distribution.

**S9:** Composite slab tested under the effect of two line loads acts at a distance of 20 % of the span from the two edges and had 50 % of dowels area put in each one quarter of the outside span while the middle part of the span was without any dowels.

The concrete compressive strength of tested slabs are shown in table (1).

**Table (1): Compressive strength of tested specimens at testing time**

| specimen | $F_{cu}$(first layer) | $F_{cu}$(second layer) | Notes |
|---|---|---|---|
| S1 | 368.5 | | Monolithic slabs |
| S2 | | | |
| S3 | | | |
| S4 | 349.8 | 366.5 | Composite pre-slabs |
| S5 | 379.2 | 391.2 | |
| S6 | 351.2 | 385.2 | |
| S7 | 379.2 | 391.2 | |
| S8 | 348.5 | 367.2 | |
| S9 | 348.5 | 367.2 | |

**Test Set-Up and Loading Arrangement:**

The specimens were tested under the effect of three types of loading; the first case of loading was the effect of uniform distributed load through a whiffel tree arrangement, the second case of loading was the effect of one line load while the third case of loading was the effect of two line loads using a hydraulic jack with increment equal to 1 ton as shown in figure (2).

Demic mechanical strain gages of 20 cm. length were used to measure the concrete strain and electrical strain gages were fixed on the steel dowels surface to measure the dowels strain.

Dial gages with 0.01 mm. accuracy were used for vertical deflection measurements. Also a horizontal dial gauge with 0.01 mm. accuracy was used to measure the slippage between the two concrete layers.

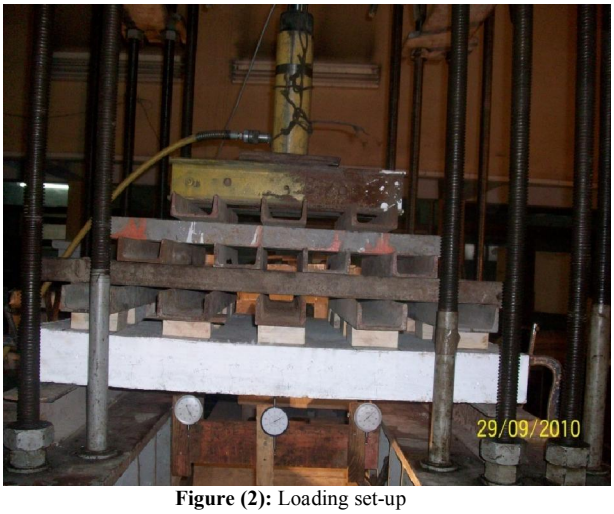



**Figure (2):** Loading set-up

**Discussion of experimental Results:**

Test results discussed here include mode of failure, cracking pattern, cracking and ultimate loads, maximum induced slippage, maximum deflection, deflection pattern, shear transfer along the interface and strains in both concrete and shear dowels.

**Cracking Pattern and Mode of Failure:**

The initiation and pattern of cracks of the tested specimens can be explained as follows:

**1- Monolithic slab (S1):**

The first crack was observed at a load of 12.8 t/m$^2$ on the bottom surface at the section of maximum moment i.e. nearly at the middle of the span. After this load level, another bottom flexure cracks appeared with the increasing of load.

The diagonal shear crack started to appear at load of 32.6 t/m², it was near the support from the two sides. Increasing the load after the diagonal shear crack led to an increase in the diagonal shear crack width till the specimen had a complete shear failure as shown in figures (3) and (4).


**Figure (3):** Crack pattern of slab S1


**Figure (4):** Shear failure of slab S1

**2- Monolithic slab (S2):**

The first crack was observed at a load of 12.6 t/m on the bottom surface at the section of maximum moment i.e. nearly at the location of applied line load. After this load level, another bottom cracks appeared adjacent to the applied line load as the increasing of load. The diagonal shear crack started to appear at load of 30 t/m and it was near the support increasing the load after the diagonal shear crack led to increasing in the shear crack width till failure in a complete shear failure as shown in figure(5).


**Figure (5):** Shear failure of slab S2

**3- Monolithic slab (S3):**

The first crack was observed at a load of 12.75 t/m on the bottom surface at the section of maximum moment i.e. nearly at the middle of the span. After this load level, another bottom flexure cracks appeared on the both sides of the first crack as the increasing of load. The diagonal shear crack started to appear at load of 21.25 t/m of each line load and it was near the support , increasing the load after the diagonal shear crack led to increasing in the shear

crack width till the specimen had a complete shear failure as shown in figure (6).


**Figure (6):** Shear failure of slab S3

**4- Pre-slab (S4):**

The first crack was observed at a load of 17.75 t/m$^2$ on the bottom surface at the section of maximum moment. After this load level, another bottom cracks appeared as the increasing of load.

The first diagonal shear crack was observed at a load of 30 t/m², it was near the support from the two sides. Increasing the load after the diagonal shear crack led to an increase in the shear crack width till the specimen failed in a complete shear failure as shown in figure (7).


**Figure (7):** Shear failure of pre-slab S4

**2- Pre-slab (S5):**

The first crack was observed at a load of 12.75 t/m$^2$ on the bottom surface at the section of maximum moment i.e. nearly at the middle of the span. After this load level, another bottom cracks appeared as the increasing of loads

The first diagonal shear crack was observed at a load of 40 t/m², it was near the support from the two sides. Increasing the load after the diagonal shear crack led to increasing in the shear crack width till a complete shear failure occurred as shown in figure (8).

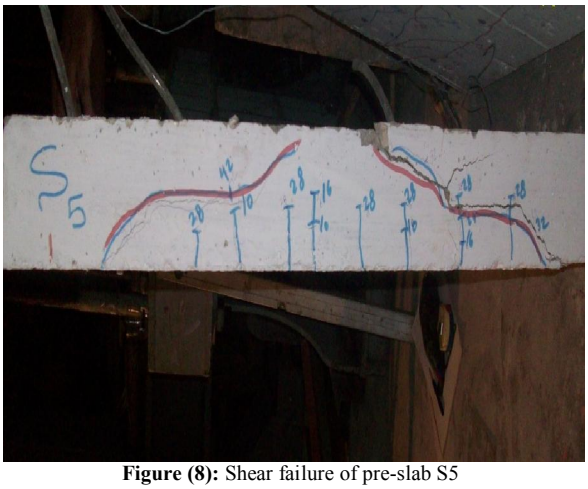



**Figure (8):** Shear failure of pre-slab S5

**6- Pre-slab (S6):**

The first crack was observed at a load of 9.5 t/m on the bottom surface at the section of maximum moment i.e. nearly at the location of applied line load. After this load level, another bottom cracks appeared adjacent to the applied line load as the increasing of load. The diagonal shear crack started to appear at load of 27.2 t/m and it was near the support, increasing the load after the diagonal shear crack appeared led to increasing in the shear crack width till failure in a complete shear failure as shown in figure (9)


**Figure (9):** Shear failure of pre-slab S6

**7- Pre-slab (S7):**

The first crack was observed at a load of 15.1 t/m on the bottom surface at the section of maximum

moment. After this load level, another bottom cracks appeared adjacent to the applied line load as the increasing of load. The diagonal shear crack started to appear at load of 35 t/m and it was near the support, increasing the load after the diagonal shear crack appeared led to increasing in the shear crack width as shown in figure (10).


**Figure (10):** Shear failure of pre-slab S7

**8- Pre-slab (S8):**

The first crack was observed at a load of 5.1 t/m of each line load on the bottom surface at the section of maximum moment. After this load level, another bottom cracks appeared on the both sides from the first crack as the increasing of load. The diagonal shear crack started to appear at load of 21.8 t/m of each line load and it was near the support. Increasing the load after the diagonal shear crack led to increasing in the shear crack width till the specimen failed in a complete shear failure as shown in figure(11).


**Figure (11):** Shear failure of pre-slab S8.

**9- Pre-slab (S9):**

The first crack was observed at a load of 5.1 t/m of each line load on the bottom surface at the section of maximum moment. After this load level, another bottom cracks appeared on the both sides from the first crack as the increasing of load. The diagonal shear crack started to appear at load of 18 t/m of each line load and it was near the support. Increasing the load after the diagonal shear crack led to increasing in the shear crack width till complete shear failure occurred as shown in figure (12).


**Figure (12):** Shear failure of pre-slab S9.

**Cracking and Ultimate Loads:**

Table (2) shows the values of the cracking load for both monolithic and pre-slabs, the first cracking load occurred at the bottom surface of the specimens at the section of maximum bending moment according to the loading type.

Table (2) also shows that for group (1) under uniformly distributed loads, the ultimate load for the pre-slab S4 with uniform dowels distribution was about 94% of corresponding monolithic slab S1, while the ultimate load for the pre-slab S5 with concentrated dowels distribution was approximately the same of corresponding monolithic slab s1.

For group (2) under uniformly one line load, the ultimate load for the pre-slab S6 with uniform dowels distribution was about 91% of corresponding monolithic slab S2 while the ultimate load for the pre-slab S7 with concentrated dowels distribution was approximately the same of corresponding monolithic slab s2.

For group (3) under uniformly two line loads, the ultimate load for the pre-slab S8with uniform dowels distribution was about 90% of corresponding monolithic slab S3 while the ultimate load for the pre-slab S9 with concentrated dowels distribution was about 98% of corresponding monolithic slab s3.

**Table (2):** Results of tested slabs.

| Specimen | | $F_{cu}$ ( kg/cm$^{2)}$ | | Cracking load $P_{cr.}$ (ton) | Ultimate load $P_{ult.}$ (ton) | Shear strength $q_u$ (kg/cm$^2$) | Vertical deflection $\delta_{max.}$ (mm) | Max. Slip. (mm) |
|---|---|---|---|---|---|---|---|---|
| | | First layer | Second layer | | | | | |
| Group (1) | S1 | 368.5 | | 10.3 | 47.1 | 23.2 | 11.41 | --- |
| | S4 | 349.8 | 366.5 | 14.2 | 44.2 | 21.4 | 11.5 | 0.06 |
| | S5 | 379.2 | 391.2 | 10.2 | 47.4 | 22.9 | 8.25 | 0.02 |
| Group (2) | S2 | 368.5 | | 10.1 | 28.6 | 41.07 | 5.6 | --- |
| | S6 | 385.2 | 351.2 | 7.6 | 27.1 | 36.61 | 5.5 | 0.05 |
| | S7 | 379.2 | 391.2 | 12.1 | 29.1 | 41.48 | 4.8 | 0.03 |
| Group (3) | S3 | 368.5 | | 10.2 | 38.9 | 33.53 | 7.6 | --- |
| | S8 | 348.5 | 367.2 | 8.2 | 35.2 | 29.9 | 6.97 | 0.125 |
| | S9 | 348.5 | 367.2 | 8.2 | 38.2 | 33 | 7.95 | 0.075 |

From these results it is clear that the concentration of the shear dowels distribution according to the shearing force distribution led to an increase in the ultimate capacity of the section which means increasing in the composite action between the two concrete layers of the pre-slabs.

**Load- Deflection Diagrams:**

The vertical deflection of the tested monolithic and pre-slabs was measured at 0.2, 0.5 and 0.8 span and the maximum deflection plotted against the applied load from zero loading up to failure as shown in figures (13) through figure (15 ).

It can be noticed that the relation between the load and deflection was nearly linear up to cracking load then it was nonlinear distribution due to excessive cracking in the concrete.

Comparing the load-deflection curve of the pre-slabs S4, S5 and monolithic slab S1, it can be noticed that the pre-slab S4 had approximately the same deflection curve of the pre-slab S5 and had a maximum deflection less with about 30% than the maximum deflection of monolithic slab S1.

On the other hand, comparing the load-deflection curves of the pre-slabs S6, S7and monolithic slab S2, it can be noticed that the pre-slab S7 had approximately the same maximum deflection of the monolithic slab S2 while the pre-slab S6 had an increase in the maximum deflection by about 18.5% of that of the monolithic slab S2.

For the load-deflection curve of the pre-slabs S8, S9 and monolithic slab S3, it can be noticed that the pre-slab S8 had a maximum deflection of about 78% of that of the monolithic slab S3 while the pre-slab S9 had a decrease in the maximum deflection by about 21% of that of monolithic slab S3, also the dowels concentrated distribution in the pre-slab S9 led to an increase in the maximum deflection by about 4% over that of S8, this is attributed to the absence of the shear dowels in the middle span zone of the pre-slab S9.

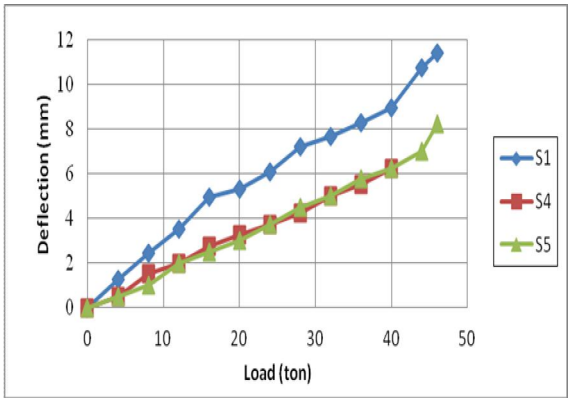



**Figure (13):** Vertical deflection at mid-spans (Group 1).

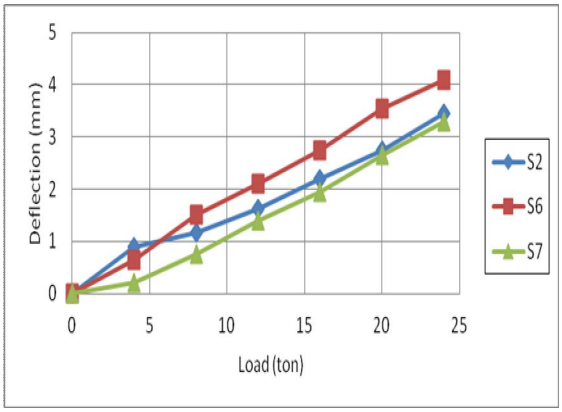



**Figure (14):** Vertical deflection at 0.2 spans (Group2).

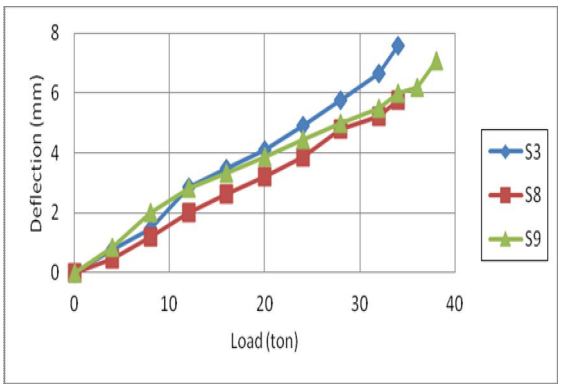



**Figure (15):** Vertical deflection at 0.2 spans (Group 3).

**Deflection Pattern:**

The deflection pattern at cracking load, as shown in figure(16), indicates that the monolithic slabs had deflection values higher than corresponding pre-slabs except in group(3) where the pre-slab S9 had deflection more than the pre-slab S3 because of the absence of shear connectors in the middle region of the pre-slab S9.While the deflection pattern at ultimate load as shown in figure(17) indicates that the monolithic slabs had deflection values less than corresponding pre-slabs except in group(1) where the monolithic slab S1 had a deflection more than the pre-slabs S4 and S5.

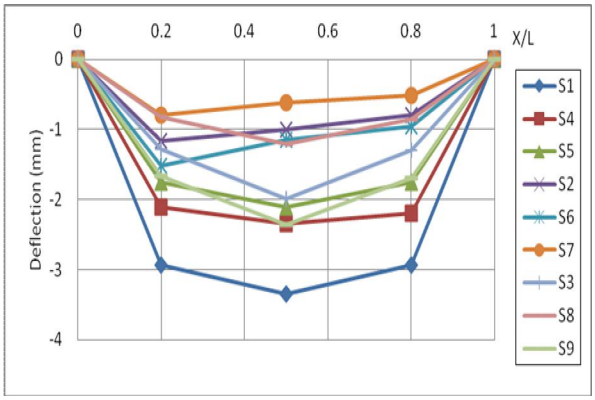



**Figure (16):** Deflection pattern of tested slabs at cracking loads.

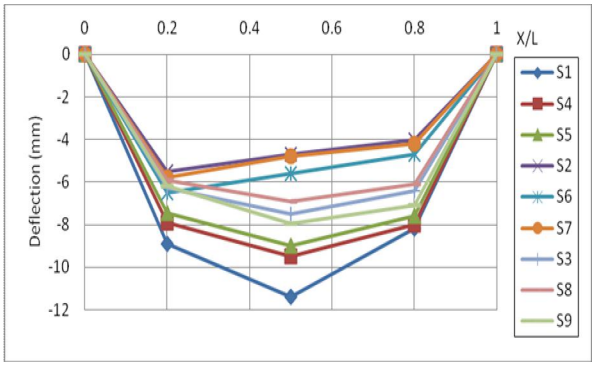



**Figure (17):** Deflection pattern of tested slabs at ultimate loads.

**Concrete Tensile Strains:**

The distribution of the tensile strains in concrete bottom fibers at the cracking load are plotted along the slabs axes as shown in figures (18) through (20).

From figure (18), it can be noticed that the tensile strain of slabs S1 and S5 was approximately the same while the tensile strain for slab S4 was less with about 17% of that for slab S5. Also, from figure (19), the maximum tensile strain was under the location of the line load (i.e. approximately at 0.2 span) and the tensile strain of the pre-slabs S6 and S7 was approximately the same and less with about 31% of that of monolithic slab S2.

The tensile strain for the last three slabs under the application of two line loads were approximately the same for S3, S8 and S9 as shown in figure (20).

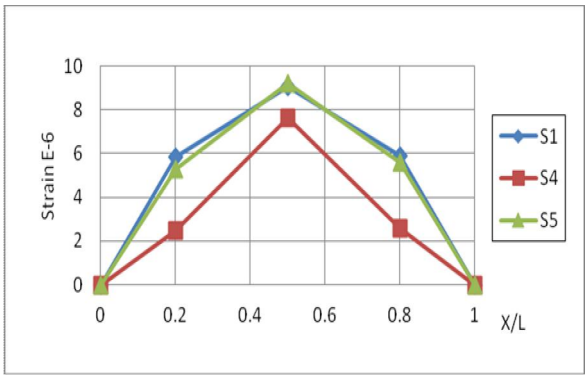



**Figure (18):** Concrete tensile strain at cracking load (group 1).

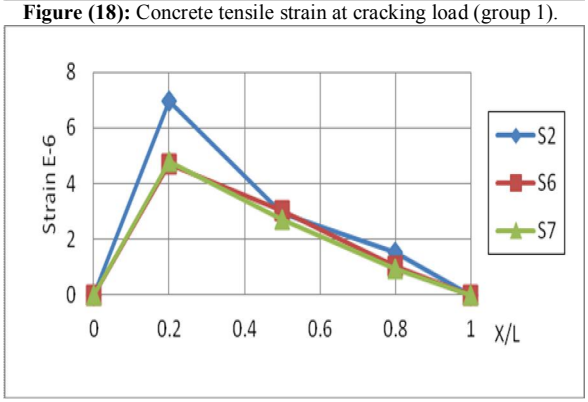



**Figure (19):** Concrete tensile strain at cracking load (group 2).

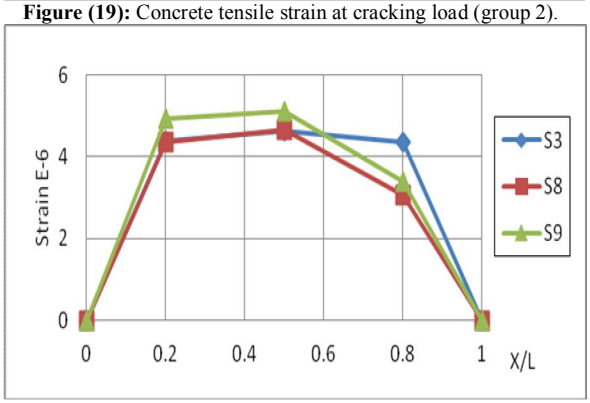



**Figure (20):** Concrete tensile strain at cracking load (group 3).

**Dowels Strains:**

The maximum strain in the shear connectors plotted against load are shown in figure (21), it is clear that the concentration distribution of dowels as done in the pre-slabs S5, S7 and S9 led to a decrease in the dowel's strain because of the large dowel's cross sectional area at the location of the maximum shear stresses along the interface.

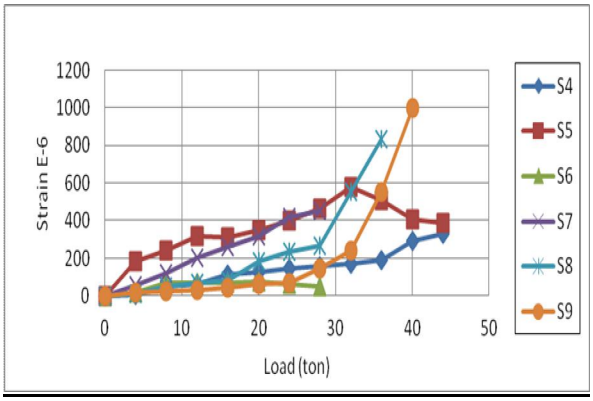



**Figure (21):** Strain in dowels of pre-slabs.

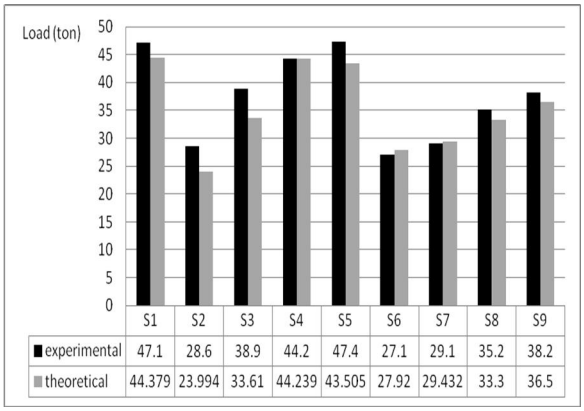
**Finite Element Program (ANSYS):**

Finite element program (ANSYS) version 11 was used in this study to simulate the behavior of the tested slabs which were modeled with finite element mesh. An eight node solid element (Solid 65) was used to model concrete and steel reinforcement bars, while the element (Beam4) was used to model the shear dowels connecting between the two concrete layers. The option (Concrete) was used to model concrete behavior and the option (Mises Plasticity) was used to model the steel behavior.

**Correlation between theoretical and experimental results:**

The comparison of the ultimate load between the theoretical and experimental values is shown in figure (22). It can be noticed that the theoretical ultimate loads were about (84%: 96%) of that of corresponding experimental results for all slabs except for slabs S4, S6 and S7 the ratio was approximately 100%.

Also, the finite element model gave a good agreement with the experimental results in vertical deflection measurements as shown in figures (23) through (25).




| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 |
|---|---|---|---|---|---|---|---|---|---|
| experimental | 47.1 | 28.6 | 38.9 | 44.2 | 47.4 | 27.1 | 29.1 | 35.2 | 38.2 |
| theoretical | 44.379 | 23.994 | 33.61 | 44.239 | 43.505 | 27.92 | 29.432 | 33.3 | 36.5 |

**Figure (22):** Ultimate load for tested slabs.

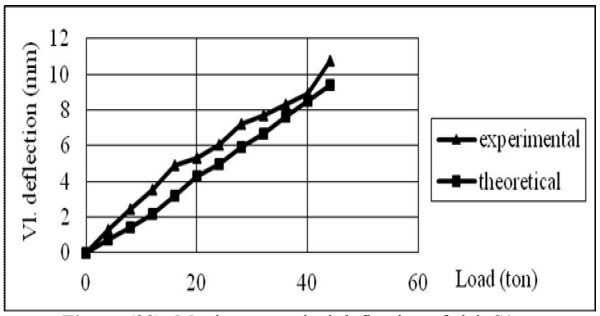



**Figure (23):** Maximum vertical deflection of slab S1.

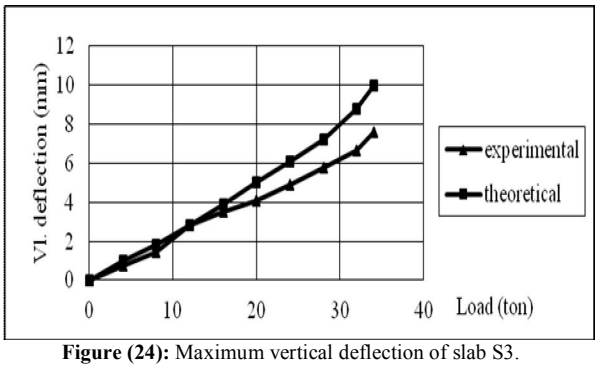



**Figure (24):** Maximum vertical deflection of slab S3.

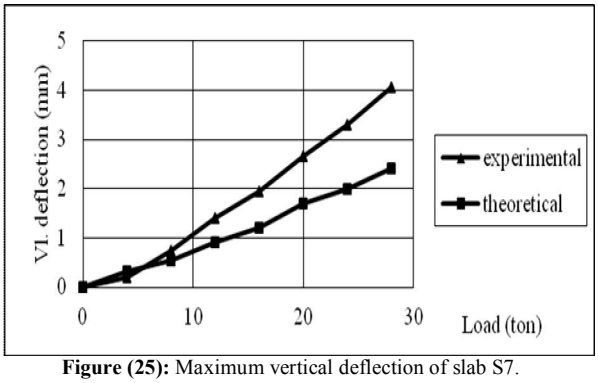



**Figure (25):** Maximum vertical deflection of slab S7.

**Conclusions:**

1- The design of the tested specimens successes to change the mode of failure from flexure failure to shear failure.
2- Shear connectors concentration in the tested pre-slabs led to the following results:
   a- Approximately the same ultimate loads for the pre-slabs as the corresponding monolithic slabs (as in case of the pre-slabs S5 and S7 and monolithic slabs S1 and S2).
   b- Increasing in shear strength of the tested pre-slabs comparing to the tested pre-slabs with uniform distribution of shear connectors (the pre-slaps S7 and S9 had an increase in shear strength with about 10% above the shear strength of the pre-slabs S6 and S8 which had a uniform dowels distribution).
   c- Approximately the same shear strength of the tested pre-slabs comparing to the monolithic slabs (pre-slabs S5, S7 and S9 had approximately the same shear strength of the corresponding monolithic slabs S1, S2 and S3 respectively).
   d- Decrease in horizontal slippage by about 67% in case of tested specimens under the effect of uniformly distributed loads and about 40% in case of tested specimens under the effect of either one or two line loads.
   e- Decrease in dowel's strains in case of two line loads as the increase in dowel's ratio happened due to the concentration of dowels on both outer quarter part as in the tested pre-slab S9.
3- The changing of loading type from uniformly distributed loads as in the tested specimens (S1, S4, S5) to concentrated one line load as in the tested specimens (S2, S6, S7) led to achieve the ultimate shear strength .

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# Relationship between Leadership; Empathy and Emotion for Junior and Senior Nursing Student

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**Abstract:** In nursing education, three concepts of: leadership, emotional intelligence and empathy are correlated and supporting each other in preparing high quality graduate of nursing students. The study aimed to assess leadership style, empathy and emotional level of nursing student. Descriptive correlation study used with non probability convenience sample. 59 students from level fourth and eight participated in this study. Three questionnaires, namely: Multifactorial Leadership Questionnaire (MLQ), the Hogan Empathy Scale (HES) and the Emotional Empathy Tendency Scale (EETS) used as data collection tools. SPSS version 17 used for statistical analysis. It was found that both of the junior and senior students perceived their leadership to be more transformational rather than transactional leadership. Furthermore, majority of the leadership domain were correlated negatively with empathy scores. It is recommended for future research to use greater sample size and various settings. In nursing practice, educators need to encourage the application of transformational leadership, emotional intelligence and empathy in order to improve the quality of graduates.
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**Key Words:** Transformational leadership, transactional leadership., empathy and emotional intelligence

## 1. Introduction:

Nowadays, the dominant topic in nursing administrative literature for past several years are the full-range theory of transformational leadership and emotional intelligence. One of the key concepts in transformational leadership is empathy. Specifically, for nursing students the three components of: leadership; emotional intelligence; and empathy are correlated. These three components are supporting each others in preparing high quality graduate of nursing students.

Leadership :Leadership defines as a process of uses interpersonal skills to influence others to accomplish specific goals. Leadership requires attending to and acknowledging others and being authentic and accountable (Sullivan & Decker, 2005). Particularly, for leaders in learning organizations, they need to master various types of leadership behavior in order improve their flexibility in adapting with different situations (Chanpoe, 1998). The teaching of leadership can enable students to develop realistic hypotheses based on contemporary leadership theories and observed behaviours (Densten & Gray, 2001). Moreover research by Chanpoe (1998) discovered that there was a significant relationship among leadership behaviour and learning organization.

In educational setting, Alger (2008) found that transformational leadership was a desirable style for school leaders involved in improvement efforts because it improve the level of awareness of workers so that they come to value organizational goals and strategies to achieve those objectives. Furthermore, the effects of transformational leaderships were closely linked with those exerted by educational structure and culture (Lam, 2002). In addition, study by Gill *et al.* (2010) concluded that student educational satisfaction was positively related to the transformational leadership used by the instructors or professors.

Emotional intelligence: Salovey and Mayer (1990) refers emotional intelligence as the subset of social intelligence that involves the ability to monitor one's own and others' feelings and emotions, to discriminate among them and to use this information to guide one's thinking and actions. In other words, Ionnidou and Konstantikaki (2008) refers it as the ability control someone's wishes and to postpone their fulfillment, to regulate others' mood, to isolate feeling from thinking, to place you into another's

shoes and to hope. Consequently, it is the process of regulating both feelings and expressions. Theorist studying emotional intelligence which is significant component for leader to establish a cooperative and effective team (Marquis & Huston, 2006).

Abilities and skills of emotional intelligence are classified into four parts, namely: the ability to (a) perceive emotion, (b) use emotion to facilitate though, (c) understand emotions, and (d) manage emotion (Mayer *et al*, 2004)

In 2002, Boyatzis *et al*, stated that emotional intelligence was significance in the educational program to prepare future leader. Moreover, Cavallo and Brienza (2001) concluded that emotional intelligence correlated with the leadership level of the manager.

Empathy: Williams and Stickley (2010) defines empathy as a series of stage that is difficult to delineate because of the interactive, dynamic and involving interpersonally through a shared understanding relationship between client and therapist. Arnold & Boggs (2003) inferred empathy as the ability to be sensitive to and communicate understanding of the client's feelings. It is closely aligned to the concept of presence and it is impossible to be fully present without being empathetic. The stages are: affective dimensions, emotional engagement and the employment of behavioural skills, respectively (Williams & Stickley, 2010).

As a nurse, empathy is an important characteristic need to be mastered in order to give a high quality nursing care for patients. Concomitantly, in the past 4 decades empathy has been touted as appropriate, desirable, therapeutic and the main component for the nurse-patient relationship (Prince & Archbold, 1997). Moreover, empathy considers as a main significant component for both emotional intelligence and leadership (Humprey, 2002). Based on this, nursing education has responsibility to facilitate education that ensures emphatic feeling (Williams & Stickley, 2010).

**Significance of the study:**

In Kingdom Saudi Arabia, researches combined three scope of concept, namely: leadership, emotional intelligence and empathy are scant to nonexistent especially in scope of nursing education. However, to improve the quality of education in the Kingdom to be a world class institution, this research is important. This research could act as a database for further leadership, emotional intelligence and empathy research in the Kingdom or other gulf countries.

**Purpose:**

This research aimed to assess leadership style and empathy level of nursing students. In addition, it also able to reflects different style of leadership between senior and junior students and correlation of it with the empathy and emotional intelligence.

## 2. Methodology

**Design:**

Descriptive correlation study used in this research to assess the leadership style, emotional intelligence and empathy level of nursing students.

**Sample & Setting:**

Non probability, convenience sample conducted in this study. Subjects were participated voluntary and no risk involve in this research. There were two groups involved in this study, junior students (n=29) and senior student (n=30) which was at the semester fourth and eight, respectively. The study conducted at College of Nursing, King Saud University.

**Data Instrument Tool:**

There were three questionnaire used in this study. First, for the leadership style, Multifactorial Leadership Questionnaire (MLQ) used to assess the leadership style of participants (Bass & Avolio, 2005). Secondly, for the cognitive dimension of empathy, the Hogan Empathy Scale (HES) used in this research. The HES is a dichotomous instrument with 39 true or false response alternatives to measure participant perception of the emotional life of others (Hogan, 1969). Thirdly, the Emotional Empathy Tendency Scale (EETS) to measure inborn trait characteristics of respondents (Mehrabian, 1972).

**Ethical Consideration:**

Permission to use the MLQ granted from the Mind Garden institution as the official institution for using this instrument. Furthermore, the study purposes and methods were described to the students by researchers. Students were assured that their response would be confidential and that it would not affect their academic success.

**Procedures:**

Directly after finishing from the ethical consideration procedures, the questionnaire distributed to students in two groups, namely: student in the fourth level semester and in the eight level semesters. The questionnaire was distributed to students by researcher during their study activities both inside college and in clinical settings.

**Statistical Analysis:**

SPSS 17 used to performed data analysis. A *p*-value < 0.05 was considered statistically significant.

**3. Results And Discussion**

Generally, participants' main characteristics were senior student, with the age more than 21 years old and single. The distribution between senior and junior student was quite equal with 48.2% and 51.8%, respectively. For the participant age, almost 45% of respondent with the age below of 21 years, whereas the other 55% were more than 21 year olds. The marital status of respondent was three fourth of them were single (80.4%).

**Table 1. Relation between empathy and leadership styles and academic level**

| | Academic level | | | | Mann Whitney test | *p*-value |
|---|---|---|---|---|---|---|
| | Junior (n=27) | | Senior (n=29) | | | |
| | Mean | SD | mean | SD | | |
| EETS | -0.44 | 8.36 | 4.00 | 13.66 | -1.00 | 0.317 |
| Hogan | 19.52 | 3.37 | 19.38 | 3.06 | -0.39 | 0.698 |
| Styles of leadership: | | | | | | |
| Transformational | 2.49 | 0.63 | 2.84 | 0.43 | -2.42 | 0.016* |
| Transactional | 2.42 | 0.64 | 2.73 | 0.46 | -2.18 | 0.029* |
| Laisser-faire | 1.96 | 0.80 | 2.85 | 0.53 | -1.00 | 0.317 |

(*) Statistically significant at p<0.05

From statistical analysis, it was found that there were statistically significance differences between junior and senior students for the transformational and transactional leadership. Both of the groups were more transformational rather than transactional. This result is similar with the study of Gunther *et al.* (2007), which found both the senior and junior student perceived their style to be transformational leadership style.

Furthermore, table (1) confirmed negative mean for the EETS of junior student. It means that when EETS score increase affect to the lower score of leadership. Moreover, it infers a low mean regarding empathy for the junior students. This result was different with study by Ozcan *et al* (2010) which inferred that the newly registered student score for empathy was higher compared with other levels. The result of the current study might occur because of less exposure with empathy subject for junior students compared with student in senior level.

**Table 2. Relation between Empathy and predominant leadership styles**

| Predominant leadership style: | Mean | ±SD | Kruskal Wallis Test | *p*-value |
|---|---|---|---|---|
| EETS score: | | | | |
| Transactional | -1.00 | 9.40 | | |
| Transformational | 1.84 | 8.90 | | |
| Laisser-faire | 4.89 | 15.30 | 2.249 | 0.325 |
| Hogan score: | | | | |
| Transactional | 18.84 | 3.63 | | |
| Transformational | 18.79 | 3.19 | | |
| Laisser-faire | 20.78 | 2.32 | 3.643 | 0.162 |

Table (2) reflects that there was no statistically significance difference for the relation between empathy and predominant leadership style. This result was in contrast with the research of Gunther *et al.* (2007) who concluded there was a weak positive correlation between the predominant transformational leadership style and empathy levels in both junior and senior students. Furthermore, the statistical analysis reveal that no statistical significance difference between the predominant leadership style and the EETS score which is regarding the emotional intelligence. This result, differ compared with study by Hur *et al.* (2011) which concluded that emotional intelligence was positively related with the transformational leadership. This result might occur due to limited sample in the present study.

There were negative correlations between empathy score and different leadership domains, namely: idealized influence (attribute); idealized influence (behavior), inspirational motivation, intellectual stimulation, individual consideration, contingent reward and active management by

exception. This result differ with study of Esfahani and Soflu (2011) found that there was a significant positive relationship between emotional intelligence and transformational leadership method (r=0.16).

**Table 3. Correlation between empathy scores and various leadership domains scores**

| | Pearson correlation | |
|---|---|---|
| | **EETS Score** | **Hogan Score** |
| Hogan score | ,091 | |
| Transformational: | | |
| Idealized Influence "Attributed" (IIA) | -,129 | -,158 |
| Idealized Influence "Behavior" (IIB) | -,007 | -,009 |
| Inspirational Motivation (IM) | -,080 | -,020 |
| Intellectual Stimulation (IS) | -,124 | ,002 |
| Individual Consideration (IC) | -,136 | -,073 |
| Transactional: | | |
| Contingent Reward (CR) | ,014 | -,077 |
| Management-by-Exception "Active" (MBEA) | -,127 | ,023 |
| Laisser-faire: | | |
| Management-by-Exception "Passive" (MBEP) | ,117 | ,094 |
| Laissez-faire Leadership (LF) | ,151 | ,051 |

**Limitation**

This study was limited to one college and result in inability to generalize results for other setting. Moreover, other factor that related with empathy, emotional intelligence and leadership were not investigated.

**Recommendation**

**Research**

It is recommended to use greater sample size and various setting for better generalization of study result. In addition, longitudinal study that follow student from junior level up to their senior level could give better illustration regarding the leadership style, empathy and emotional intelligence of nursing students during their study period.

**Nursing Practice**

In nursing practice, educators need to encourage transformational leadership for both junior and senior students in order to improve the quality of learning outcomes. Moreover, it is advisable for teaching staff to conduct learning and teaching session that able to improve empathy and emotional intelligence of their nursing students.

**Conclusion**

Both of the junior and senior students perceived their leadership to be more transformational rather than transactional leadership. In addition, there was no statistically significance difference for the relation between empathy and predominant leadership style. Consequently, most of the leadership domain were correlated negatively with empathy scores.

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# Histological and Hormonal Changes in Rat Endometrium under the Effect of Camphor

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**Abstract:** Camphor is prescribed in traditional medicine for the treatment of inflammation-related diseases and skin care products. The present study aims at finding out the effects of Camphor on the endometrium structures of Sprague–Dawley female Rats. 40 animals (3 months old) were divided into 4 subgroups (n = 10), 3 experimental groups were given daily intraperitoneal injection of 5, 10 and 20 mg/kg of Camphor watery solution and the control group was given distilled water. All groups were kept in the same environmental conditions. At the end of 6 weeks, all rats were killed and their uteri were removed for histological analysis. Comparing with the control group, an increase in the body and reproductive system weight, less uterine glands, degeneration of luminal epithelium and enlargement of uterus lumen were recorded. All the treated groups showed an increase in estrogen concentration. Furthermore, the highest dose caused an increase in progesterone concentration. The present study showed that Camphor could alter both hormonal and structural aspect of uterus that ultimately reflected on fertility of exposed animals.

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**Keywords:** Camphor, female rats, endometrium, progesterone, estrogen

## 1. Introduction

Several plants are now being used in part or as a whole to treat many diseases, active components of these plants are now being investigated, extracted and developed into drugs with little or no negative effects or contra-indication (Oluyemi *et al.,* 2007). Camphor is taken from the Camphor tree *Cinnamomum camphora* family Lauracae , it is extracted through the oil produced by distillation of the flowers and leaves of the camphor tree. However the bigger portion of camphor is extracted from the wood present in the stem and roots (Health Council of the Netherlands, 2001).

Camphor is used as a secretolytic medicine for relieving respiratory symptoms (Ciuman, 2012).

Nowadays camphor is synthetically produced from turpentine oil and is present in many non-prescription medicines such as Tiger Balm, Vick's vaposteam, Bayer Muscle and Joint cream and many other medicines (Ashby *et al.,* 2004). Camphor oil contains many compounds such as camphor, safrol, eugenol, terpeniol, cineol and ligans (Oudi, 2004).

Many studies were performed on animals to study Camphor toxicity, one study showed that the lethal dose of Camphor for dogs was 9-14 grams where this dose caused gradual paralysis of the central nervous system and suffocation .It was also shown that exposing animals to lethal doses of camphor caused nervous convulsions and oedema in the digestive system, kidney and brain (Clarke *et al.,* 1981).

Studies on the effect of camphor during pregnancy did not show any teratological effects, when rats were orally given different doses of camphor (Leuschner, 1997). It was also shown that continuous exposure of mice to camphor caused the appearance of cancer symptoms (Cincinnati , 2001).

In another study it was shown that volatile oils are strong inhibitors for bone metabolism and bone reabsorption in rats (Mühlbauer *et al.,* 2003). Jamshidzadeh and Sajedianfard, (2006) showed that several doses of Camphor affected all parts of the rat male reproductive system such as testis, seminal vesicles and vas deference.

Several medical reports have shown toxic effects of camphor on humans, it was shown that a dose of 0.06-4 grams caused vision disturbance, shivering weakness and paralysis (Grant and Charles, 1974). Camphor is easily absorbed through skin, small intestine and the respiratory tract, when taken orally, it causes very high hypertension within 5-10 min) Litovitz *et al.,* 1993). When using dermatological products containing camphor for a long period several symptoms were reported as rash and skin irritation. In cases of large doses acute toxicity might result leading to epilepsy, coma and death might occur of suffocation (Ford *et al.,* 2001).

In children the lethal dose was found to be 0.7-1 gram of camphor where it caused kidney and liver malfunction leading to urinary retention and albuminuria (Gosselin *et. al.,* 1984). Medical report has shown that 9 to 19 children were epileptic 14-120 min after swallowing 0.07 to 0.6 gm of camphor

(Ford *et. al*., 2001) and Camphor can be detected in the mother blood after 15 minutes from swallowing and through delivery after 36 hours. It was also detected in the amniotic fluid, the umbilical cord and the fetus blood where the fetus was unable to breathe after delivery (IPCS, May 1989).

Camphor compounds 3-Benzylidene camphor (3-BC) and 4-Methyl benzylidene Camphor (4MBC) are used as UV filters in many products such as hair and skin care products, household products, optical materials, textiles, fabrics and transdermal drug delivery systems. It was demonstrated that 3BC had adverse significant effect on the reproduction of fathead minnows in a dose-dependent manner (Health &Consumer protection directorate-General., 2006).

Camphor is widely used in Saudi Arabia with grave clothes (cerements), and it is traditionally known that pregnant women should not come near dead relatives because of camphor odors and its effect on the mother and embryo. As this information is traditional and there is a dearth of publications on the effects of this plant on the uterine tissues, this research was carried out to prove its reality and to study the effect of pure camphor on the female reproductive system using rats as a model animal.

## 2. Material and Methods

This study was given approval for the methodology and other ethical issues concerning the work by King Fahad Medical Research Center at King Abdulaziz University.

Camphor blocks were obtained from the traditional medicines market; it's used as aromatic substances added to dead bodies' wash in Saudi Arabia.

### Experimental Animals and Route of Administration:

Forty cyclic female Sprague-Dawley rats (3 month old) (200-300g) were sorted randomly from the animal house of the King Fahad Medical Research Center. The rats were kept in the animal control room and acclimatized for two weeks. The rats were fed on standard rat pellet produced by Bendel Feed and Flour Mills Limited. They were allowed access to water ad libitum and maintained under standard conditions.

The animal room was well ventilated with a temperature range of (22 ± 2 ◦C) under day/night 12-12 hours photoperiodicity. The rats were randomly grouped into four groups of 10 rats each, G1 (5mg/kg body weight), G2 (10mg/kg body weight), G3 (20mg/Kg body weight) and G4 (control) (Jamshidzadeh and Sajedianfard ,2006). The three experimental groups received intraperitoneal injections of camphor solution dissolved in distilled water, control group were injected by distilled water with the same doses and route of administration.

### Methodology:

All rats in this experiment were weighed before the first injection, and every Saturday and Tuesday of each week during the experiment. The estrous cycles were monitored by a method described by Marcondes *et al.* (2002) where vaginal smear was collected with a glass pipette filled with 10ml of normal saline (NaCl 0.9%). The vaginal fluid was placed on clean glass slide and observed under a light microscope at x 1OO and x400 magnification. The three types of cells recognized were epithelial, cornfield and leucocytes cells. The proportions among these cells were used to determine the estrous phases according to Long & Evans (1922).

### Histological studies:

At the end of 6 weeks, polyestrous female rats were sacrificed at proestrus stage of estrous cycle, the uteri of the animals from each group were dissected out by laparatomy immediately and both the length and size of them were measured. Also, anatomical photos were taken for them using Nikon coolpix s10 digital camera .The removed uteri were per fused in normal saline, blotted dry and weighed in electronic weighing balance and then fixed in 10% buffered formalin for histological assessment ,7 micron thickness paraffin sections were cut and stained with hematoxylin-eosin, examined and photographed using digital camera connected to computer. The thickness of endometrium was measured from the histological photos.

### Hormonal assays:

Animals were slightly anaesthetized and their eyes were bled within two minutes by using a heparinized syringe and blood samples were taken from each rat by the end of the experiment (after six weeks). The blood samples (10 ml) were collected in EDTA tubes, then centrifuged at 1200 rpm for 10 minutes to separated the plasma from the blood and kept at -20 ° C. Hormonal assay for estrogen and progesterone serum levels was done using (Estradiol-E2, Elecsys and cobase analyzers, Roche Diagnostic Gmbh.D-68298 Mannheim: US Distributor), (Progesterone 12145383122, Elecys 1010/2010 and MODULAR ANALYTICA E170, Roche Diagnostic Gmbh.D-68298 Mannheim: US Distributor) and correlated with histological endometrial changes.

### Statistical analysis:

The whole body weight, weight of reproductive system, the length and width of uterus .Also thickness of endometrium and estrogen &

progesterone concentration data were compared at appropriate confidence intervals. Values are recorded as mean ± S.E.M. and all data were statistically analyzed using SPSS 13 for windows. The normality test was done using One way Anova , Test of Homogeneity of Variances, then if data was normally distributed Student-Newman-Keuls test and Tukey test were performed to see if there were any significant differences between the treated and control groups. Whenever the data was not normally distributed nonparametric tests were done and these were Mann-Whitney U test and Kolmogrov-Smimov Z test to see if there were any significant differences between the treated and control groups. In all cases difference was considered significant if ($p$<0.05).

## 3. Results

During the experiment, all animals survived and this meant that the doses administered the laboratory conditions of water, food and shelter was appropriate.

Whole body weight:

We noticed an increase in the whole body weight of all experimental rats and this increase was significant ($p$<0.05) with the high dose G3 (20 mg/kg) only on the days 3,10,14,21 ($p$=0.049, 0.036, 0.039, 0.044) of the experiment one compared to the controls (Fig.1).

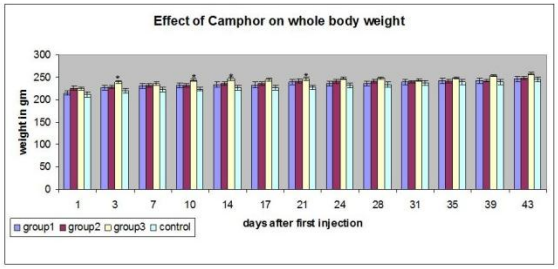


**Figure. (1):** Showing the effect of Camphor on the whole body weight. Values are means ±SD, n =5. *$P$<0.05

**Whole weight of reproductive system:**

The average of reproductive system weight of those animals treated with Camphor dissolved in distilled water was somewhat more than for those given water alone, as there were increase in the whole weight of the reproductive system in all experimental groups but this increase was not significant in the highest Camphor dose G3 (20 mg/kg) compared to the controls (Fig. 2).

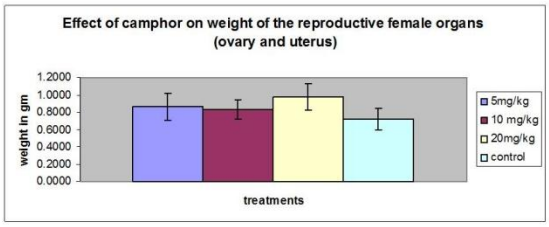


**Figure (2):** Showing the effect of Camphor on the female reproductive system weight. Values are means ±SD, n =5.

**The length and the width of uterus horns:**

There was a non-significant decrease in the length of the uteri horns in all experimental groups and this decrease was indirectly proportional with the injected doses as the most noticeable decrease was recorded in the lowest Camphor dose G1 (5 mg/kg) compared to the controls (Fig. 3), but the width of these uteri increased non significantly in the experimental groups as the most widest one was G2 (10 mg/kg) compared to the controls (Fig. 4).

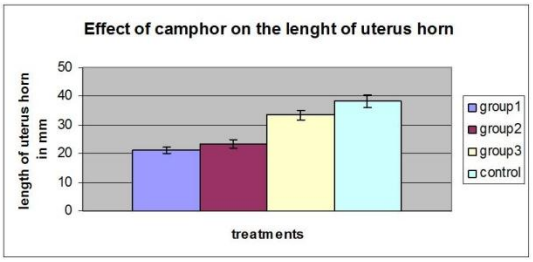


**Figure (3):** Showing the effect of Camphor on the length of uterine horn. Values are means ±SD, n =5.

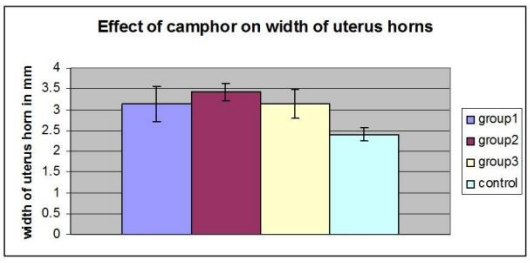


**Figure (4):** Showing the effect of Camphor on the width of uterine horn. Values are means ±SD, n =5.

**The endometrial thickness:**

There was a slight insignificant increase in the thickness of endometrium between the treated and the control groups and this increase was noticeable in G2 (10 mg/kg) agree with the anatomical measurements (Fig. 5).

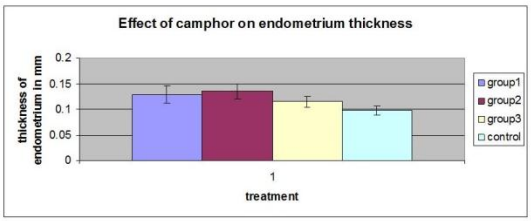


**Figure (5):** Showing the effect of Camphor on the endometrium thickness. Values are means ±SD, n =5.

**The level of estrogen and progesterone concentration:**

In the present study hormonal assay revealed a significant dose dependant increase in serum estrogen in Camphor injected animals (Fig.6). On the other hand significant decrease in progesterone levels was observed in G1 (5mg/ kg) and G 2 (10mg /kg) while an increase was observed in G3 (20 mg /kg) compared to control group (Fig.7).

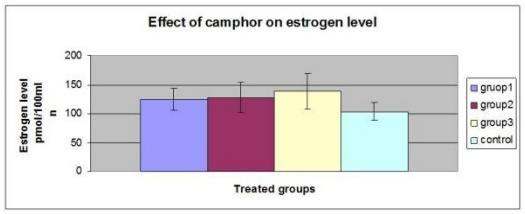


**Figure. (6):** Showing the effect of Camphor on estrogen level in blood. Values are means ±SD, n =5.

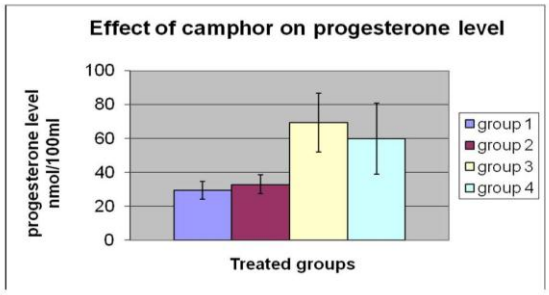


**Figure (7):** Showing the effect of Camphor on progesterone level in blood. Values are means ±SD, n =5.

**Histological analysis:**

Upon examining the histological sections of the different parts of the uterus of the treated rats and comparing them with the controls, several remarks were noted as follows:

1-G1 (5mg/kg body weight):

The examination of treated rat uterus histological sections and comparing it to the controls revealed that the number of the uterine glands was fewer with an increases in the endometrial thickness; also the lumen cavity became wider (Fig. 9) .In the basal portion of the endometrium the vascular channels were distended and the stroma was denser (Fig. 10). Many of the luminal epithelial cells were undergoing supranuclear vacuolation, exhibited small, hyper chromatic nuclei and contained in-between necrotic cells (Fig. 11).

**2- G2 (10 mg/kg body weight):**

The lumen of uterus in G 2 became wider in compared with the other treated groups and the control group and the endometrial thickness increased with a significant lower in the uterine glands (Fig.9), also more dilated vascular channels and hyperplasia of the stroma cells in the basal portion of the endometrium were seen (Fig.10). The luminal lining epithelial cells were reduced in height with rounded nuclei i. e. the endometrial epithelium displayed cubical epithelium and there is cytoplasmic vacuolation. Noticeable stroma esinophilic infiltration was indicated (Fig. 11).

3- G3 (20gm/kg body weight):

Sections of G 3 (20 mg/kg) showed large lumen and the endometrial thickness increased as a result of endometrial stromal cell proliferation with very small endometrial glands in compared to the control( Fig. 9). The examination of the basal portion of the endometrium revealed distended vascular channels with stasis of red blood corpuscles and the connective tissue fibers in the stroma were densely packed Fig. 10). There was a marked decrease in the height of the uterine epithelium, with malformation in the lining cells where lytic cells with degenerated nuclei in between the low height luminal epithelial cells which contained irregular nuclei (Fig.11).

**4. Discussions**

Camphor is used nowadays as an active ingredient in many substances such as cosmetics especially creams protecting from UV (Schlumph *et al.*, 2001, Tinwell *et al.*, 2002, Schlumph *et al.*, 2004 and Wagner, 2006), medicines, rubs for muscles and colds (Manoguerra *et al.*, 2006) .It is also used as moth repellent and an artificial flavoring to give acceptable odor to insecticide (Wagner, 2006).

In this study Intraperitoneal injection of rats with Camphor caused a significant increase in the whole body weight which agrees with the study of Ttinwell *et al.*, (2002) where immature female rats were subcutaneously or orally injected with 500 – 800 mg/kg 4MBC, both groups had significantly increased whole body weight compared to the controls. The increase in body weight in response to Camphor involves increase in the reproductive system weight which was explained as due to more

liquid ambition as shown before with 4MBC which caused a slight increase in uterus weight (Seidlová-Wuttke *et al.*, 2006).

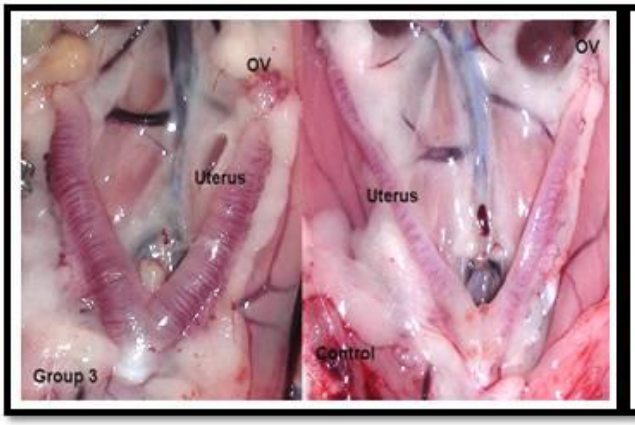


**Figure (8):** Photographs of female reproductive system, showed the increase in uterine width in response to Camphor in G3 (20 mg/kg) compared to the control.

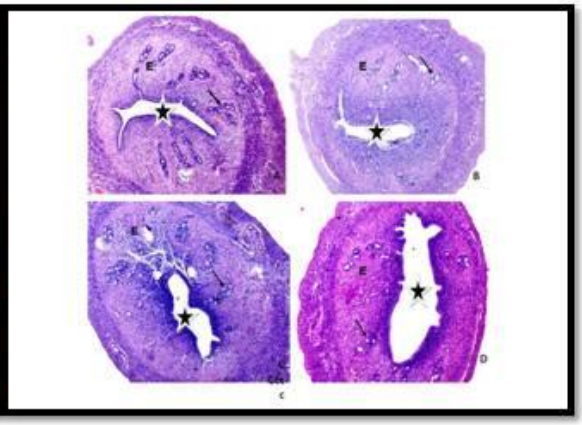

**Figure. (9):** Showing a difference in the pro-estrous uterus cavity (*) between the control and treated groups. (A) Control, (B) G1(C) G2 (D) G3. As seen the lumen cavity becomes wider and a significant lower in the uterine glands (arrow) with more treatment compared to the control. G2 shows the most increase in the endometrial thickness (E) (X40) (H&E).

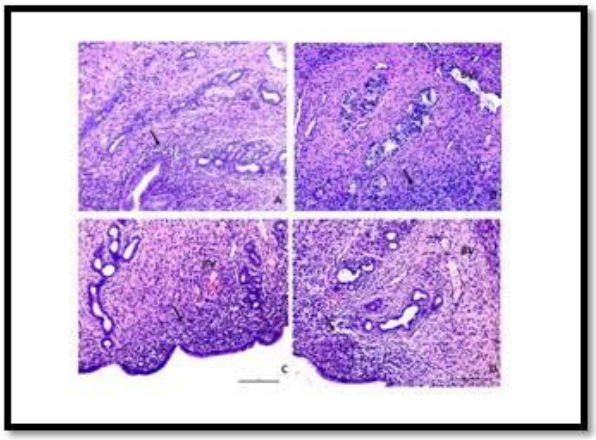

**Figure. (10):** Showing a difference in the pro-estrous endometrium between the control (A) and treated groups, G1 (B) G2 (C) G3 (D). In the basal portion of the endometrium the vascular channels (BV) were distended, the stroma became denser (arrows) with more treatment compared to the control (X100) (H&E). 2

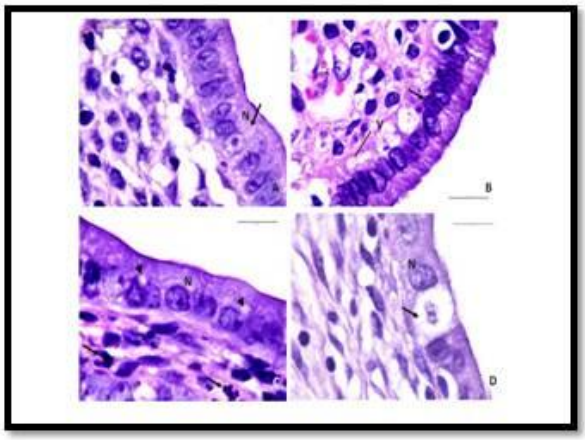

**Figure. (11):** Showing a difference in the pro-estrous endometrium between the control (A) and treated groups, G1 (B) G2 (C) G3 (D). In control (A) the uterine lumen is lined by tall columnar epithelium (arrow) with oval nucleus(N), in G 1(B) many of the luminal epithelial cells undergo vacuolar degeneration (V) and necrosis (arrows), in G2(C) the luminal lining epithelial cells are reduced in height with rounded nucleus (N) , there is cytoplasmic vacuolation (head-arrow), the stroma esinophilic infiltration (arrow),in G3 lytic cell ( arrow ) in between the low height luminal epithelial cells with deformed nucleus (N) (X1000) (H&E).

On the other hand, Falodun *et al.* (2006) reported the toxic effects of Aspiliaafricana which have anti-fertility effects on the function of isolated uterus from female rats, he found a decrease in the weight of the uteri which could be due to the ability of the extract to contract smooth muscle fibers as reported by Dimo *et al.* (2002), they reported that, extract of *Aspilia africana* increases *in vitro* vascular smooth muscle contraction in rats' aortic ring preparations. These effects are most probably due to imbalances in hormonal level caused by high level of saponins and other phytoestrogens found in this plant.

In this study there was a difference in the lumen width of the uterus where it seemed wider with more Camphor treatment although both control and treated uteruses were in proestrus stage when fixed as shown by the vaginal smears. This dilation led to uterus swelling as seen in the anatomical photos of the treated female rats.

Camphor is considered as an endocrine disruptor and an estrogen agonist (Caserta, 2008). Therefore it caused hormonal imbalances which led to increase in endometrium thickness as the thickness of endometrium varies considerably according to the individual's hormonal state (Spornitz, 1992) and hormonal balance is required for implantation and proper development of concepts (William, 1999) and this might explain the toxic effect of Camphor on the histological appearance of the uterus.

During proestrus in rats, the uterine lumen is distended with clear fluid and the lumen is lined by large low columnar cells (Yuan and Foley, 2002) and all cellular components of the uterus respond to steroid hormones such as estrogen, which stimulates DNA synthesis and cellular proliferation in the uterus of mammals (Mendoza-Rodriguez *et al.*, 2003). In the current study, for treated rats in the proestrus stage, the highest degree of cellular proliferation was seen in the stromal cells only and many of the luminal and glandular epithelial cells undergo vacuolar degeneration and necrosis as mentioned before (Radi and Khan, 2006).

Mendoza-Rodriguez *et al.*, (2003) illustrated that when progesterone levels drops significantly, there is a corresponding decrease in glandular and luminal epithelial proliferation and increased apoptosis in these cells .In corroboration, our data demonstrated that cellular proliferation was minimal in the glandular and luminal uterine compartments during this study, as shown before when 4MBC inhibited slightly and non-significantly endometrium proliferation (Seidlovă-Wuttke *et al.*, 2003) and the luminal lining epithelial cells are reduced in height, the stroma becomes denser, and mitotic activity decreases (Yuan and Foley, 2002).

Studies in several species have addressed the role of progesterone (P4) in modulating estrogen (E2) activity and maintaining the uterus in a state of quiescence or inactivity (Gimple and Fahrenholz, 2001). Progestrone is secreted for only a limited time by the rat unless a leuteotropic signal from the pituitary is received (Carson *et al.*, 2000). . Appropriate E2/P4 synergism is governed by co activators and repressors of steroid receptors (Chen *et al.*, 2005). For example, estrogen receptor negative uteri are hypoplastic while progesterone receptor negative uteri are hyperplastic.

The results of this research showed that Camphotr possess negative influences on histo-architecture of the uterus of female rats suggesting negative influences on the reproductive health of the animals. Therefore more studies have to be done on the effect of camphor on the histology of the female reproductive system in mammals, and its effect on placenta formation and pregnancy continuation.

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# Effect of Humic Acid Isolated by IHSS-$N_2$/Mn Method and P Fertilization on Yield of Pepper Plants

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**Abstract:** A field experiment was carried out at Ismailia Agriculture Research Station, Agriculture Research center, during summer season of 2010, to benefit from composting of remnants of food factories and then humic acid (HA) was extracted from mature compost and using it with P fertilization to study their impact on yield and nutrients content of pepper plants. Extraction and purification of humic acid (HA) was based on the IHSS –$N_2$ method and IHSS –$N_2$/Mn method (IHSS, "International Humic Substances Society"). Total acidities and phenolic hydroxyls contents are the highest for HA isolated by modified method (IHSS-$N_2$/Mn) which indicates that the HA posse less altered eases oxidisable phenolic hydroxyl groups. Humic acid isolated by IHSS-$N_2$/Mn method and applied with drip irrigation water as it was added every two weeks starting from the stage of germination till harvest. Treatments were representing at all the combinations of humic acid (1, 2 and 3 ml $L^{-1}$) and P fertilizer rates 60, 90 and 120 kg $P_2O_5$ $fed^{-1}$ (fed. Equal 4200 $m^2$). Results showed that, increasing the rates of HA and P fertilization increased pepper yield (quality and quantity) compared with that of control (without humic acid or P fertilization). The most promising treatments for production of marketable and unmarketable pepper yield could be: Those of (3 ml $L^{-1}$ of HA+ 120 kg $P_2O_5$ $fed^{-1}$) which showed an increment of (+10.0%) and (+7.22%), respectively. This high rate could be recommended for obtaining the highest rate of income from the marketable yield of pepper.

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**Key word:** Humic acid (HA), IHSS –$N_2$/Mn method, P fertilization, Pepper yield.

## 1. Introduction

Food processing is a major industry that is rapidly growing because of a demand for packaged foods in urban areas. The companies of food processing have large processing facilities where vegetables, such as lettuce, cabbage, onions, peas, artichoke, etc. are cleaned, chopped, mixed and packaged. In a typical operation, the amount of wastes generated equal in quantity (by weight) to the amount of product shipped. Presently, these wastes are land disposed or land filled. Vegetable wastes do not provide any known concerns relating to pathogens or human health issues, however, they are prone to potential odors during decomposition and are expensive to dispose because of their high moisture content leading to high landfill tip fee and transportation cost.

Composting can be defined as being the breakdown of organic materials by large numbers of microorganisms in a moist, warm and aerated environment leading to the production of carbon dioxide, water, minerals and stabilized organic matter. The process generally starts by stacking the organic wastes in piles. The mixture is then composted in the presence of air for a period of 4-12 weeks depending on the type of system used, followed by a maturation phase (curing) of approximately the same duration ( Diaz *et al*., 2002). Jovičić *et al*., (2009) showed that the creation of compost has become a more popular option of waste management as a waste and reduce pressure on landfill. Because of the importance of composting in order to achieve the objectives of waste management in the world. As necessarily, regional composting plants need to be built. Reusing this materials will remarkable reduce final quantity of deposited wastes. This is the main benefit, but compost is very useful product in agricultural business, that's why material retrieves validity. Remarkably, (McGuckin *et al*., 1999) reported that the sulfur content of lettuce and onion wastes were 0.2 and 0.7 %, respectively. Discarded components of lettuce and onions have an effective carbon to sulfur ratio of 215 and 62, moisture contents of 96.2 and 91.1% and carbon to nitrogen ratios of 10.3 and 11.5, respectively. The low C/S ratio of onions indicates that mixes with high fractions of onions can result in release of odorous sulfur compounds. High water content, most of which is bound within the vegetable fiber, results in significant leachate formation during composting and collapse of the composting matrix from initial height of e.g. 1.5 m to a lower value of e.g. 0.5 m resulting in reduction in air space and oxygen availability within the pile.

Humic acid (HA) is a major fraction of humic substances, forms strong complexes with metal and plays a vital role in metal ion mobilization and transportation in the subsurface soil and aquatic environments. Humic acids capability for binding metal ions is largely due to diverse functionalities in the HA structure (Osman *et al*., 2009). Humic acid is one of the most active fractions of organic matter, it improves the absorption of nutrients by

plants and soil microorganisms, have a positive effect on the dynamic of N and P in soil, stimulate plant respiration and the photosynthesis process, and favor the formation of soil aggregates, etc. (Hernandez et al., 2001 and Brunetti et al., 2007).

There are several of methods for extraction of HA, using different extraction reagents, which are summarized in Table (1):

Table (1): Methods of HA extraction.

| Type of material | Extractant | Organic matter extracted |
|---|---|---|
| Humic substances | NaOH | to 80 % |
| | Mild extractants: | |
| | $Na_4P_2O_7$ and other | to 30 % |
| | Organic chelates: acetyloacetone, cupferron, hydroxyquinoline | to 30 % |
| | Formic acid (HCOOH) | to 55 % |

In general, the disadvantages of alkali extraction are as follows: a) alkali solutions dissolve silica from the mineral matter and this silica contaminate the organic fractions separated from the extract; b) alkali solutions dissolve protoplasmic and structural components from fresh organic tissues and these become mixed with the humified organic matter; c) under alkaline conditions, autoxidation of some organic constituents occurs in contact with air both during extraction and when the extracts are allowed to stand; d) in alkaline solution condensation between amino acids and aldehydes or quinines can occur (Andelkovic *et al.*, 2001).

Because of the contact air with humic substances material under alkaline condition, new method like IHSS was established (Sparks, 1996). However, for isolation of even less altered material, the presence of $O_2$ in the alkaline extraction solution (AES) should not be neglected, although it usually is. Andelkovic *et al*., (2001) modified standard IHSS procedure by using $MnSO_4$ for deoxygenation of AES. Dissolved oxygen present in the AES, rapidly oxidizes dispersed divalint manganous hydroxide precipitate to hydroxide of higher valence state, as shown in the equation (1):

$$Mn^{2+} + OH^- \rightarrow Mn(OH)_2 + O_2 \rightarrow MnO(OH)_2 \ldots\ldots\ldots\ldots \text{Equation (1)}$$

This deoxygenized AES is, after filtration in $N_2$ atmosphere, used for humic acid extraction. Therefore, the aims of this research were: (i) comparison between the two extraction methods of HA isolated from compost to choose the best way of extraction using FT-IR and E4/E6 ratio; (ii) study the effect of humic acid and P fertilization rates on nutrients content and yield of pepper.

**2. Materials and Methods**

A field trial was conducted on a loamy sand soil at Ismailia Agricultural Research Station, by cultivating pepper (*Capsicum annum L.*, cv Marrkony) at summer season of 2010. Main and interaction effects of different rates of HA and super phosphate (as P source) on yield components and nutrients content of pepper plant were investigated. The experiment was carried out following the randomized complete block design, with three replicates for each experimental unit. Humic acid was added with drip irrigation water as it was added every two weeks starting from the stage of germination until harvest, which was combined with three $P_2O_5$ rates of (60, 90 and 120 kg $P_2O_5$ $fed^{-1}$) in the form of superphosphate (15 % $P_2O_5$). N fertilization rate (80 kg N $fed^{-1}$) was added in the form of ammonium sulfate (20 % N). K fertilization rate (24 kg $K_2O$ $fed^{-1}$) was added in the form of potassium sulfate (50 % $K_2O$). The N and K fertilization was run entirely through preparing the soil before planting, at the recommended doses of mineral N and K fertilization (ammonium sulfate = 400 kg $fed^{-1}$ as source of N and potassium sulfate = 300 kg $fed^{-1}$ as source of K) and also without HA addition to act as a control treatment which were compared to the other treatments.

Humic acid were extracted from the compost, which was made from remnants of food factories, where the waste was a peel fruits of pea and artichoke leaves fruit and broccoli plant residues. Composting process was over within two months. Compost has been added to the experience at one of 10 ton/$fed^{-1}$.

Extraction and purification of HA from compost was based on two methods to choose the best of HA, one of those methods was traditional extraction of HA (International humic acid substances society, IHSS- $N_2$ method), while the other was the modified method of IHSS (IHSS-$N_2$/Mn method). It is known that, both of the two methods are the same technique; however, $MnSO_4$ is added in the modified method. In initial treatments, phosphoric acid was used rather than

HCl, which is recommended by IHSS, because $H_3PO_4$ would prevent the oxidation of organic substances by the Fe (III) ion. Also, alkaline extraction solution was deoxygenized by dissolving 2.0 g $MnSO_4$ in 1000 ml KOH solution at pH 11. After 12h, the solution was filtered, under $N_2$ atmosphere, in order to decrease $O_2$ level as much as possible. HA was dissolved in 0.1 M KOH (prepared with distal water in which $N_2$ was purged in order to remove dissolved $O_2$), in a separating funnel of appropriate volume so that no air was left over the solution surface. HA was passed over Dowex and collected in a flask (in which a minute volume of $H_2SO_4$ was added) connected with $N_2$. HA rapidly coagulates in acid solution. The protonated HA was centrifuged, this method was described by Andelkovic *et al*., (2001).

The experimental soil plots were sampled initially before pepper planting to determine some physical and chemical properties according to the standard procedures outlined by Cottenie (1980) (Table, 2).

Chemical properties of the tested humic acid was measured according to the standard methods described by Cottenie (1980). The infrared spectra (FT-IR) were recorded from pellets containing 2 mg of the dried humic acids with 250 mg of dry KBr. The instrument used was PerkinElmer 1600 FTIR spectrophotometer covering a wave number range of 400-4000 $cm^{-1}$. The E4/E6 parameter was determined by UV\VIS method on a spectrophotometer by dividing the absorbance of 2 mg dried humic acid in 25 ml 0.025 M $NaHCO_3$ at 465 nm by absorbance at 665 nm.

Plant samples were collected from mature pepper plants at harvest stage for analysis. Plant samples were dried at 65C° for 48 hrs, ground and wet digested using $H_2SO_4$: $H_2O_2$ method (Cottenie, 1980). The digests were then subjected to measurement of N using Micro-Kjeldahl method; P was assayed using molybdenum blue method, while, K was determined by Flame Photometer (Chapman and Pratt, 1961). Ascorbic acid content was assayed using oxalic acid method (Jacobs, 1951).

Table (2): Some physical and chemical properties of the soil used.

| Soil property | Value | Soil property | Value |
|---|---|---|---|
| Particle size distribution % | | pH (1:2.5 soil suspension) | 7.52 |
| Coarse sand | 69.9 | ECe (dS $m^{-1}$) | 1.26 |
| Fine sand | 14.2 | Soluble ions (meq $L^{-1}$) | |
| Silt | 5.70 | $Ca^{++}$ | 5.66 |
| Clay | 10.2 | $Mg^{++}$ | 4.08 |
| Texture | Loamy sand | $Na^{+}$ | 1.94 |
| $CaCO_3$ % | 2.10 | $K^{+}$ | 0.22 |
| Saturation percent | 23.3 | $CO_3^{--}$ | nd* |
| Organic carbon % | 0.02 | $HCO_3^{-}$ | 1.99 |
| Available N (mg $kg^{-1}$) | 9.36 | $Cl^{-}$ | 4.78 |
| Available P (mg $kg^{-1}$) | 1.81 | $SO_4^{--}$ | 5.13 |
| Available K (mg $kg^{-1}$) | 65.9 | CEC (meq 100 $g^{-1}$ soil) | 6.50 |

nd : not detected

## 3. Results and Discussion

### 3.1. Comparison between the two humic acid extraction methods (IHSS -$N_2$ and IHSS-$N_2$/Mn)

Results in (Fig, 1) indicated that, the main common and different FT-IR features of HA isolated from compost by IHSS-$N_2$ and IHSS-$N_2$/Mn methods, and their corresponding assignments, according to Stevenson (1994); Plaza *et al.* (2002); Senesi *et al.* (2003) they found: a common, intense broad band at about 3400 $cm^{-1}$ usually attributed to O-H stretching, the intensity of 3373 cm-1 with the IHSS-$N_2$ increases in the other way of extraction. This line is observed in spectra of distillate water.

In this situation links in the range of 2923 $cm^{-1}$ are reduced which are related to $CH_2$ bond. These links are weakened and with some displacement are observed in the range of 2921-2923 $cm^{-1}$.The bands observed at 2923 cm-1 and at 2845 $cm^{-1}$ are due to the asymmetric and symmetric stretching modes of the methylene chain in the membrane lipids. The band at about 1445 $cm^{-1}$ assigned to the aliphatic C-H deformation, corresponding to HA obtained from IHSS-$N_2$/Mn method similar for HA obtained from IHSS-$N_2$. The absorption at about 1700 $cm^{-1}$ due to C=O stretching of COOH and other carbonyl groups are more intense for HA obtained from IHSS-$N_2$/Mn method than for this HA obtained from IHSS-$N_2$ method, where they appear at 1732 and 1710 $cm^{-1}$ respectively. The band at 1642 and 1632 cm-1 are related to c=c aromatic in the HA extracted by IHSS-$N_2$ and IHSS-$N_2$/Mn respectively. The band at about 1500 $cm^{-1}$, preferentially ascribed to N-H deformation and C=H stretching of amides, are more evident in FT-IR spectra of HA obtained from IHSS-$N_2$/Mn method than in spectra of HA obtained from IHSS-$N_2$ where it appears at 1515cm-1. The band about 1280 $cm^{-1}$ is generally ascribed to C-O stretching and deformation of carboxyl and C-O stretching of

ethers and phenols has medium intensity in the spectra corresponding to HA obtained from IHSS-$N_2$/Mn method only, and did not appear in spectra corresponding to HA obtained from IHSS-$N_2$ method. The absorption at about 1000-1200 $cm^{-1}$, ascribed to C-OH stretching of aliphatic O-H, corresponding to HA obtained from IHSS-$N_2$/Mn method similar for HA obtained from IHSS-$N_2$. While, it is common to find P=O group (phosphen oxide) at wavenumber 1172$cm^{-1}$ for HA obtained from IHSS-$N_2$/Mn.

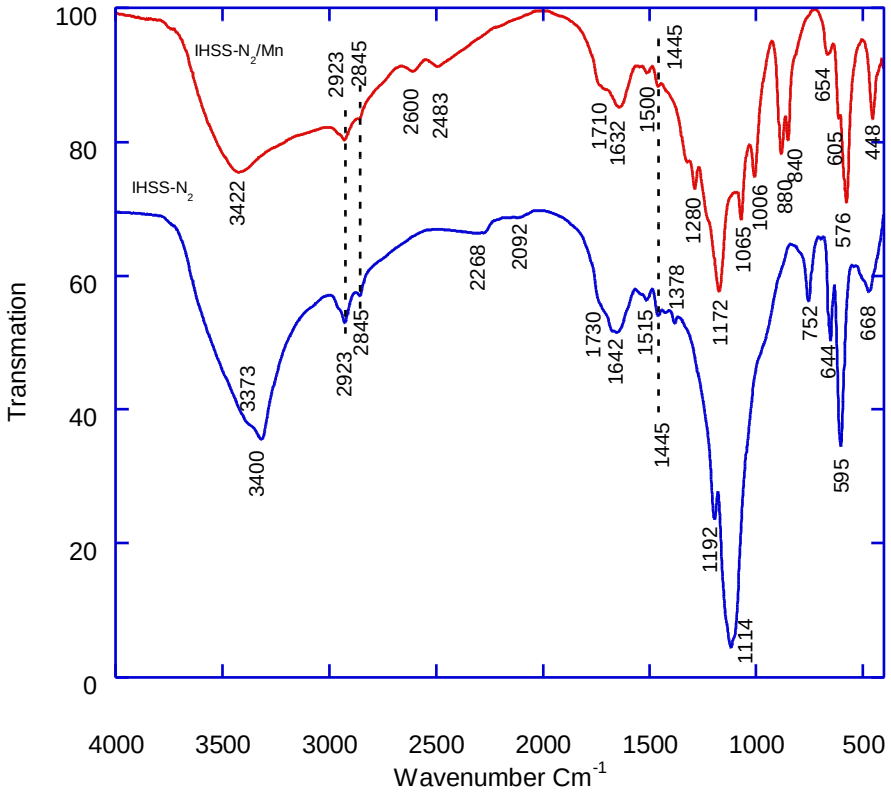


Fig. 1: FT-IR spectra of the humic acid isolated from compost by IHSS-$N_2$ and IHSS-$N_2$/Mn methods

The band in the range from 1580-1650 cm-1 are related to the group function Si-O-Si which obvious found in the HA extracted from IHSS-$N_2$, while, it is not found in IHSS-$N_2$/Mn method of extraction. The band ranges below 1000cm-1 is related to metal ion complex, it is obvious to be more in the HA extracted by IHSS-$N_2$/Mn due to the presence of Mn.

Nitrogen, phosphorus and potassium content, C/N ratio and *E*4/*E*6 ratio of HA studied are shown in Table 3. Two methods extraction did not differ in N, P and content as well as C/N ratio since this ratio is an indicator of source of humic acid in natural system. Therefore, as long as the source of HA from the same source (compost), the C/N ratio of HA did not differ of two methods (Meyers and Ishiwatari, 1993 and Chai *et al*., 2007).

From the above mentioned discussion it is obvious that the best HA extraction methods method, is related to the IHSS-$N_2$/Mn. This may be due to the ability to make metal complexes with Mn which affect the absorption of the nutrient over the colloids and increase the nutrient transfer to the plant. Furthermore, the total acidities and phenolic hydroxyls contents are the highest for HA isolated by modified method (IHSS-$N_2$/Mn) which indicates that the HA posse less altered eases oxidisable phenolic hydroxyl groups.

E4/E6 ratio of HA extracted from IHSS-$N_2$/Mn method was more than E4/E6 ratio of HA extracted from IHSS-$N_2$ method, so HA extracted from IHSS-$N_2$/Mn method is a low content of carbon, a low molecular weight, a high total of oxygen and COOH groups and a high total acidity. Polak *et al*., (2007) reported that UV/VIS spectroscopy is the source of information on the composition of molecules of HA and on their origin. For this purpose *E*4/*E*6 ratio value was determined. The low E4/*E*6 ratio for HA may be attributed to the absorption by aromatic C=C functional groups. Additionally, the high degree of condensation of the aromatic rings as well as the large molecular weight of HA are believed to contribute to its relatively high absorption in the visible range (Chen *et al*., 2004). On the other hand, the high value of *E*4/*E*6 ratio points to a low molecular weight of HA, a low content of carbon, a high content of oxygen and COOH groups. Morever it also indicates a high total acidity and a high degree of aromatization of humic substances

(Polak *et al.*, 2011 ). Accordingly, the method of HA extraction by modified method of IHSS (IHSS-$N_2$/Mn). Andelkovic *et al.*, (2001) showed that HAs were isolated and purified by three method: by modified method, IHSS-$N_2$/Mn method, IHSS method and IHSS-$O_2$ method. Concerning differences in the obtained data of all three HAs, it is reasonable to assume that significant alteration occurs in the cases when $MnSO_4$ is not used in the isolation procedure. So, it is recommended to use this modified, IHSS-$N_2$/Mn method in isolation of HA with more preserved structure.

Table (3): Nitrogen, phosphorus and potassium content, C/N ratio and E4/E6 ratio of HA isolated by IHSS-$N_2$ /Mn and IHSS-$N_2$ methods.

| Source | pH (1:2.5 ) | N% | P% | K% | Organic carbon % | C/N | $E_4/E_6$ ratio |
|---|---|---|---|---|---|---|---|
| Humic acid (IHSS -$N_2$/Mn method) | 7.65 | 2.11 | 1.36 | 3.27 | 51.8 | 24.5 | 2.02 |
| Humic acid (IHSS -$N_2$ method) | 7.66 | 2.10 | 1.36 | 3.27 | 53.5 | 25.5 | 1.87 |

**3.2. Effect of humic acid and P fertilization on yield components of pepper plants.**

Results in (Table, 4) indicate that increasing P fertilization rate under humic acid (HA) rates significantly increased for yield components. The mostly induced parameters, i.e., marketable, unmarketable yields (unmarketable yield was mean first and second packing), fruit length and diameter, ascorbic acid content, and total chlorophyll all of which under the highest rates of applied HA (3 ml $L^{-1}$) as well as the highest P fertilization rate (120 kg $P_2O_5$ $fed^{-1}$).

In other words, the dual synergistic effect probably was mutual for HA and P fertilization rates. However, the average values of yield and yield parameters increased significantly under highest HA compared with the lower HA one. Türkmen *et al.*, (2005) reported that humic acid application increased the plant growth, nutrient uptake and pepper yield and quality. Increasing the rate of both P and organic fertilizer treatments significantly enhanced fresh fruit yield per plant when compared with the control treatment. This also enhanced significantly the yield per hectare and yield components such as the fruit length and diameter (Alabi, 2006). Mesut *et al.*, (2010) reported that high levels of HA and P applications increased the growth and growth parameters, as well as pepper yield components than each separate effect. Results in Table (5) indicate decrements in both marketable and unmarketable yield of pepper under all treatments as compared with control. The rate of reduction was partially compensated by increasing the added humic acid rate from 60 to 120ml $L^{-1}$ and the addition of highest rates of P rates consistently. The high reduction in marketable and unmarketable yield amounted to (-18.42% and -7.222%), respectively, under lower rate of humic acid (1 ml $L^{-1}$) and P fertilization rate (60 kg $fed^{-1}$). This reduction of both yield reduced by increasing humic acid and P fertilization rates.

The most promising treatment (3 ml $L^{-1}$ of HA + 120 kg $P_2O_5$ $fed^{-1}$) which showed an increments of (+10.0%) and (+7.222%) of marketable and unmarketable yield. Account these values into net income by taking into considerations the price of added fertilizer and expected price of marketable yield, the calculations reveal that the net income for the treatment could be higher than that of control treatment by 400$. The treatment of (3 ml $L^{-1}$of HA + 120 kg $P_2O_5$ $fed^{-1}$) could be recommended for obtaining the highest rate of income from the marketable yield of pepper.

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Table (4): Interaction effect between humic acid and P fertilization rates on yield and yield components of pepper plant.

| Humic acid rates ml \L | $P_2O_5$ kg fed$^{-1}$ (P) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 60 | 90 | 120 | mean | 60 | 90 | 120 | mean |
| | marketable yield ( ton fed$^{-1}$) | | | | Unmarketable yield ton fed$^{-1}$ | | | |
| 1 | 7.750 | 7.990 | 9.270 | 8.337 | 1.670 | 1.750 | 1.860 | 1.760 |
| 2 | 8.660 | 9.380 | 9.630 | 9.223 | 1.680 | 1.810 | 1.900 | 1.797 |
| 3 | 9.000 | 9.380 | 10.45 | 9.610 | 1.750 | 1.850 | 1.930 | 1.843 |
| Mean | 8.470 | 8.917 | 9.783 | | 1.700 | 1.803 | 1.897 | |
| L.S.D.$_{0.05}$ humic acid = 0.243 P = 0.240 Humic acid *P = 0.342 Control = 9.500 | | | | | L.S.D.$_{0.05}$ humic acid = 0.058 P = 0.039 Humic acid *P = 0.055 Control = 1.800 | | | |
| | Fruit length ( cm) | | | | Fruit diameter (cm) | | | |
| 1 | 9.560 | 9.630 | 10.00 | 9.730 | 8.110 | 8.310 | 8.390 | 8.270 |
| 2 | 10.55 | 11.49 | 11.73 | 11.26 | 8.410 | 8.710 | 8.960 | 8.693 |
| 3 | 11.60 | 11.74 | 11.84 | 11.73 | 8.700 | 8.790 | 9.120 | 8.870 |
| Mean | 10.57 | 10.95 | 11.19 | | 8.407 | 8.603 | 8.823 | |
| L.S.D.$_{0.05}$ humic acid = 0.284 P = 0.502 Humic acid *P = 0.711 Control = 11.64 | | | | | L.S.D.$_{0.05}$ humic acid = 0.112 P = 0.230 Humic acid *P = 0.326 Control = 8.620 | | | |
| Vitamin C (%) | | | | | Total chlorophyll | | | |
| 1 | 71.52 | 72.15 | 72.52 | 75.77 | 45.36 | 48.80 | 50.60 | 48.25 |
| 2 | 74.19 | 79.91 | 81.38 | 51.93 | 53.25 | 54.60 | 55.90 | 54.58 |
| 3 | 81.60 | 83.04 | 83.97 | 82.87 | 55.38 | 55.97 | 56.50 | 55.95 |
| Mean | 75.77 | 78.37 | 79.29 | | 48.25 | 50.12 | 54.33 | |
| L.S.D.$_{0.05}$ humic acid = 2.139 P = 1.227 Humic acid *P = 1.733 Control = 82.10 | | | | | L.S.D.$_{0.05}$ humic acid = 2.113 P = 1.201 Humic acid *P = 1.633 Control = 54.50 | | | |

Table (5): Surplus (+) or deficit (-) values for yield relating the different experimental treatments over or under those obtained by the control treatment.

| Treatment | | Percentage of Yield | |
|---|---|---|---|
| Humic acid ml/L | $P_2O_5$ (kg fed$^{-1}$) | Marketable | Unmarketable |
| 1 | 60 | -18.42 | -7.222 |
| | 90 | -15.89 | -2.778 |
| | 120 | -2.421 | +3.333 |
| 2 | 60 | -8.842 | -6.667 |
| | 90 | -1.263 | +0.556 |
| | 120 | +1.368 | +5.556 |
| 3 | 60 | -5.263 | -2.778 |
| | 90 | -1.263 | +2.778 |
| | 120 | +10.0 | +7.222 |

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# An Evaluation of Anti-Diabetic and Anti-Lipidemic Properties of *Momordica charantia* (Bitter Melon) Fruit Extract in Experimentally Induced Diabetes

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**Abstract:** Aim: *Momordica charantia* is reported to possess hypoglycemic activity. This study aims at investigating the effect of *Momordica charantia* extract on glucose tolerance and some biochemical parameters in alloxan induced diabetes, comparing it to the effect of rosiglitazone maleate, an oral hypoglycemic drug, and to suggest the possible mechanisms of its action. Main methods: Rats were divided into 5groups: normal control, rats received bitter melon, diabetic control, diabetic treated with rosiglitazone (4mg/kg BW), and diabetic received *Momordica charantia* (300 mg/kg BW). After 4weeks, OGTT, serum insulin, lipid profiles, glycohemoglobin% (HbA1c%), liver enzymes activity and glycogen content, intestinal absorption and diaphragm uptake of glucose and histopathological studies on the pancreas were evaluated. Key findings: Bitter melon (BM) induced a significant improvement of OGTT and induced a significant decrease in HbA1c% ($p$<0.05), significantly increased insulin release from the pancreas and serum insulin level, increased glucose uptake by rat diaphragm and decreased intestinal glucose absorption ($p$<0.05). BM improved lipid profile. In addition, BM significantly increased liver glycogen content and reduced liver enzyme activity compared to the diabetic control. BM treatment of diabetic rats resulted in significant hypoglycemic and hypolipidemic effects as compared to rosiglitazone ($p$<0.05). Significance: Results demonstrated anti-diabetic effects of bitter melon may be through increasing insulin release and serum insulin, increasing glucose uptake by muscles and decreasing intestinal glucose absorption and a hypolipidemic effect and this recommend its therapeutic use in diabetes.
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**Key words:** *Momordica charantia*, diabetes, glucose absorption, rat diaphragm glucose uptake, rosiglitazone maleate

## 1. Introduction

Diabetes mellitus is the most common endocrine disease. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 (1). Diabetes mellitus leads to metabolic abnormalities and is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (2).

Although, oral hypoglycemic agents and insulin are the mainstay of treatment of diabetes, they have prominent side effects and fail to significantly alter the course of diabetic complications (3), The common side effects associated with oral hypoglycemic agents are hypoglycemia, weight gain, gastrointestinal disorders, peripheral edema and impaired liver function, in addition to the cost of treatment (4).

Since natural remedies are somehow safer and more efficacious than pharmaceutically derived remedies, herbalism has become mainstream worldwide (5).

*Momordica charantia*, also known as bitter melon, bitter gourd, or balsam pear, is a plant widely cultivated in many tropical and subtropical regions of the world and is frequently used in South Asia and the Orient as a food stuff and medicinal plant. Extracts from various components of this plant have been reported to possess hypoglycaemic activity (6). Thus, bitter melon can be an alternative therapy used for lowering glucose level in diabetic patients (7).

The hypoglycemic activity of *Momordica charantia* fruit juice is demonstrated in animals with experimental diabetes and also in humans in both type 1 and type 2 diabetes mellitus (8).

Scientists have identified 3 groups of constituents thought to be responsible for blood sugar lowering action of bitter melon; one of these, a compound called charantin which is composed of sitosteryl glucoside & stigmasteryl glucoside and can potentially replace treatment by insulin (9). Another compound, polypeptide p (plant insulin) found in seeds and fruits of bitter melon is similar to insulin in composition, so it can be of a great benefit in therapy of type 1 diabetes (10). Third compound is alkaloids which have also been noted to have a blood sugar lowering effect. Compounds known as oleanolic acid glycosides have been found to improve glucose tolerance in type 2 diabetes (11).

**Aim of work**:

The present study aims at investigating the effect of *Momordica charantia* (bitter melon) fruit extract on body weight, oral glucose tolerance test, serum insulin, blood glycohemoglobin percentage [HbA1c%], liver glycogen content, serum ALT and

AST and lipid profile (triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol) in alloxan induced diabetes, comparing it to the effect of rosiglitazone maleate. The possible mechanisms of the hypoglycemic action of such agents was investigated by studying peripheral glucose uptake by rat diaphragm in vitro, insulin release from the isolated islets of Langerhans *in vitro* and intestinal glucose absorption in situ. Histopathological examination of the rat pancreas was examined.

## 2. Materials and Methods

### Experimental animals

Fifty adult male albino rats weighing about 120-160g were divided into five groups (ten rats in each group) as follow:

Group I: rats of this group served as control group and were fed standard rat chow and pure water (NC).

Group II: this group included normal rats received bitter melon (*Momordica charantia*), at a daily dose of 300 mg/kg BW, dissolved in distilled water and given by gavage for 4weeks (NBM).

Group III: this group included diabetic control rats those were given pure distilled water (DC).

Group IV: this group included diabetic rats treated with Avandia® (rosiglitazone). The drug was purchased from Smith Kline Beecham Pharmaceuticals (U.S.A). The tablets were crushed, suspended in distilled water and was administered by gavage daily in a dose of 4 mg/kg BW (12) (DAV).

Group V: this group comprised of diabetic rats received, bitter melon (*Momordica charantia*) at a daily dose of 300 mg/kg BW, dissolved in distilled water and given by gavage for 4weeks (DBM).

Forty alloxan- induced diabetic rats were added to the diabetic group for the in vitro and in situ studies.

The rats were obtained from the animal house of Faculty of Medicine, Cairo University, Egypt. Rats were housed in separate cages temperature 25 ± 5C◦ and were given free access to water and food.

### Experimental protocol

In the current study diabetes was induced experimentally in fasted rats by intra-peritoneal injection of a single dose of 100 mg/kg BW alloxan monohydrate (Sigma Company) dissolved in citrate buffer at pH 4.5 (13). Animals were given 5% glucose solution to drink instead of tap water for a few days until sustained hyperglycemia was established. Rats having serum glucose ranging from 180-300 mg/dl after 2 hours of glucose intake were only included in the experiment.

Preliminary testing of hypoglycemic activity of different doses of bitter melon was done for a week using diabetic rats to select the most potent dose which was used in the subsequent studies.

The effect of alloxan induced diabetes, as well as rosiglitazone maleate and *Momordica charantia* treatments, were investigated on: body weight, oral glucose tolerance test, serum insulin, blood glycohemoglobin [HbA1c] percentage, liver glycogen content, and serum ALT and AST activity, and lipid profiles. Peripheral glucose uptake by rat diaphragm, insulin release from isolated islets of Langerhans *in vitro* were performed and intestinal glucose absorption in situ was estimated. The present study also includes the histopathological changes in the pancreata of normal, diabetic control and diabetic treated rats.

At the end of the experimental period (4 weeks), OGTT was done to the fasted rats in the five groups. Twenty four hours later, fasted rats were sacrificed under diethyl ether anesthesia, and blood samples were collected from the rats. Pancreata and livers were excised quickly after dissection of the sacrificed animals. Fresh liver samples were used for determination of glycogen content. Pancreas was fixed in 10% neutral buffered formalin for paraffin section preparation.

### Preparation of freeze-dried bitter melon BM juice

According to the methods of **Chen and Li**, (14) unripe BM fresh fruit was cut open and the seeds were removed. The extracted juice from the edible portion was frozen and completely lyophilized by continuous freeze-drying operation for 72hrs. The powder was kept in airtight containers at -70C$^0$ until used.

### Biochemical analysis

1-Serum glucose levels and oral glucose tolerance test were performed according to the method described by **Leatherdale *et al.*** (15), using reagent kits purchased from Bio Merieux Chemicals (France).

2-ALT and AST activity in serum were determined according to the method of **Moss and Henderson** (16) using reagent kits purchased from Randox Company (United Kingdom).

3-Serum triglycerides concentration was determined according to the method of **Nauk *et al.*** (17), using reagent kits obtained from Reactivos Spinreact (Spain).

4-Serum LDL-cholesterol concentration was determined according to **Friendewald *et al.*** (18).

5-Liver glycogen content was determined according to the method of **Seifter *et al.*** (19)

6-Blood HbA1c% was estimated according to the method of **Abraham and Rao**(20), using reagent kits purchased from Stanbio Company (Texas).

### Peripheral glucose uptake

Peripheral glucose consumption was studied in preparations from diabetic, 24 hrs fasted rats prior to sacrifice and exsanguinations according to **Zarzuelo *et al.*** (21). Diaphragms were incubated in a nutrient solution at 37°C with constant oxygenation for 1 hr. The preparation was used to compare between the

effect of rosiglitazone maleate and *Momordica charantia* on glucose uptake by the muscle, at their low concentrations (0.45mg/ml&0.2mg/ml respectively) and high concentrations (0.9mg/ml &0.4mg/ml respectively), in absence and presence of 50 μIU/ml insulin.

**Intestinal glucose absorption**

An intestinal perfusion in situ technique (21) was used to study the effects of rosiglitazone and *Momordica charantia* at their low & high concentrations on intestinal glucose absorption in diabetic 24 hrs fasted rats. First 10 cm of jejunum was perfused by a Kreb`s solution. Results were expressed as percentage glucose absorption calculated from the amount of glucose in solution before and after perfusion with rosiglitazone and *Momordica charantia* compared with a control study.

**Histopathological study**

The pancreas was immediately removed from each animal after sacrificing, fixed in 10% neutral buffered formalin and transferred to the National Cancer Institute, Cairo, Egypt for preparation. Pancreata were stained with modified aldehyde fuchsine stain method (22).

**Isolation of islets of Langerhans and incubation techniques:**

Pancreatic islets were isolated from diabetic rats, using the collagenase digestion technique (23). Collagenase (Type V) was purchased from Sigma Company, USA. To study the effect of different treatments on insulin release, 0.35 ml of rosiglitazone and *Momordica charantia*, both at their low (0.45,0.2 mg/ml) and high (0.9,0.4 mg/ml) concentrations respectively, were added to the isolated islets separately and incubated for 1 hr at 37° C. Another preparation was kept without treatments and used as a control study.

**Statistical analysis of the results:**

The data were analyzed using one way analysis of variance ANOVA, followed by least significant difference LSD analysis to compare various groups with each other. Results were expressed as mean ± standard deviation and values of $P<0.05$ were considered statistically significant.

**3. Results**

Figure 1 shows that the three doses of bitter melon BM (150, 300, and 600mg/Kg) produced varying significant hypoglycemic effects compared to the control group. However, the most potent dose was 300 mg /kg BW.

Table 1 and figure 2 show that treatment of diabetic rats with BM or Avandia induced a significant increase in BW, decrease in fasting blood glucose levels than those of the diabetic untreated group. Bitter melon and Avandia induced a significant hypoglycemic effect throughout the OGTT, decreased HbA1c% and increase in serum insulin in diabetic rats ($p<0.05$) compared to the diabetic untreated group.

Bitter melon induced a significant hypoglycemic effect, decrease in Hb A1c % and increase in serum insulin in diabetic rats as compared to Avandia (Table 1 and Figure 2).

Both bitter melon and Avandia significantly increased liver glycogen content, decreased liver enzymes of diabetic rats as compared to the diabetic untreated group ($p<0.05$).. Effect of bitter melon treatment was significant when compared to Avandia treated group (Table 1).

Bitter melon induced a significant decrease in serum total cholesterol, triglycerides and LDL but a significant increase in HDL as compared to the diabetic untreated group ($p<0.05$), while its effect on normal rats was insignificant as compared to the normal control group($p>0.05$). Avandia® induced an insignificant decrease in total cholesterol, triglycerides ($p>0.05$), but a significant decrease in LDL($p<0.05$), in addition to an insignificant increase in HDL ($p>0.05$) as compared to the diabetic untreated group. Effect of BM on diabetic rats was significant as compared to Avandia® (Figure 3).

Table 2 shows that, in the absence and presence of insulin, bitter melon caused a significant increase in percentage of glucose uptake by rat diaphragm at low and high concentrations ($p<0.05$), while values obtained with Avandia® were insignificant at low concentration($p>0.05$) and significantly increased at higher concentration ($p<0.05$) as compared to their controls.

Bitter melon induced a significant increase in insulin release from the pancreas of diabetic rats at both low and high concentrations in a dose dependent manner ($p<0.05$) as compared to control values. Avandia® showed no significant effect on insulin release (Table 3). Table 3 shows a significant decrease in % glucose absorption in situ at both low and high concentrations of BM in a dose dependent manner ($p<0.05$), while values obtained with Avandia were insignificant ($p>00.5$)as compared to control values.

Compared to the normal appearance of pancreas shown in figure (4A,B),bitter melon treatment had no effect on normal pancreas (Fig.4C). Intra-peritoneal injection of alloxan, at a dose of 100 mg/kg B. W. resulted in morphological alterations of pancreatic islet cells and showed destructed β cells with decreased number and vacuolated cytoplasm (Fig4D).

Treatments for 4 weeks of diabetic rats with either Avandia® (Fig.4E) or bitter melon (Fig.4F)

stimulated recovery of the islet cells. The islets approximately regained their normal appearance with a marked increase of β cell number and fewer vacuolated cells when compared to the pancreas of untreated diabetic rat.

Table (1): Effect of Avandia® and bitter melon on % change in body weight, serum insulin and HbA1c % and liver glycogen and liver enzymes of normal and alloxan diabetic rats compared to their controls after 4 weeks experimental period.

| Groups | % change in body weight | Insulin µIU/ml | HbA1c % | Liver glycogen mg/g. fresh tissue | ALT U/l | AST U/l |
|---|---|---|---|---|---|---|
| NC | 10.3±2.07[a] | 19.6±2.37[a] | 4.5±0.72[c] | 10.5±1.70[a] | 43.9±4.06[d] | 40.2±4.38[d] |
| NBM | 8.4±2.20[a] | 19.8±1.75[a] | 4.9±0.19[c] | 10.7±2.26[a] | 43.6±4.74[d] | 40.2±4.91[d] |
| DC | -12.3±2.81[c] | 6.3±0.87[d] | 13.6±0.53[a] | 3.2±1.00[c] | 74.6±4.40[a] | 63.7±4.5[a] |
| DAV | 5.7±2.11[b] | 7.9±0.97[d] | 6.2±0.62[b] | 5.9±0.93[b] | 62.3±3.95[b] | 56.9±4.36[b] |
| DBM | 6.4±2.22[b] | 12.5±2.12[b] | 4.7±0.58[c] | 9.8±1.29[a] | 55.1±3.86[c] | 48.5±5.23[c] |

-Data are expressed as mean ±SD. -Number of samples in each group is 10.
-Means with different superscript letters in the same raw differ significantly ($P < 0.05$) and those with same superscript letter do not have a significant difference (P>0.05).
- For % change in B. Wt, LSD at 5% is 2.739 and LSD at 1% is 3.706.
- For insulin, LSD at 5% is 2.057 and LSD at 1% is 2.783.
- For glycol Hb %, LSD at 5% is 0.647 and LSD at 1% is 0.912.
- For liver glycogen, LSD at 5% is 1.808 and LSD at 1% is 2.446.
- For ALT, LSD at 5% is 5.018 and LSD at 1% is 6.789. - For AST, LSD at 5% is 5.586 and LSD at 1% is 7.558.

% change in BW = $W_x - W_o \times 100 / W_o$
$W_o$: Body weight at the beginning of the experiment. -$W_x$: Body weight at the end of the experiment.

Table (2): Effect of different concentrations of Avandia® and bitter melon on % glucose uptake by rat diaphragm of diabetic rats in presence and absence of insulin compared to normal control values.

| Group / Studies | control | Avandia® | | Bitter melon | |
|---|---|---|---|---|---|
| | | Low 0.45mg/ml | High 0.9mg/ml | Low 0.2mg/ml | High 0.4mg/ml |
| In absence of insulin | 12.0±1.38[c] | 12.9±1.57[b,c] | 14.1±0.63[b] | 14.2±1.26[b] | 17.2±1.52[a] |
| In presence of insulin | 12.7±1.57[c] | 13.1±1.98[b,c] | 14.7±1.44[b] | 14.9±1.86[b] | 19.7±2.45[a] |

-Data are expressed as mean ±SD. -Number of samples in each group is 5.
- Means with different superscript letters in the same raw differ significantly ($P < 0.05$) and those with same superscript letter do not have a significant difference (P>0.05).
- For % glucose absorption values in absence of insulin, LSD at 5% is 1.883 & at 1% is 2.568.and in presence of insulin, LSD at 5% is 2.345 and at 1% is 3.199.

Table (3): Effect of different concentrations of Avandia® and bitter melon on % intestinal glucose absorption in situ and insulin release from isolated islets of diabetic rats compared to control values.

| Group / Studies | Control | Avandia® | | Bitter melon | |
|---|---|---|---|---|---|
| | | Low 0.45mg/ml | High 0.9mg/ml | Low 0.2mg/ml | High 0.4mg/ml |
| % glucose absorption | 29.2±1.57[c] | 28.7±1.53[b,c] | 28.6±2.20[b,c] | 26.7±1.89[b] | 24.4±1.19[a] |
| Insulin release (µIu/islet/hour) | 7.3±0.86[c] | 7.5±1.14[c] | 7.3±1.70[c] | 9.5±1.85[b] | 11.3±1.57[a] |

-Data are expressed as mean ±SD.
-Number of samples in each group is 8 for insulin release studies and 10 for % glucose absorption.
-Means with different superscript letters in the same raw differ significantly ($P < 0.05$) and those with same superscript letter do not have a significant difference ($P$>0.05).
-For % glucose absorption values, LSD at 5% is 2.04 and at 1% is 2.76.
-For insulin release values, LSD at 5 % is 1.754 and at 1% is 2.374.

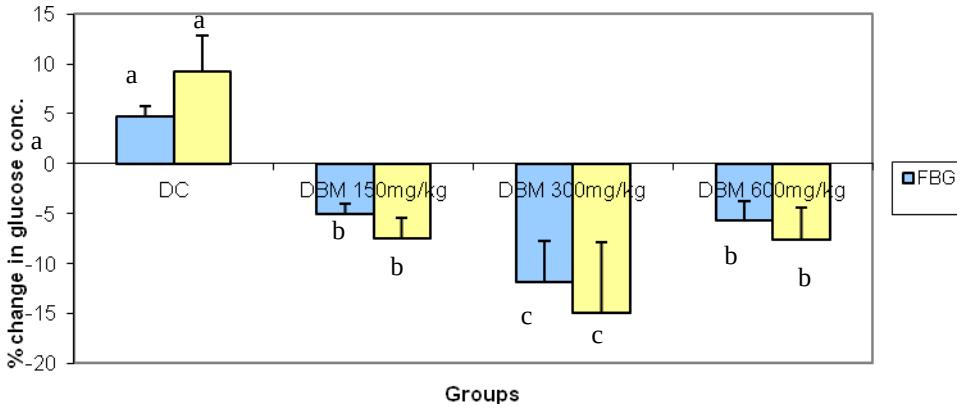


Figure1: Hypoglycemic effect of different doses of bitter melon in diabetic rats treated for one week. Results are expressed as mean±SD. Means with different letters differ §ignificantly.

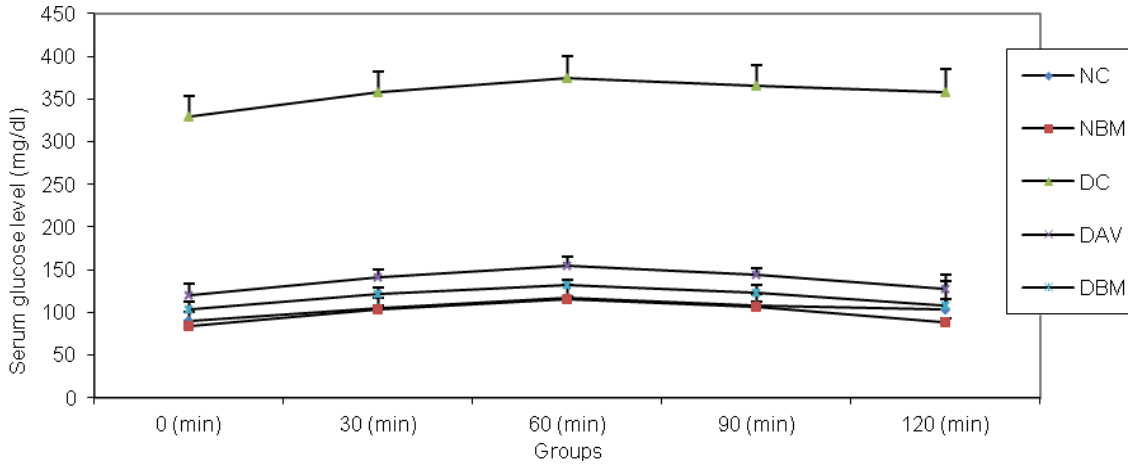


Fig. (2) Effect of Avandia® and bitter melon on OGTT of normal and alloxan diabetic rats compared to their controls after 4 weeks experimental period. Results are expressed as mean±SD.

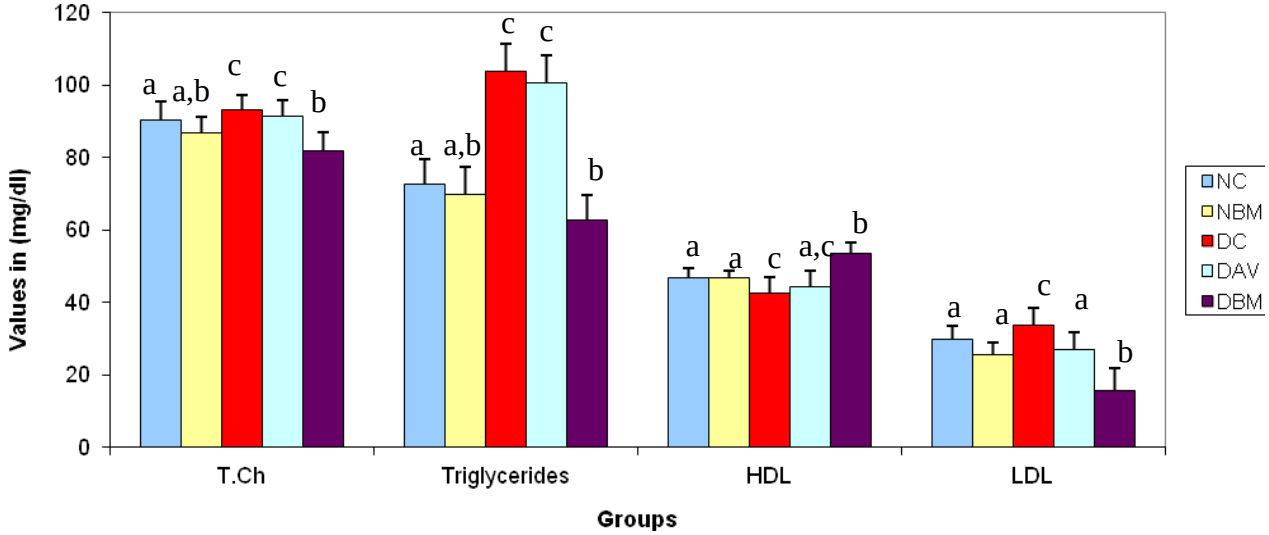


Fig. (3) Effect of avandia and bitter melon on serum total cholesterol, triglycerides, HDL, LDL of normal and alloxan diabetic rats compared to their controls after 4 weeks experimental period. Results are expressed as mean±SD. Means with different letters differ significantly.

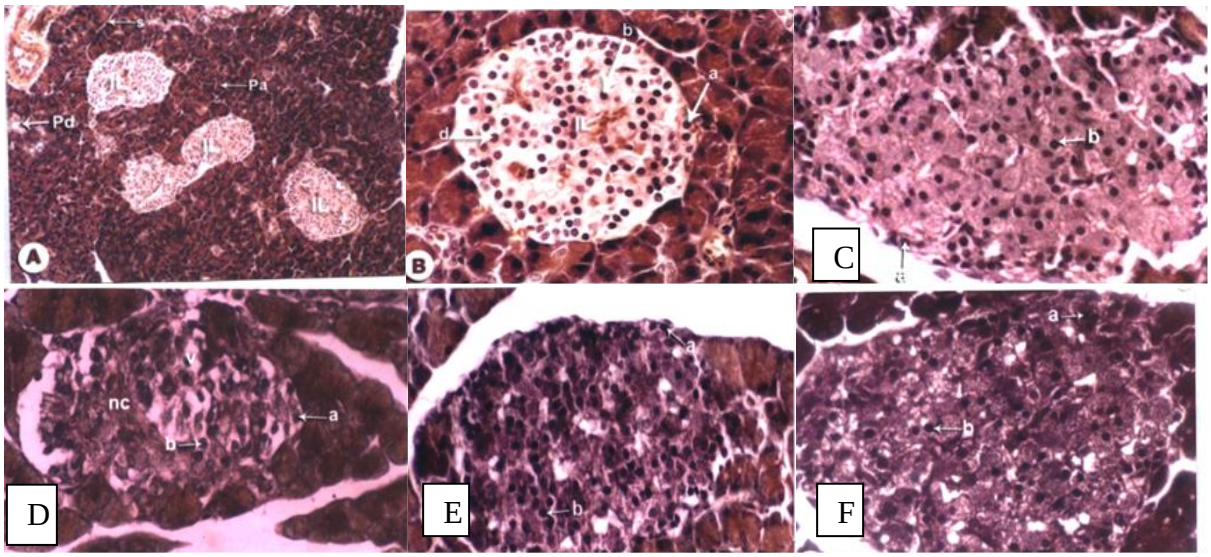


**Figure (4)(A)**: Light micrograph of the pancreas of a normal male albino rat consist of exocrine and endocrine portions. The exocrine portion is subdivided by septa (S) into pancreatic acini (Pa) and ducts (Pd).The endocrine portion consists of the islets of Langerhans (IL). **(B)** Higher magnification of an islet of Langerhans which consists of three types of cells, alpha (a), beta (b) and delta (d). All cell types reveal a normal appearance. **(C)** Pancreas of a normal male albino rat treated with bitter melon for 4 weeks. The islets seemed to have a normal architecture.
**(D)** Pancreas of untreated diabetic rat after 4 weeks experimental period. The islets showed necrosis (nc) and vaculations (v) **(E)** Pancreas of a diabetic rat treated with rosiglitazone for 4 weeks. The number of alpha and beta cells per islet was increased and there were less vaculations. **(F)** Pancreas of a diabetic rat treated with bitter melon for 4 weeks showing little damage, less vaculations and the number of beta cells per islet was increased.

**4. Discussion**

The present study revealed that intra-peritoneal injection of a single dose (100 mg/kg B.Wt.) of alloxan to adult male albino rats was suitable to induce histopathological changes in the islets of Langerhans characterized by a marked decrease of β cells and vacuolar appearance, a significant decrease in fasting serum insulin level, decrease in the body weight and a significant increase in serum glucose level in OGTT of diabetic untreated rats, and a significant elevation in blood HbA1c% and an increase in the activity of both transaminases (ALT & AST), with significant decrease in liver glycogen content. The present findings are in agreement with **Lashin and Andrea** (24) and **Umrani *et al*.**(25).

Alloxan decreases body weight due to depressed synthesis of DNA and RNA in diabetic animals (26,27,28).

The hyperglycemia could arise due to destruction of β cells and reduced uptake of glucose to peripheral tissues as evidenced by the decrease rat diaphragm glucose uptake, glycogenolysis (29), and gluconeogenesis (30) as a result of insulin deficiency and may be due to the loss of glycogen synthetase activity, increased activity of glycogen phosphorylase (31) and / or increased activity of glucose-6-phosphatase (32).

This finding is supported by our results that revealed an enormous depletion in hepatic glycogen content and the detected elevation in liver enzymes in diabetic control rats as compared to control rats.

The elevated levels of both transaminases in the serum of diabetic rats of the present study may be ascribed to induced synthesis of these enzymes (33) and or destructive changes in hepatic cells as a result of toxaemia (34).

Treatment of diabetic rats with bitter melon induced a significant increase in body weight as compared to diabetic control rats. These results are in agreement with the findings of **Fernandes *et al***.(35) and **Yuan *et al***.(36), but disagree with **Dans *et al***. (37), who found that bitter melon had no significant effect on body weight of diabetics. This increase in body weight of diabetic rats as a result of bitter melon treatment may be ascribed to the increase in insulin release.

Treatment of diabetic rats with bitter melon produced a significant increase in fasting serum insulin as compared to the diabetic untreated group. The present finding is in agreement with the results of **Fernandes *et al* .** (35), **Yuan *et al*** .(36), **Sundaram and Kumar** (38), **Garau *et al*** .(39), **Yibchok *et al*** .(40), and **Hui *et al*** .(41).

On contrary, **Toshihiro *et al***.(42) and **Subratty *et al.*** (43) reported that treatment of diabetic rats with bitter melon decreased serum insulin. **Dans *et al*** .(37) reported that bitter melon had no effect on serum insulin.

The significant increase in serum insulin concentration of diabetic rats after bitter melon treatment in the present study might be ascribed to the ability of this agent to stimulate the spontaneous recovery of β cells of the islets of Langerhans**.** In vitro studies using isolated islets of Langerhans demonstrated that bitter melon induced a significant increase in insulin release. The work of **Fernandes *et al.***(35), **Garau *et al.*** (39) and **Singh and Gupta** (44) supports this finding. Treatment of diabetic rats with bitter melon showed a significant increase in β cell number. This indicates that bitter melon has a regenerative effect on β cells. On the other hand, **Sundaram and Kumar** (38) reported that treatment of diabetic rats with bitter melon did not restore β cells of islet of Langerhans destroyed by alloxan, however, viable β cells were found to be more active and granulated on bitter melon treatment.

Bitter melon may exert its effect by either preventing the death of beta cells by decreasing the oxidative stress caused by alloxan in diabetic rats since bitter melon contains vitamin C (anti-oxidant). Antioxidants act by neutralizing the free radicals released (45). **Xiang *et al.*** (46) suggested that bitter melon may act as a growth factor for pancreatic beta cells.

Regarding serum glucose level (OGTT), treatment of diabetic rats with bitter melon caused significant decreases in fasting and post- prandial serum glucose levels as compared to the diabetic untreated group. These results are in accordance with the findings of **Jayasuriya *et al.*** (7), **Fernandes *et al.*** (35) **Yuan *et al.*** (36) and **Chatuvedi *et al.*** (47). The present finding disagrees with the finding of **Dans *et al.*** (37) who reported that bitter melon had no significant hypoglycemic effect in alloxan diabetic rats.

In an attempt to gain an insight on the underlying physiological mechanisms of the hypoglycemic effect of bitter melon, we assayed its effect on peripheral glucose uptake by rat diaphragm (*in vitro*) and intestinal glucose absorption in situ.

Regarding peripheral glucose uptake of rat diaphragm, the obtained data indicated that, in both absence and presence of insulin, bitter melon induced a significant increase of glucose uptake as compared to a control study.

The present results are in agreement with the results of **Fernandes *et al.*** (35),**Garau *et al.***(39), **Ahmed *et al.*** (48), and **Shih *et al.*** (49). The mechanism by which bitter melon increases glucose uptake by skeletal muscle and adipose tissue was suggested by **Shih *et al.*** (49) and **Chuang *et al.*** (50) who demonstrated that bitter melon significantly increases mRNA expression and protein of glucose transporter 4 (GLUT4) in skeletal muscle. Bitter melon extract may stimulate GLUT4 translocation on the cell membrane in both myocytes and adipocytes (51).

These results on peripheral glucose uptake give evidence that bitter melon also have insulin-mimetic effects in addition to its insulin secretogogue or insulinotropic effect.

Concerning intestinal glucose absorption, the obtained data revealed that bitter melon produced a significant decrease of intestinal glucose absorption in diabetic rats compared to a control study. These results are in agreement with the findings of **Garau *et al.*** (39), **Ahmed *et al.*** (48) and **Mahmoodally *et al.*** (52).

It is hypothesized that bioactive phytochemicals such as saponins in bitter melon extract inhibit the active transport of d-glucose, l-tyrosine and fluid across rat intestine by inhibiting the ATPase responsible for the active transport of these molecules (52). This positive influence of feeding bitter melon on intestinal glucose absorption may also be through affecting disaccharidase activity (53). Also, it has been shown that oleanolic acid glycosides isolated from bitter melon suppress gastric emptying in alloxan diabetic rats and decrease glucose absorption in small intestine *in vitro* (54).

Based on the above mentioned data, it is worth mentioning that an enhancement of insulin release, increase of peripheral glucose uptake, and suppression of intestinal glucose absorption are involved in the mechanisms of hypoglycemic action of bitter melon in alloxan diabetic rats.

Treatment of alloxan diabetic rats in the present study with bitter melon induced a significant decrease of HbA1c% as compared to the diabetic untreated group. Such decrease may be ascribed to the insulinotropic effect of this agent. This finding is supported by **Fernandes *et al.*** (35) and **Garau *et al.*** (39), but disagrees with **Dans *et al.*** (37) who reported no significant effect of bitter melon on blood glycohemoglobin (%) in alloxan diabetic rats.

The present study revealed that administration of bitter melon to diabetic rats induced an increase in hepatic glycogen concentration. This finding is in agreement with **Garau *et al.*** (39), **Singh & Gupta** (44), and **Rathi *et al.*** (55) but disagrees with **Fernandes *et al.*** (35) and **Yuan *et al.*** (36) who reported decreased glycogen content of the liver of alloxan diabetic rats treated with bitter melon.

Stimulated insulin release induced by bitter melon treatment, as shown in the current study, may be responsible for increasing glycogen synthetase activity (56).

Regarding liver enzymes, the present study revealed a significant decrease in the activities of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in diabetic rats treated with bitter melon as compared to the diabetic untreated group. These findings are in agreement with studies of **Garau *et al.*** (39). Results of **Dans *et al.*** (37) on

diabetic rats treated with alloxan showed no effect on serum ALT and AST.

The present results elucidated a significant increase of total cholesterol, triglycerides and LDL-cholesterol concentrations in the serum of diabetic control rats as compared to normal control group. These results are in agreement with **Newairy *et al*.** (57). On the other hand, HDL-cholesterol level was significantly decreased in serum of diabetic control rats in the present study as compared to the normal control group. This finding parallels that of **Nakura *et al*.** (32), and disagrees with **Wasan *et al*.** (58) who reported a significant increase of HDL-cholesterol in alloxan diabetic rats.

The markedly increased level of triglycerides and LDL-cholesterol in the serum of diabetic rats of the present work may be a consequence of either overproduction by the liver or defective removal from the circulation or both secondary to insulin deficiency (59).

Mechanisms by which HDL decreases in diabetes may be due to the impaired metabolism of triglycerides rich lipoprotein with decreased activity of lipoprotein lipase and impaired transfer of materials to the HDL components, in addition to the high level of hepatic lipase among diabetics (60). Finally, insulin resistance may be a direct cause of decrease of HDL concentration (61).

In a view of the present results, it was found that treatment of diabetic rats with bitter melon produced marked decreases of serum total cholesterol, triglycerides and LDL-cholesterol concentrations and an increase in serum HDL-cholesterol concentration as compared to the diabetic control group. These obtained data are concomitant with the results of **Fernandes *et al*.** (35), **Yuan *et al*.** (36) and **Chatuvedi *et al*.** (47), The present findings disagree with the results of **Dans *et al*.** (37) who found that the addition of bitter melon to hypercholesterolemic diet of rats had no effect on serum lipid profiles.

Bitter melon may affect the break down of specific lipoprotein (e.g LDL) or it may enhance fat oxidation in the body. The saponins and plant sterol in bitter melon also reduce blood triglyceride level and they also reduce the absorption of cholesterol from the intestine. In addition, the insulin like molecule in bitter melon may, like insulin, prevent the increase in triglyceride level due to the movement of fat from body cells into the blood stream (7).

In this study, bitter melon did not have a hypoglycemic or hypolipidemic effect on normal rats treated for 4 weeks. **Toshihiro *et al*.** (42) and **Ouviña *et al*.** (62) supported these findings. On the other hand, these findings disagree with those obtained by **Yibchok *et al*.** (40) and **Ojewole *et al*.** (63) who found that bitter melon fruit extract had significant hypoglycemic and hypolipidemic effects in normal rats.

In the current study, results obtained from diabetic rats treated with rosiglitazone (Avandia), an oral hypoglycemic drug, revealed that rosiglitazone decreased serum glucose, blood glycohemoglobin %, increased liver glycogen, and decreased liver ALT and AST significantly, but had insignificant effects on serum insulin and lipid profiles except LDL which decreased significantly with rosiglitazone treatment. These results except for lipid profiles agree with **Al-Salman *et al*.** (64) and **Leibowitz and Cerasi** (65) who demonstrated that rosigliazone had significant hypoglycemic and hypolipidemic effects in diabetic rats.

Regarding the in vitro and insitu studies, rosiglitazone had no significant effect on insulin release from isolated beta cells of the pancreas or on intestinal glucose absorption, while, it increased glucose uptake significantly by rat diaphragm only at high concentration. In addition, rosiglitazone treatment was found to increase number of beta cells in the pancreas of diabetic rats. These findings are in agreement with findings of **Finegood *et al*.** (66) and **Smith *et al*.** (67).

Bitter melon treatment of diabetic rats resulted in significant hypoglycemic and hypolipidemic effects as compared to rosiglitazone.

**Conclusion:**

In conclusion, the present study calls attention to the therapeutic use of bitter melon in diabetes mellitus. The results of the current study demonstrated that bitter melon has numerous anti-diabetic effects such as, decreasing serum glucose concentration, increasing serum insulin level, increasing glucose uptake by the peripheral tissues and decreasing intestinal glucose absorption. In addition, it showed hypolipidemic and thus cardiac protective effects. It was shown in this study that bitter melon did not cause hypoglycemia when given for normal rats, this indicates that it is safe if utilized by normoglycemic persons for its other beneficial effects.

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# Efficiency of Peppermint Oil Fumigant on Controlling *Callosobruchus maculatus* F. Infesting Cowpea Seeds

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**Abstract:** Fumigation tests were carried out to evaluate the efficiency of peppermint oil on controlling *C. maculatus* by studying its effect on survival of developmental stages, mating and oviposition behavior. Also, the effect of peppermint oil on the antennal segments and their sensilla was studied by using scanning electron microscope. The obtained data reveal the susceptibility of all stages of *C. maculatus* to fumigation with peppermint oil. The egg stage was the most susceptible stage. Mating frequency, fecundity and survivorship of the next generation progenies were significantly decreased by oil treatment. The effects of peppermint oil were always greater on treated male pairs than on treated female pairs. Fumigation of early pupae with 5 µl peppermint oil resulted in malformation and disorientation in the antennae and their associated sensilla in the emerged adults.
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**Key words:** Peppermint oil, fumigant, control, *Callosobruchus maculatus*, cowpea seeds

## 1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp. is one of the most nutritious grain legumes (**Ehlers and Hall, 1997**) where it is valuable as a source of dietary protein as well as vitamins and minerals (**Singh *et al*., 2003**). Loss of seed yield in legumes crops during storage due to bruchid beetles is a very serious problem for farmers and traders (**Ress, 2004**). Cowpea weevil, *Callosobruchus maculatus* is one of the most destructive bruchid species to several legumes including cowpea. Their larvae being internal feeders are hard to control with insecticides. It was also not advisable to mix insecticides with food grains. Fumigation was being the most effective method for controlling stored grains insects. Many researches conducted managing cowpea weevil by fumigation with various essential oils (**Shaaya *et al*., 1997; Ketia *et al*., 2001; Braga *et al*., 2007 and Manzoomi *et al*., 2010**). Oil of *Mintha piperita* L. (peppermint oil), a widely used essential oil was evaluated for its insecticidal activity against several stored grain insects. **Klingauf *et al*. (1983)** concluded that the exposure to 6µl/liter, through fumigation with essential oil of *M. piperita* for 3 hours, has led to 100% mortality in *Sitotroga cerealella* and 50% in adults of *Acanthoscelides obtectus*. Also fumigation with essential oil of *M. piperita* in a concentration of 15 µ l/liter for a 3 hours-period, has caused mortality above 75% in adults of *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis* and *Sitophilus oryzae* (**Shaaya *et al*.,1991**).

It has become evident that the antenna is a major channel of sensory input, including receptors for volatile odors and pheromones, contact chemoreceptors, sound perception and touch (**Ehmer and Gronenberg, 1997 and Renthal *et al*., 2003**). Moreover, antennal sensilla can play an important role in the insect pest control. **Leal (2005**) reported that the odorant substances including sex pheromones and host plant volatiles diffuse through the wall of the pore of antennal sensilla into the sensillar lymph and transferred to olfactory receptors on the dendrites of olfactory sensillae and odorant binding proteins. Volatile oil can disrupt communication in mating behavior of cowpea weevil by blocking the function of antennal sensilla and unsuccessful mating could lead to a lower fecundity and ultimately lower the population of insect pest (**Ahmed *et al*., 2001**).

The objective of this study is to evaluate the efficiency of peppermint oil on controlling *C. maculatus* by studying its effect on the survival of different developmental stages, and on mating and oviposition behavior of *C. maculatus* reared under laboratory conditions. The Scanning Electron Microscopy was also used to detect the effect of peppermint oil on the morphology of adults' antennae and their associated sensilla when early pupae were treated.

## 2. Material and Methods

### Insect culture:

Cowpea weevil, *Callosobruchus maculatus* F. was reared for several generations on cowpea seeds, *Vigna unguiculata* L. under fluctuating relative humidity of 70± 5% and temperature of 27± 3ºC. All the experiments were carried out under these conditions in the Department of Entomology, Faculty of Science, Ain Shams University.

**Volatile Oil:**

Peppermint oil was purchased as pure oil (Branded in Egypt) from Katue Aromatic Company. The oil was extracted by steam distillation from the leaves of aromatic plant *Mintha piperita* L.

**Effect of peppermint oil on *C. maculatus* developmental stages:**

The efficacy of peppermint oil in controlling *C. maculatus* was tested by fumigation on different developmental stages. One- day old adults were placed on clean cowpea seeds for egg laying. After few hours, the adults were removed from the seeds and the number of eggs was standardized to one egg /seed. Insects were treated when they were 0-1 day old (eggs), 13-14 days old (third larval instars), 18-19 days old (early pupae), and 20-22 days old (late pupae).

All fumigation tests were carried out in small glass vials (6 ml long, 2.5 ml in diameter). Amounts of peppermint oil (1, 3 and 5µl) were spread on 2 cm diameter filter paper discs. Seeds containing each insect stage were put in the vials which then were covered with nylon mesh clothes. Treated filter paper discs were put on the nylon clothes under the caps of the vials which then were screwed tightly onto the vials. So, the oil vapors saturated the atmosphere of the vials around the seeds containing the insect stage. Untreated filter paper discs were used in the controls. Five replicates of each treatment and control were set up. Ten seeds were used for each replicate of the egg, larval and pupal tests. The vials were kept under laboratory conditions till adult emergence and the mortality of eggs, larvae and pupae were calculated. Any morphological changes resulted from oil treatment during the development of different stages were observed and photographed.

**Effect of peppermint oil on mating and oviposition behavior of *C. maculatus*:**

To ensure the virginity of each adult, few days prior to the emergence, each seed containing single developing insect was placed in an individual 1.5 ml eppendroff tube. The emerged unmated males and females were differentiated according to criteria given by **South gate (1958)**. The unmated males and females were exposed separately to 5µl peppermint oil for different exposure periods (100, 150 and 200 minutes) by fumigation test as mentioned above. The effect of peppermint oil on mating behavior was tested at different combinations (T♂ X T♀, T♂ X N ♀, N♂ X T♀ and N♂ X N ♀). The mating was observed in 10 pairs of adults for each combination and 5 minutes observation of mating behavior was carried out for each pair and the numbers of mating pairs were counted. To study the oviposition behavior of the treated females, fifty fresh cowpea seeds were put in vials containing each combination pair and covered with fine nylon cloth then left for oviposition and adult emergence. Five replicated pairs were made for each combination and the number of eggs deposited by each female was counted, also the emerged adults were counted daily and the emergence rate was estimated for each combination.

Preliminary tests were carried out after which the amount of oil used and the testing times were chosen.

The relation between data was examined by analysis of variance (ANOVA).

**Examination with the Scanning Electron Microscopy (SEM):**

Fresh specimens of males and females from a colony maintained in the laboratory (untreated) and adult males and females resulted from treatment of early pupae with 5 µl peppermint oil were used. They were dried in the chamber of the scanning electron microscope, SEM (Jeol – JSM – 5600 LV in SEM Unit, Egypt) in the low vacuum mode, and then the micrographs were taken. Identification of antennal sensilla was carried out according to **Hu *et al.* (2009)**.

**3. Results and Discussion**

**Effect of peppermint oil on developmental stages of *C. maculatus*:**

Results of the present study reveal the susceptibility of all stages of *C. maculatus* to fumigation with peppermint oil. Adult emergence decreased significantly as peppermint oil amount increased (Table 1). Statistical analysis showed that the numbers of adults that emerged from treated eggs, larvae and pupae (early and late) were significantly lower than the control (Fig. 1). The most susceptible stage of *C. maculatus* was the youngest stage, 0-1 day old eggs. After exposure of this stage to 1µl oil, adult emergence decreased by 37.66% from control, and complete inhibition (100% reduction) was recorded after exposure to 3 and 5µl oil (Table 1). The ovicidal effect of peppermint oil may be due to infiltration of oil particles under the egg cover that may block respiration or disrupt the water balance of eggs and developing embryo (**Credland, 1992**). The effect of oil on the instars developing inside the seeds showed that at 5µl oil, percentage adult emergence was 20% compared with 84% in the control (Table 1). When early pupae were treated with 5µl oil, only 4% developed inside the seeds till adult emergence proving to be highly susceptible compared to 24% of the late pupae which were more tolerant. These results agree well with **Arti and Sujoita (2009)** who stated that some of the phytochemical substances act

as general toxicant which generally kills the different life stages of the insect or interfere with growth. The results obtained are also in confirmation with the works of (**Eman and Abass, 2010**) who reported that the essential oils proved to be the most effective in reducing the population of *C. maculatus*. **Mbata *et al*. (2000)** reported that higher oxygen uptake was found in early pharate adults of *C. subinnotatus*, so that saturation of the atmosphere surrounding infested cowpea seeds with peppermint oil fumigant may cause suffocation and inhibition of various biosynthesis processes of the insect.

**Evaluation of the morphological abnormalities in *C. maculatus* after fumigation of the developmental stages with 5µl peppermint oil:**

In the present study treated eggs were observed to be dry while treated cowpea seed with larvae inside showed pores with various sizes on the surface of the seeds (Figs. 3 b, c and d). The adults that were formed from treated early and late pupae with 5 µl peppermint oil also showed various abnormalities like malformation on the morphology of the antennal segments, absence of melanization of the antennae or incomplete melanization with dark parts on the antennal segments, incomplete development and collapse of legs with attached exuvia and enlarged abdomen (Fig. 3f, g and h). Similar results were recorded by **Aly *et al.* (2010).** They found that extracts of the wild plant, *Fagonia bruguieri* caused adult deformities of *Schistocerca gregaria*. **Lee *et al.* (2002)** concluded that the insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoides. Monoterpenoides are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions.

Peppermint oil is one of the monoterpenes that can be toxic via penetration of the insect cuticle or the respiratory system (**Prates *et al.*, 1998**). Application of peppermint oil probably disrupted the delicate balance of timing and concentrations of hormones in the intact insect, resulting in the formation of abnormal forms. Hence, peppermint oil shows effective Insect Growth Regulators (IGR) activity and exhibits great promise in suppression of population of insects. **Mesbah *et al.* (2006)** reported that all the efficiently tested essential and/or volatile oils acted principally as Insect Growth Inhibitors (IGIs) rather than antifeedants causing disruption of the insect development, abnormal larvae, pupae and adults that were lead finally to death. Also peppermint oil may interfere in ecdysis and resulted in formation of malformed individuals. Peppermint oil may cause inactivation or inhibition of the tyrosinase which result in incomplete cuticle hardening and darkening. The aromatic compounds act as inhibitors of tyrosinase, interact with the binuclear copper active site of enzyme, so disrupt the tertiary structure of the enzyme **(Kubo, 1997)**. Tyrosinase is the key enzyme involved in the biosynthesis of melanin. It catalyzes the rate limiting step, the oxidation of the aromatic amino acid tyrosine to 3, 4 dihydroxy phenyl alanine (DOPA) and subsequently to DOPA –quinone which converted by multi step reactions to melanine pigments (**Kramer and Hopkins, 1987**).

**Effect of peppermint oil on mating and oviposition behavior of *C. maculatus*:**

Results presented in Figure (2) and statistical analysis (Anova) reveal that oil treatment had a significant effect on the mating of males and females *C. maculatus.* In the control pair, more than 90% of cowpea weevils were mated within five minutes, while the insects in all treated combinations (T♂ X T♀, T♂ X N ♀ and N♂ X T♀) completely failed to mate during the five minutes after all the exposure time of treatments (100, 150 and 200 minutes). **Ahmed *et al*. (2001)** came to the same conclusion after treatment of *C. chinensis* with neem oil. They reported that mating success of unmated pairs depend on the chemical activity of females (production of sex pheromones) and the physical behavior activity of males (response to sex pheromones). In the present study, adult males' failure to perform normal mating may be attributed to the binding of odor molecules of peppermint oil to the olfactory receptors on the antennae. The vapor of the oil may chemically hamper the pheromone produced by the females and cause confusion to males; therefore, they may fail to recognize the pheromones. **Leal (2005)** reported that the odorant substances including sex pheromones and host plant volatiles diffuse through the wall of the pore of antennal sensilla into the sensillar lymph and transferred to olfactory receptors on the dendrites of olfactory proteins and odorant binding proteins.

Also fumigation of unmated females with peppermint oil disrupt communication in mating behavior, the vapor of the oil may prevent the female from perception of sex pheromones, so this disrupt the intraspecific communication between males and females. This is in accordance with **Chapman (1972)**.

In case of oviposition behavior, results in table (2) and statistical analysis (Anova) reveal that females in treated groups (T♂ X T♀, T♂ X N ♀ and N♂ X T♀) deposited a significantly lower number of eggs than females in untreated groups (N♂ X N ♀). The present findings get support from the earlier works of **Kamakshi *et al*. (2000)** who reported a significant reduction in the number of eggs laid by *C.*

*maculatus* when treated with *Mentha arvensis* and *Ocimum sanctum* as compared with control. **Raja *et al.* (2001)** also concluded that egg laying by *C. maculatus* was significantly influenced by treatments with volatile oils. Similar results were reported by **Kétoh *et al.* (2000)** after treatment of *C. maculatus* with essential oils. This reduction of egg laying could be attributed to the early death of adults of *C. maculatus* due to the effect of the oil vapors. Similarly, **Schmidt *et al.* (1991)** and **Mazibur and Gerhard (1999)** showed that the effect of essential oils of *Acorus calamus* on *Callosobruchus phaseoli* could involve ovarian changes similar to those caused by the chemosterilants by blocking females egg laying. This assumption was also put into view by **Aboua *et al.* (2010)** who studied the effect of three aromatic plants, *Ageratum conyzoides* L., *Citrus aurantifolia* and *Melaleuca quinquenervia* L. on *C. maculatus.*

Results also reveal that fecundity was always significantly lower in males treated pairs than in females treated pairs (Table 2). This tendency was also found in the adult emergence rate. These results suggest that an insufficient number of sperms were transferred to females, this may be due to short copulation period, or the oil may have some spermicidal effect on males so; treated males may produce lower number of sperms (**Ahmed *et al*., 2001**).

Data in table (2) show that, generally, the effect of oil on number of eggs laid and emergence of adults increased with increasing exposure time.

**General description of antennae of *C. maculatus* :**

According to **Hu *et al*. (2009)** both female and male has serrated flagellar antennae. The antennae consist of scape (Sc), pedicle (Pe) and nine flagellomeres. All flagellomeres, except for the first one, have acute angled wedge-shaped ventral extensions.

As shown in Figs. (4a and 5a, b and 6a), there are one type of Bohm bristles (BB), two types of sensilla trichoid (ST1,ST2), one type of sensilla chaetica (SC), two types of sensilla basiconica (SB1,SB2) and one type of grooved pegs (GP).

**Bohm bristles (BB)**

Each sensillum is a triangular peg-like structure inserted into wide sockets. The BB sensilla occur on the base of the scape and pedicel, at the joints between the scape and the head and between the scape and the pedicle.

The location of the BB on the scape and pedicel only suggests that these might be mechanoreceptors (**Schneider, 1964; Zacharuk, 1985**). Concentration of Bohm bristles at the intersegmental joints between the scape and the head as well as between the scape and the pedicel, in many insects, indicates that these sensilla probably perceive the antennal position and movements (**Merivee *et al*., 2002**). Absence of these sensilla or reduction in its number resulted in disorientation of the antennae (Fig. 4b).

**Sensilla trichoid 1 (ST1)**

The ST1 sensilla are sharp-tipped hairs with strong longitudinal grooves and are nearly straight or slightly curved toward the antennal shaft (Figs. 5a, b and 6a). The ST1 is the most abundant sensilla type on the whole antenna; it might indicate that they have an olfactory function (**Keil, 1999).**

**Sensilla trichoid 2 (ST2)**

The ST2 sensilla are blunt-tipped straight hairs with cuticle wall (Figs. 5a and b). With the increasing of antennal antennomere, the number of sinsilla is also increasing. They probably function as sex pheromone receptors (**Merivee *et al*., 1999**).

**Sensilla chaetica (SC)**

The SC occurs on each antennomere of the antennae. This type of sensilla is characterized by grooved surface and straight hairs with blunt tip. They are inserted into a wide socket (Figs. 4a, 5a and 6a). The (SC) sensilla are believed to have a dual function of mechanoreception and contact chemoreception (**Jourdan *et al*., 1995**).

**Sensilla basiconica (SB1)**

The SB1 are characterized by smooth cuticle and a straight blunt tip (Figs. 5a, b and 6a). They are distributed on the flagellomeres of the antennae, with the exception of the first and the second segments (**Hu, *et al*., 2009**). Most of the SB1 are located on the lateral side of the apex of the above flagellomeres.

**Sensilla basiconica (SB2)**

The SB2 are characterized by a blunt tip which curved at the distal end. They are inserted into wide sockets (Fig.5a). (SB1) and (SB2) sensilla may have a sex-pheromone receptor role and olfactory function.

**Grooved pegs (GP)**

This type of sensilla is characterized by grooved surface and straight pegs with blunt tip (Figs.5a and 6a). GP are bulb- like structures projecting from a depression in the center of raised area of cuticle. Grooved pegs are situated on the posterior sides of the dorsal extensions of the flagellomeres and near the distal margins, often close together. They can also be found on the front side of the terminal flagellomeres. The probable function of these sensilla is chemo or thermoreception (**Zacharuk, 1985**).

In the present study, treatment of early pupae with 5 µl peppermint oil caused a reduction in the number of BB bristle in the emerged adults, malformation and disorientation in the direction of antennal sensilla, fusion of sensilla trichodea (ST1, ST2).

Sticky malformed mouth parts with attached pupal skin, enlarged membranous joint and malformed swollen between the antennal segments were also observed after treatment (Figs. 4b, 5c and 6b).

The present results reveal that peppermint oil caused abnormalities in the shape of antennal sensilla especially trichoid sensilla which are specific for the female sex pheromones. The results obtained are in confirmation with the works of **Reda *et al.* (2010)** who reported that peppermint oil caused malformation and disorientation in the antennal structures and their associated sensilla in the museum pests. Similarly, **Soryia, *et al.* (2009)** observed morphogenic defects on antennae due to treatments of adults of *C. maculatus* with lufenuron.

The undesirable effects of peppermint oil on the structure of antennae and their associated sensilla may cause failure of treated males to mate with females and ultimately lower the population of insect pest.

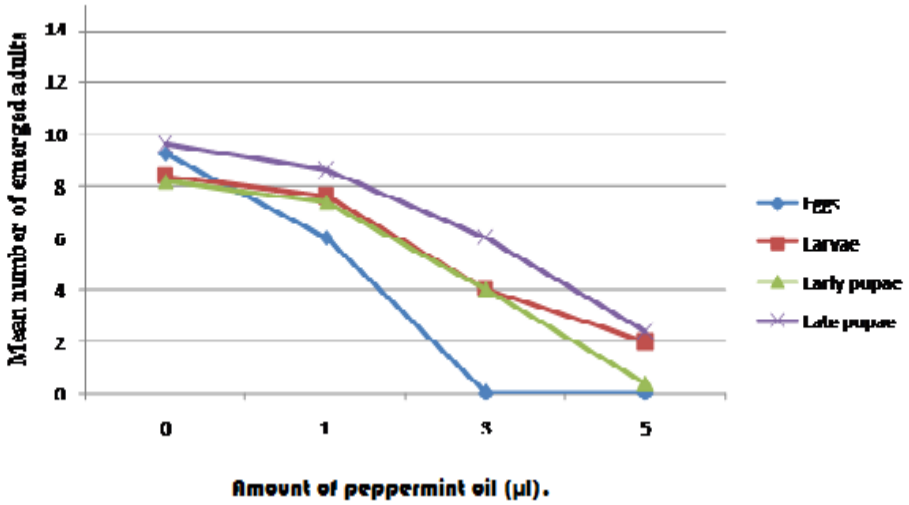


**Fig. 1: Susceptibility of different developmental stages of *C. maculatus* to peppermint oil fumigation (estimated from the number of emerged adults).**

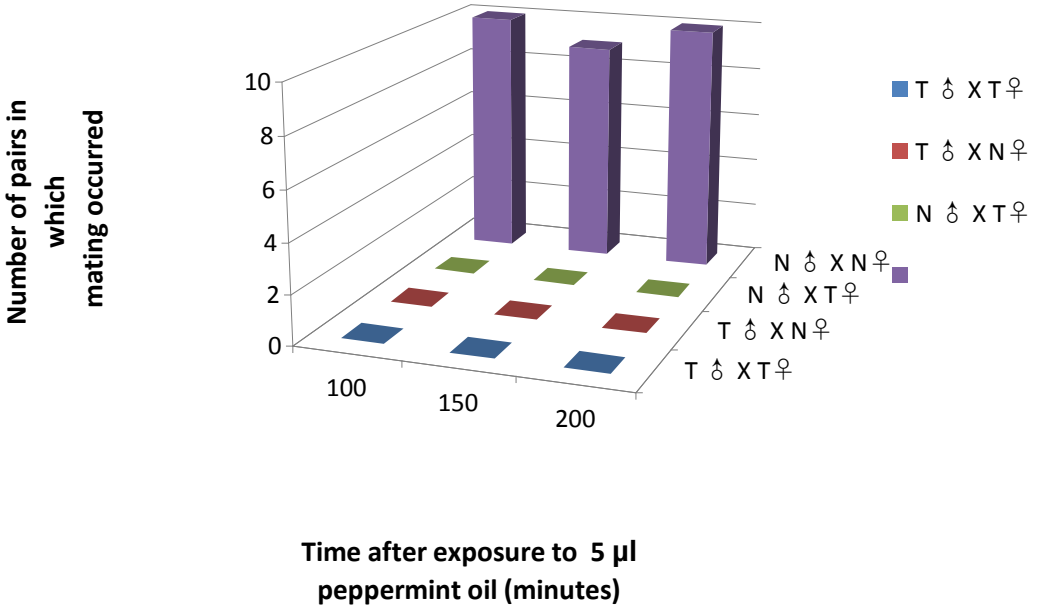


**Fig. 2: Effect of peppermint oil on mating behavior for different combinations of pairs (observations within five minutes) after different exposure times** (T: treated insects; N: untreated insects).

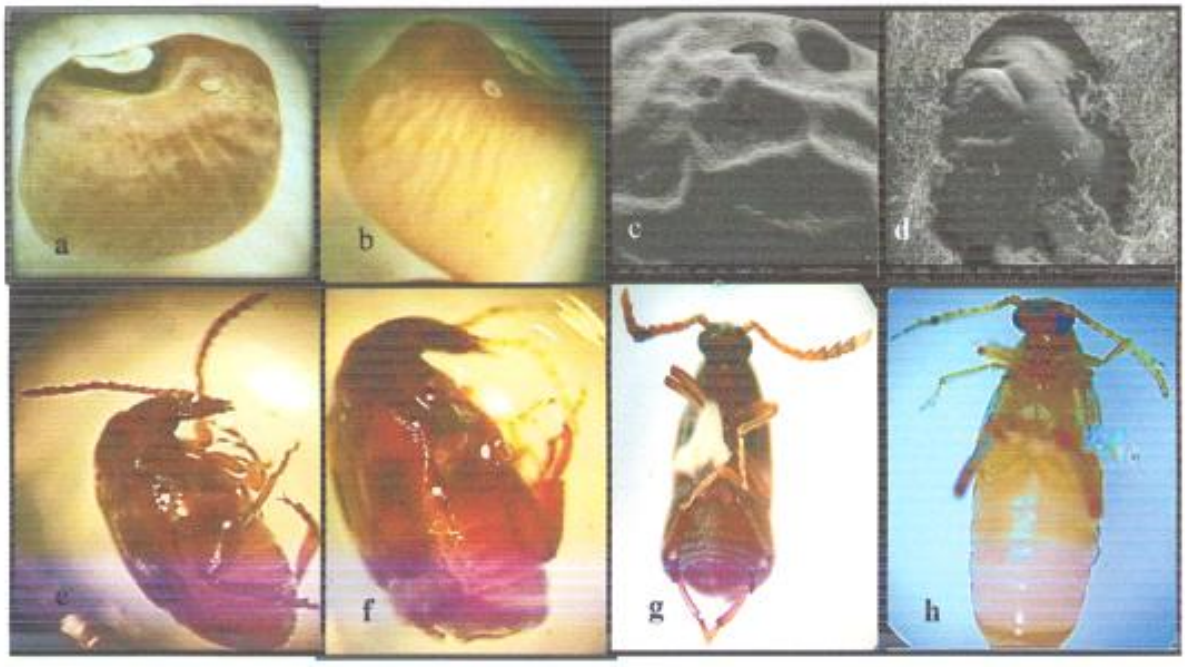


Fig. (3): Morphological changes induced on developmental stages of *C. maculatus* due to fumigation with peppermint oil.

(a): Photograph of normal untreated egg on cowpea seed (30x).

(b): Photograph of treated egg (30x).

(c): Scanning electron micrograph of treated cowpea seed with larvae inside showing pores on the surface of the seed. Bar 500 µm.

(d): Scanning electron micrograph showing treated larva making pores on the surface of a cowpea seed. Bar 300 µm.

(e): Photograph of normal adult *C. maculatus* showing normal structure of antennae and legs (30x).

(f): Photograph of adult *C. maculatus* resulted from treatment of early pupa with 5µl peppermint oil, showing disoriented unmelanized antennae and collapse of legs (30x).

(g): Photograph of adult *C. maculatus* resulted from treatment of late pupa with 5µl peppermint oil, showing different degrees of melanization of antennae, collapse of legs and attachment of exuvia to the legs (30x).

(h): Photograph of malformed *C. maculatus* resulted from treatment of early pupa with 5µl peppermint oil, showing some black color in antennae, collapse of legs with attached exuvia and enlarged abdomen (30x).

**Table (1): Effect of fumigation with different amounts of peppermint oil on survival of developmental stages of *C. maculatus*.**

| Amount of oil (µl) | Developmental stages | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Eggs | | Larvae | | Early pupae | | Late pupae | |
| | % Adult emergence | *% Reduction | % Adult emergence | *% Reduction | % Adult emergence | *% Reduction | % Adult emergence | *% Reduction |
| 0 | 96.25 | - | 84.00 | - | 82.00 | - | 96 | - |
| 1 | 60.00 | 37.66 | 76.00 | 9.52 | 74.00 | 9.76 | 86 | 10.42 |
| 3 | 0.00 | 100.00 | 40.00 | 52.38 | 40.00 | 51.22 | 60 | 37.50 |
| 5 | 0.00 | 100.00 | 20.00 | 76.19 | 4.00 | 95.12 | 24 | 75.00 |

***Reduction of adult emergence from control (0 µl)**

**Table (2): Effect of fumigation with peppermint oil (5 µl) on fecundity and adult emergence of *C. maculates* after different exposure times.**

| Exposure time (minutes) | Crossing pairs | Mean no. of eggs ±S.D | % Reduction | Mean no. of emerged adults ±S.D | % Reduction |
|---|---|---|---|---|---|
| 100 | N♂ XN ♀ | 61.6±5.85 | | 51.2±3.56 | |
| | T♂X T♀ | 30.0±29.06 | 51.29 | 0.0±0.0 | 100.0 |
| | T♂XN ♀ | 43.0±30.72 | 30.19 | 2.6±5.81 | 94.92 |
| | N♂ X T♀ | 53.75±12.72 | 12.74 | 4.2±6.9 | 91.79 |
| 150 | N♂ XN ♀ | 61.6±5.85 | | 51.2±3.56 | |
| | T♂X T♀ | 5.4±10.9 | 91.23 | 0.0±0.0 | 100.0 |
| | T♂XN ♀ | 11.2±12.47 | 81.81 | 0.6±1.34 | 98.83 |
| | N♂ X T♀ | 19.6±18.22 | 68.18 | 3.6±5.68 | 92.97 |
| 200 | N♂ XN ♀ | 61.6±5.85 | | 51.2±3.56 | |
| | T♂X T♀ | 2.2±3.89 | 96.42 | 0.0±0.0 | 100.0 |
| | T♂XN ♀ | 2.0±2.91 | 96.75 | 1.4±1.51 | 97.27 |
| | N♂ X T♀ | 3.0±5.19 | 95.12 | 1.0±2.23 | 98.05 |

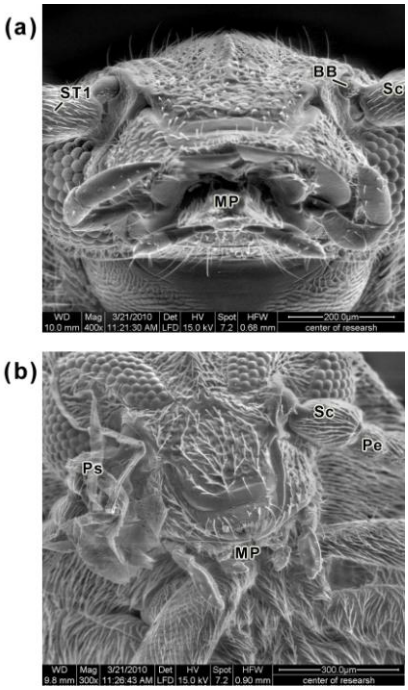


Fig.4(a):Scanning electron micrograph of frontal view of head of normal *C. maculatus* showing Bohm Bristles (BB) on joint between head and scape (Sc) Sensilla Trichoid (ST1) and normal structure of mouth parts (MP). Bar 200 µm.
(b) Scanning electron micrograph of frontal view of head capsule of adult resulted from treatment of early pupae with 5µl peppermint oil, showing absence of Bohm bristles (BB) on the base of the scape, malformation in the direction of antennal sensilla, fusion of sensilla and sticky malformed mouth parts (MP) with attached pupal skin (Ps). Bar 300 µm.

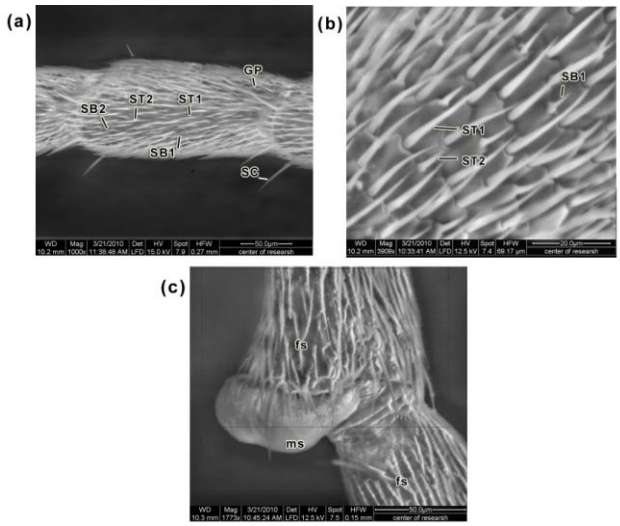


Fig. 5 (a): Scanning electron micrograph of dorsal view of antennal sensilla in $FL_6$ of normal *C. maculatus* showing Sensilla chaetica (SC), Sensilla trichoid1 (ST1), Sensilla trichoid2 (ST2), Sensilla basiconica1 (SB1), Sensilla basiconica2 (SB2) and grooved pegs (GP). Bar 50 µm.
(b): Scanning electron micrograph of dorsal view of antennal sensilla in $FL_6$ of normal *C. maculatus* showing sensilla trichoid1 (ST1), Sensilla trichoid2 (ST2) and Sensilla basiconica1 (SB1). Bar 20 µm.
(c): Scanning electron micrograph of joint between $Fl_5$ and $fl_6$ of treated *C. maculatus* showing malformed swollen (ms) and fused Sensilla (fs). Bar 50 µm.

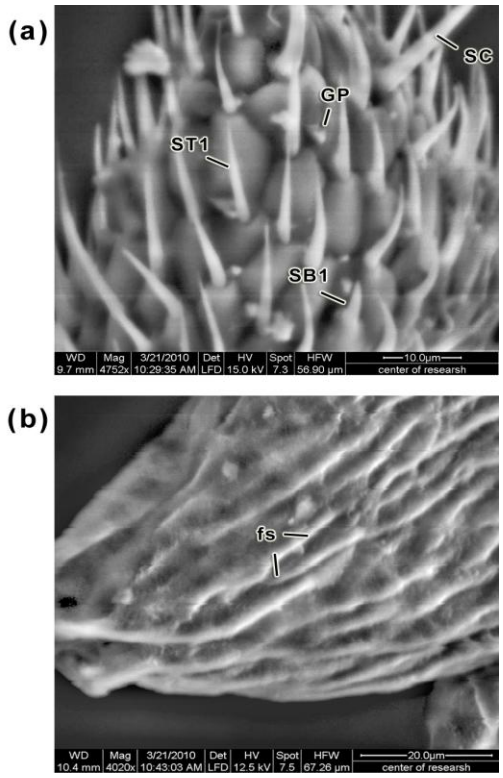


Fig. 6: Scanning electron micrograph of terminal antennal segment 9th of :
(a): untreated adult *C. maculatus* showing Sensilla chaetica (SC), Sensilla trichoid1 (ST1), Sensilla basiconica1 (SB1) and grooved pegs (GP). Bar 10 µm.
(b): adult resulting from treatment of early pupa with 5 µl peppermint oil showing fused sensillae (fs) Bar 20 µm.

This study shows that the peppermint volatile oil has an insecticidal activity; it may be toxic by penetrating the insect body via the respiratory system. The use of essential oils extracted from plants will have purely to be advised for the safeguarding of the environment and the health of the user. Essential oils could be used as biodegradable and natural bio protector for controlling stored product pests.

Results obtained in this study suggest an effect of the oil used on melanization of antennae. Further study on the effect of volatile oils on the amino acids involved is recommended.

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# Assessment of Heavy Metals Accumulation in Native Plant Species from Soils Contaminated in Riyadh City, Saudi Arabia

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**Abstract:** The second industrial area located south of Riyadh City – Saudi Arabia was selected for detailed study as pollution of this area with heavy metals has accelerated dramatically during the last decades. The concentrations of eight metals (Cd, Cr, Co, Cu, Fe, Ni, Pb and Zn) in soils and different plant organs of seven native plant species (*Calotropis procera* , *Citrullus colocynthis* , *Rhazya stricta* , *Cassia italic*, *Phragmite australis* , *Cyperus laevigatus and Argemone Mexicana*) collected from studied area were investigated. The bioaccumulation and transfer of metals from soil to roots and from roots to shoots was evaluated in terms of Bioaccumulation factor (BAF) and translocation factor (TF). The results showed that the concentrations of heavy metals in the soils have the sequence of (Fe > Zn >Cr >Cu > Pb > Ni > Co > Cd) while in plants the trend was (Fe > Zn >Cu >Cr > Ni > Co > Pb > Cd). Generally, leaves of the studied species accumulated less heavy metals than the corresponding roots except for Cd that could be accumulated in all plant organs (leaves, stems and roots). Based on BAFs and TFs values, most of the studied species have potential for phytostabilization and phytoextraction. *Calotropis procera* is suggested for phytostabiliation of Cu, Cd and Zn whereas *Rhazya stricta*, *Phragmite australis* and *Cyperus laevigatus* for Ni phytostabiliation. Among the plant species screened for Cd, Cu, Ni, Co, Pb and Zn, most of the species were efficient to take up and translocate more than one heavy metal from roots to shoots. According to accumulation capability of the investigated species for most metals, both *Phragmite australis* and *Cyperus laevigatus* are found to be the best candidates for biomonitoring and phytoremediation programs of polluted soils.
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**Keywords:** Heavy metals, Pollution, Phytoremediation, Metal accumulators

## 1. Introduction

Heavy metal pollution is considered to be one of the most dangerous hazards affecting both developing and developed countries. The large-scale industrialization and production of variety of chemical compounds has led to global deterioration of the environmental quality [1]. In Saudi Arabia, the pollution of main cities, especially Riyadh City, has increased in the past few decades because of increases in population and industrial activities that is likely to increase the volume of pollutants discharged to this area.

Metal persistence in soil for much longer periods than in other compartments of the biosphere is a matter of serious concern. According to Beyermann and Hartwig [2] heavy metals like As, Cd, Cr, Ni, Pb, etc, has classified to be carcinogenic to humans and wildlife.

Numerous efforts have been undertaken recently to find cost-effective technologies for remediation of heavy metal-contaminated soil [3]. Therefore, plants can be used to ameliorate heavy metal pollutants from the soil. This cost effective approach is called phytoremediation which also referred as green solution [4]. Phytoremediation has recently become a subject of public and scientific interest and a topic of many researches [5-7]. For chemically polluted lands, vegetation plays an increasingly important ecological and sanitary role [5]. Proper management of plants in such areas may significantly contribute to restoring the natural environment.

Plants growing in metalliferous soil can be grouped into the following three categories according to Baker [8]: a) excluders, in which metal concentrations in the shoots are maintained at low level up to a critical value across a wide range of soil concentrations; b) accumulators, in which metals are concentrated in above-ground plant parts from low to great soil concentrations; and c) indicators, in which the internal concentration reflects external levels. Moreover, the bioavailability of trace elements for plants is dependent on many environmental factors: concentrations in the environment, a biotic factors, exposure time, growth form of the plant, type of absorption mechanisms, affinity of trace elements for the adsorption sites and element speciation [9].

The identification of metal hyperaccumulators, plants capable of accumulating extra- ordinary high metal levels, demonstrates that plants have the genetic potential to clean up contaminated soil. Hyperaccumulators are also characterized by a shoot-to-root metal concentration ratio (i-e. the

translocation factor (TF)) of more than 1, whereas non-hyperaccumulator plants usually have great metal concentrations in the roots than in the shoots. Several authors [10-11] include the bioaccumulation factor (BAF) as an element for classification as a hyperaccumulator species. The BAF refers to the plant metal concentration in root and the soil metal concentration ratio. This ratio should be greater than one for inclusion into the hyperaccumulator category. Importance of hyperaccumulators has emphasized on further research in exploring the contaminated sites and finding new hyperaccumulator plants. Many plant species have become metal tolerant due to the adaptive responses of plant species to heavy metals, as these species are growing in contaminated sites from a long period. According to Antonsiewicz et al., and Yoon et al. [12-13] , native plants should be preferred for phytoremediation because these plants are often better in terms of survival, growth and reproduction under environmental stress than plants introduced from other environment. Therefore, the search for native plants that are tolerant to heavy metals is of particular importance. Few studies evaluated, under field conditions, the potential for phytoremediation of native plants [14]. With this idea, and public concern over soil contamination by heavy metals in industrialized area in Riyadh City, Saudi Arabia, searching for plant species with the potential for phytoremediation is necessary because no metal-tolerant and metal hyperaccumulator plants with potential application to this area have been reported. Therefore, the aim of this study was to: **1)** evaluate the concentrations of Cd, Co, Cr, Cu, Fe, Pb, Ni and Zn in soils and different plant organs (leaves, stems and roots) of seven native plant species (*Calotropis procera* (1), *Citrullus colocynthis* (2), *Rhazya stricta* (3), *Cassia italic* (4), *Phragmite australis* (5), *Cyperus laevigatus* (6) *and Argemone Mexicana* (7)), **2)** define which species and which plant organ exhibit the greatest accumulation, **3)** evaluate whether these species could be usefully employed in biomonitoring studies. Moreover, BAF and TF indices were determined to assess the tolerance categories developed by these species and to evaluate their potential for phytoremediation purposes.

## 2. Site Description

The Second Industrial City that located 12 Km south of Riyadh City, capital of Saudi Arabia, was established in 1976. It has been developed on four stages of a total area more than 18 million square meters. It houses more than 1050 of different industrial units with 120 thousand workers. The most important industries in this area are: food industries, metal industries, electrical and control equipment industries, and chemical industries. Plants growing in the nearby zone of industrial areas along various industrial units exhibiting increased concentrations of heavy metals, serving in many cases as biomonitors/ accumulators of pollution load. The area of collected plants and soils extended about 3 Km around metal and chemical industries.The climate in this area is continental with extremes of heat in summer and markedly cold in winter with low rainfall distributed mainly from December to March. The dried soil are similar to natural one, sandy clay, but with different metal concentration. Our observation showed that the vegetation was few and non-compact. Plant species collected were the most common/dominant species at the contaminated area. A total of seven plants and soils (at 0-20 cm depth from rhizosphere of each plant were taken from each site from where plant sample was rooted) were collected in August and September 2010, and their scientific names and characteristics were determined. The concentration of heavy metals was determined in the soil and in plant organs. The plants with high concentration of heavy metals were chosen as accumulators.

## 3. Materials and Methods

### 3. 1. Sampling

Soils as well as seven abundant and dominating native plants (*Calotropis procera, Citrullus colocynthis, Rhazya stricta, Cassia italica, Phragmite australis, Cyperus laevigatus and Argemone mexicana*) were collected from the second industrial area, south of Riyadh city, Saudi Arabia. For each soil sample, pH, texture and heavy metals were measured. pH of soil was measured immediately after collection using suspension of soil and water at a ratio of 1:2.5; additionally, this suspension was stirred for 5 min. For plant sampling, at least three whole plants of each species of current year were collected. To remove only soils, roots and rhizomes were washed with tap water while leaves were not washed before analyses. Therefore, the element concentrations in the roots and rhizomes refer to their tissue and surficially adsorbed elements. The native plant species were identified according to Alfarhan and Thomas[15]. Leaves, rhizomes and roots of the collected plants were separated to identify the different accumulation capability and selectivity of each organ. .

### 3.2 Analytical techniques

Soil samples (a composite mixture) were wet-sieved through a 63-mm sieve, washed with De-ionized water, dried at 105°C and homogenized. A representative portion of the sample (About 20 g) was used for grain size analysis using the standard dry sieving and sedimentation techniques [16]. For

heavy metals analysis, one gram of homogenized samples was digested using $HNO_3$-HF-$H_3BO_4$ acids according to Wade et al. [17]. Plant materials were oven-dried at 75 $^oC$ and grounded to a fine powder. In this way, homogeneous samples were obtained for each plant organ. Approximately 0.2 gram of leaves, rhizomes and roots powder were weighed and digested according to method described by Allen[18]. Soil and plant samples were analyzed for heavy metals by inductively coupled plasma optical emission spectrometry (ICP-OES) using a perkin Elmer Model Optima 5300 DV spectrometer. All the analyses were carried out on three subsamples.

Standard Reference Material (SRM) of National Institute and Technology (NIST, 2709 San Joaquin Soil and 1547Peach leaves) and internal reference materials were used for precision, quality assurance and control (QA/QC) for selected metal measurements. Average values of three replicates were taken for each determination. The precision of analytical procedures was expressed as Relative Standard Deviation (RSD) which ranged from 5-10% and was calculated from the standard deviation divided by the mean. The recovery rates of studied metals were within 90±10%. Chemicals, stock solutions and reagents were obtained from Merck and was of analytical grade. All glassware before use were washed with distilled water, soaked in nitric acid (30%) overnight, rinsed in de-ionized water and air-dried.

Biological Concentration Factor (BCF) was calculated as metal concentration ratio of plant roots to soil given in equation 1 [14]. Translocation Factor (TF) was described as ratio of heavy metals in plant shoot to that in plant root given in equation 2 [19;20].

BAC = [Metals] root/[Metals]soil ……(1)
TF = [Metals] shoot/ [Metals] root ……(2)

## 4. Results and Discussion

### 4.1. Soil properties

The topsoil from the different sampling sites, in the area under investigation, had small differences in texture and pH (Table 1). The results revealed that all sites characterized by sandy texture (88%-93%) except soil collected in the area located with *Phragmite australis* where mud percentage reaches 76% as this area affected by direct outfall of industrial wastes. The uniform grain size distribution obtained along the area indicated a stable depositional environment for a long period of time.

**Table 1. Characteristics of soils from studied sites**

| Soil properties | Soil sites | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| pH | 6.7±0.05 | 6.9±0.06 | 7.3±0.07 | 7.3±0.07 | 7.6 ±0.06 | 7.1±0.04 | 7.5±0.08 |
| Sand % | 90.1 | 92.5 | 88.6 | 93.2 | 33.2 | 90.7 | 86.5 |
| Mud % | 9.9 | 7.5 | 11.4 | 6.8 | 76.8 | 9.3 | 13.5 |

As indicated from table 1, the pH of soil was alkaline in nature throughout the studied area and varies from 6.7-7.6.

### 4.2 Plant and soil metal composition

Heavy metals contamination of arable soil showed several problems, including phytotoxic effects of certain elements such as Cd, Pb, Zn and Cu, which are well known as micronutrients and cause several phytotoxicities if critical endogenous levels are exceeded [21-22]. Another and even a more serious problem is posed by the up taking of potentially noxious elements through food or forage plant species and their being transferred to the food chain and, finally, to humans [23]. All heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants [23]. The use of plants for environmental restoration is an emerging technology. In this approach, plants capable of accumulating high levels of metals are grown in contaminated soils [24]. Interest in phytoextraction has significantly grown following the identification of metal accumulator plants.

According to the results of this study, the native plants and soil can well present further information about the metal content of their environment. Plant and soil analyses revealed that the accumulation is considerably the consequence of a kind of elements [25]. The concentrations of the investigated heavy metals in soil possess the sequence of (Fe > Zn >Cr >Cu > Pb > Ni > Co > Cd) while in plants the trend was (Fe > Zn >Cu >Cr > Ni > Co > Pb > Cd). However, the investigated native plants exhibited different element concentrations, depending on plant organ and the sampling site.

Cadmium (Cd) is a toxic element and exists along with Zn in nature. Average Cd concentrations of the seven plants and soils are given in Fig.1. Generally, The Cd concentration in the soils was relatively low (1 $\mu g.g^{-1}$ d.w). The highest Cd concentration was recorded at associated with *Argemone Mexicana* (site 7) and *Cassia italic* (site 4). This is may be attributed to the relatively high pH

value (Table 1) which enhance Cd precipitation at this sites [26]. The results indicated that Cd could be accumulated in all plant organs (leaves, stems and roots). The distribution of Cd within plant organs is quite variable and clearly illustrates its rapid translocation from roots to shoots [27]. The highest uptake of Cd was attained by *Calotropis procera* stem followed by *Argemone Mexicana*.

Chromium (Cr) is one of the toxic metals widely distributed in nature. It has two forms found in the environment, trivalent and hexavalent. The latter form is considered to be the greatest threat because of its strong oxidizing ability as well as high solubility and availability to penetrate cell membranes [28]. Chromium (Cr) is a non-essential metal to plant growth, and may be possible that plants do not have any specific mechanism and transport of Cr [29]. Generally, soils of all selected sites in the area under investigation acquired low concentrations of Cr except in site associated with *Phragmite australis* (Fig. 1), with the highest value recorded (528 ug.$g^{-1}$ d.w.). This is due to its location in place of highly polluted drain affected by industrial discharges. Results from the present study showed that roots of all plants attained higher Cr concentrations than other organs, with the highest value of 628.8 528 ug.$g^{-1}$ d.w attained by *Phragmite australis* root. This could be because Cr is immobilized in the vacuoles of the root cells and showed less translocation, thus rendering it less toxic. This may be a neutral toxicity response of the plants [30]. According to Macnicol and Bekett [30], the toxic levels of Cr in plants range from 1 to 10 µg.$g^{-1}$ dry weight.

Copper (Cu) is an essential element for plants and animals. However, excessive concentrations of this metal are considered to be highly toxic. The distribution pattern of Cu in the soil of studied sites (Fig. 1) indicated that sites 3, 5and 7 were enriched with this element (> 80 µg.g-1 d.w) compared with other sites. The average concentrations of Cu in all examined species are comparable (Fig.1). Generally, roots of most plants attained higher Cu concentrations than other organs, with maximum value of 741 µg.$g^{-1}$ d.w attained by *Phragmite australis* root. However, leaves and stems of both *Cassia italica* and *Cyperus Laevigatus* were found to accumulate considerable amounts of Cu (Fig. 1). Cu concentrations in plants above 10-30 µg.$g^{-1}$ d.w are regarded as poisonous [30]. Within roots, Cu is associated mainly with cell walls and is largely immobile. However, higher concentrations of Cu in shoots (leaves and stems) are always in phases of intensive growth and at the luxury Cu supply level [31]. High concentrations of Cu in the roots of. *Phragmite australis* with relatively high pH values in soil (Table 1) may be attributed to the presence of plaque, a metal-rich rhizo-concentrations composed of iron hydroxides and other metals that are mobilized and precipitated on the root surface [32]. This is in agreement with the finding of Weis and Weis [33] who reported that at higher pH conditions (> 7.0) the presence of plaque enhanced Cu uptake into roots.

Iron (Fe) is an essential micronutrient for plants and animals [34]. However, excessive Fe uptake can produce toxic effects. Fe is the most abundant metal in the studied area. The highest Fe concentration (Fig. 1) was determined in the soil of site 3, affected by industrial discharges from a nearby industrial complex. The results obtained from plant analysis asserted that roots of all seven plants are found to be highly capable of Fe accumulation (Fig.1). The highest concentrations were recorded in roots of *Rhazia stricta* (29160 µg.$g^{-1}$ d.w) followed by *Cyperus laevigatus* (27398 µg.$g^{-1}$ d.w). According to Allen [18], Fe concentrations above 40-500 µg.$g^{-1}$ d.w are considered as toxic to plants. As indicated by Tiffin [31], roots tend to absorb $Fe^{+2}$ cation more than $Fe^{+3}$. The ability of roots to reduce $Fe^{+3}$ to $Fe^{+2}$ is believed to be fundamental in the absorption of this cation by most plants [35]. Moreover, some bacteria species (e.g. *Metallogenium* sp.) are involved in Fe reduction and are known to accumulate this metal on the surface of living cells [36]. Higher concentrations of Fe in the roots of the investigated species could be due to its precipitation in iron- plaque on the root surface [37-38].

Lead (Pb) is the least mobile among the heavy metals. It is not essential but toxic to plants. The highest Pb concentration in soils was detected at sites 3 and 5. As regards to Pb accumulation in plants, Pb is believed to be the metal of least bioavailability and the most highly accumulated metal in root tissue while Pb shoot accumulation is much lower in most plant species [27]. This is in agreement with the results obtained from plant analysis in our study. The highest Pb concentration was detected in roots of all studied plants (Fig. 1) except in case of *Cassia italic* and *Cyperus laevigatus* where leaves exhibited more concentrations than roots. Recent results of Pb translocation and uptake studies showed that Pb is mobile within the plant under certain conditions [39].. Also, Blaylock and Huang [40] indicated that shoot Pb concentrations reached a value similar to the concentration found in intact roots of the same species, when it is immersed in a nutrient solution containing Pb. Generally, Pb concentrations in all seven plants were notably higher at sites 2, 3 and 6. This is could be related to airborne Pb deposition emitted from a heavily traffic high way affected the open area under investigation. Airborn Pb is readily taken up by plants through foliage [41]. As such, it

may be suggested that the habitually occurrence of *Cassia italic* and *Cyperus laevigatus* in an open desert area make it capable of receiving higher amounts of airborn Pb (32 and 40.8 µg.g$^{-1}$ d.w, respectively). According to Ross [42], 30-300 µg.g$^{-1}$ Pb concentrations are considered toxic to plants. Plants with higher Pb translocation will yield a higher shoot Pb concentration. These plants are considered promising for Pb phytoremediation programs because only shoots should be harvested in Pb phytoextraction which highlights the importance of the selected species as Pb accumulators [43].

Although Zn is essential trace element, high levels can cause harmful health effects. Toxicity of high level Zn concentrations in man is well known, [44]. Zn concentrations in soils in the studied area attained highest values of 820 and 680 µg/g d.w at sites 5 and 2, respectively (Fig.1). According to [23], toxicity level of this element is around 300 µg. g -1d.w.

The upper toxic levels of Zn in various plants range from 100 to 500 µg/g d.w [45]. The results demonstrated that roots often contain more Zn than shoots. The highest Zn root concentration,15060 µg. g$^{-1}$, was attained by *Phragmite australis*. The roots are thought to be important for zinc uptake [46]. It was noted that the highest zinc concentrations in roots of *Phragmite australis* and *Citrullus colocynthis* were associated with high concentrations in soils at the same place. Previous studies on the accumulation of various metal ions by native plants have shown that the deposition of most metals was higher in roots than the other parts of plants [47-48]. This is in line with the findings of the present study. *Phragmite australis* was tested for concurrent removal of Zn. This plant has removed the metal successfully without production of toxicity.

The mean concentration in normal plants (aboveground tissues) is 66 µg/g [49], and the toxic level is up to 230 µg/g [50-51]. The ranges of Zn in plants presented here were generally higher than the levels reported for other plants [52]. The results obtained by Aboulroos et al. [53] indicated that Zn content of plant increased with increasing levels of Zn in the soils. The research done by Kandil et al. [54] found highly significant correlations between the soil content of macro, micro-nutrients and heavy metals and its accumulation in roots of plants.

Both, cobalt and nickel are used in the metallurgical industry, for the production of high quality iron-based alloys. They are also, used extensively as catalysts in the chemical and food industry, as prime materials for the reduction of paints and batteries, and in the electroplating industry [55]. The highest Co and Ni concentrations in soils were detected at sites 6 and 5. As regards to Co and Ni accumulation in plants, they are believed to be highly accumulated in root tissues of *Cyperus laevigatus* ( 24.4 and 66.36 ug.g$^{-1}$ for Co and Ni, respectively) and *Phragmite australis,*(378.6 and 489 ug.g$^{-1}$ for Co and Ni, respectively). According to Kabata-Pendias,and Pendias[56] , the normal Ni content of terrestrial plants growing in uncontaminated soils was found to be in range of 0.1-3.7 ug.g$^{-1}$ for Ni. Our results showed that concentrations of Ni in the investigated species were higher than the normal plant, and this shows that these plants had a strong ability to tolerate this element. Heavy metal concentrations in roots of *Cyperus laevigatus* and *Phragmite australis* increased in the following pattern: Cu > Cr > Ni > Co. This is may indicate that all four metals come from similar sources of contamination. Moreover, increased concentrations of four metals in roots system were due to the presence of plaque, [32] with high pH conditions (> 7.0) which enhanced metals uptake into roots [33].

**4.3 Bioaccumulation and translocation in plants**

Accumulation of selected metals varied greatly among plants species and uptake of an element by a plant is primarily dependent on the plant species, its inherent controls, and the soil quality [57]. Large number of factors control metal accumulation and bioavailability associated with soil and climatic conditions, plant genotype and agronomic management, including: active/passive transfer processes, sequestration and speciation, redox states, the type of plant root system and the response of plants to elements in relation to seasonal cycles [56]. Structure of the sediment has also been considered very important that affect the extent of the metals taken up by the plants. Clay particles also play an important role in availability of the metals. Metal solubility in soils is predominantly controlled by pH, and oxidation state of the system [58]. The results indicated that soils of study area were sandy texture and were neutral in nature with pH greater than 6.7. Neutral and high soil pH can stabilize soil toxic elements, resulting in decreased leaching effects of the soils toxic elements. Moreover, toxic elements may also become stabilized due to slightly basic soil pH which may result in less element concentrations in the soil solution. This may restrain the absorbability of the elements from the soil solution and translocation into plant tissues [59]. Phytostabilisation is a process which depends on roots ability to limit the contaminant mobility and bio-availability in the soils which occurs through the sorption, precipitation, complexation or metal valance reduction [58]. Most of plant species under investigation had BAF >1, although the concentration

of heavy metals remained below 1000 ug. $g^{-1}$ (except for Fe and Zn). In general, BAF values of Cd, Cu, Ni and Zn were highest as compared to other metals (Table 2). The BAF values of *Calotropis procera, Citrullus colocynthis* and *Cassia italica* were highest for Cu (49.0, 58.9 and 55.9) and *Calotropis procera* for Cd (41.5). *Rhazya stricta*, *Phragmite australis* and *Cyperus laevigatus* had highest BAF for Ni while *Calotropis procera* and *Cyperus laevigatus* had highest BAF for Zn (191.0 and 27.6, respectively). Heavy metals tolerant species with high BAF can be used for phytostabilisation of contaminated soils as these species retains metals in their roots and limit metal mobility from roots to shoots once absorbed by roots of plants [19].

**Table 2. Bioaccumulation factor (BAF) of native plant species of selected metals**

| Heavy metal concentrations | | | | | | | | Species |
|---|---|---|---|---|---|---|---|---|
| Zn | Pb | Ni | Fe | Cu | Co | Cr | Cd | |
| 191.0 | 8.8 | 21.3 | 2.4 | 49.0 | 12.7 | 7.8 | 41.5 | *Calotropis procera* |
| 15.0 | 9.6 | 20.2 | 3.1 | 58.9 | 4.1 | 4.9 | 18 | *Citrullus colocynthis* |
| 11.2 | 0.7 | 31.8 | 3.3 | 6.8 | 8.4 | 5.8 | 27.3 | *Rhazya stricta* |
| 12.8 | 2.1 | 20.5 | 4.0 | 55.9 | 0.6 | 7.8 | 1.7 | *Cassia italica* |
| 18.4 | 0.7 | 29.9 | 3.8 | 8.2 | 11.2 | 1.2 | 5.3 | *Phragmite australis* |
| 27.6 | 2.5 | 30.6 | 3.8 | 13.4 | 19.3 | 2.1 | 20.1 | *Cyperus laevigatus* |
| 7.22 | 2.3 | 29.3 | 3.2 | 6.2 | 9.8 | 4.7 | 3.7 | *Argemone mexicana* |

The translocation factors (TF) generally showed the movement of metal from soil to root and shoot, indicating the efficiency to uptake the bio-available metals from the system. TF gives an idea whether the native plant is an accumulator, excluder or indicator. Among the plant species screened for Cd, Cu, Ni, Co , Pb and Zn, most of the species were efficient to take up and translocate more than one heavy metal from roots to shoots (Table 3) with a noticeable variations between TF values. The highest TF value (6.38) was found for Cd by *Calotropis procera*. Moreover. *Cassia italica* was efficient in translocation Co and Pb from roots to shoots with TF values of 7.2 and 4.43, respectively. According to Ghosh and Singh [58], high root to shoot translocation of heavy metals indicated that these plants have vital characteristics to be used in phytoextraction of these metals. It is easy for plants species with TF > 1 to translocate metals from roots to shoots than those which restrict metals in their roots.

**Table 3. Translocation factor (TF) of native plant species of selected metals**

| Heavy metal concentrations | | | | | | | | Species |
|---|---|---|---|---|---|---|---|---|
| Zn | Pb | Ni | Fe | Cu | Co | Cr | Cd | |
| 0.60 | 1.02 | 1.23 | 0.48 | 1.90 | 0.27 | 0.54 | 6.38 | *Calotropis procera* |
| 1.40 | 0.64 | 1.10 | 0.93 | 1.90 | 2.26 | 2.46 | 1.90 | *Citrullus colocynthis* |
| 1.70 | 0.74 | 0.60 | 0.70 | 1.40 | 0.50 | 0.96 | 1.10 | *Rhazya stricta* |
| 1.57 | 4.43 | 1.78 | 0.84 | 2.40 | 7.20 | 1.43 | 2.50 | *Cassia italica* |
| 0.76 | 1.12 | 1.00 | 0.84 | 1.20 | 2.10 | 0.37 | 1.85 | *Phragmite australis* |
| 1.16 | 1.98 | 1.16 | 0.90 | 2.40 | 0.43 | 0.67 | 1.08 | *Cyperus laevigatus* |
| 1.20 | 1.86 | 1.77 | 0.93 | 1.80 | 1.60 | 1.54 | 3.30 | *Argemone mexicana* |

High metal accumulation may be attributed to well develop detoxification mechanism based on sequestration of heavy metal ions in vacuoles, by binding them on appropriate ligands such as organic acids, proteins and peptides in the presence of enzymes that can function at high level of metalicions [19] and metal exclusion strategies of plant species [58]. Plant species with high TF values were considered suitable for phytoextraction generally requires translocation of heavy metals in easily harvestable plant parts i.e. shoots [13]. According to Gosh and Singh [58] phyto-extraction is a process to remove the contamination from soil without destroying soil structure and fertility.

The results of the present study highlighted that all plants had relatively low BAF (2.4-4.0) and TF < 1 for Fe in comparison to other metals. The elevated concentration of Fe in roots of plants under investigation and low translocation in above ground parts indicated their suitability for phytostabilisation of this element in the study area.

## Conclusion

Results of this research work indicated that all seven plants namely *Calotropis procera* , *Citrullus colocynthis* , *Rhazya stricta* , *Cassia italic*, *Phragmite australis*, *Cyperus laevigatus, and Argemone Mexicana* are accumulator for the studied heavy metals. The concentrations of heavy metals in soils have the sequence of (Fe > Zn >Cr >Cu > Pb > Ni > Co > Cd) while in plants the trend was (Fe > Zn >Cu >Cr > Ni > Co > Pb > Cd. Roots of all seven plants with the highest concentrations of all studied metals, except Cd, are the best biomonitors for heavy metals

contamination in the studied area. The bioaccumulation factor (BAF) values of Cd, Cu, Ni and Zn were highest as compared to other metals**.** According to translocation factor (TF)**,** the highest value was found for Cd by *Calotropis procera.* While *Cassia italic* was efficient in translocation of Co and Pb from roots to shoots. Those species could be considered as hyper accumulators and suitable for phytoextraction. However, All plants had relatively low BAF and TF < 1 for Fe in comparison to other metals. The elevated concentration of Fe in roots of studied plants and low translocation in above ground parts indicated their suitability for phytostabilisation of this element in the study area. The present study shows that some plant species can be suitable option for phytoextraction and phytostabilization. Growing factors important to phytoremediation can provide a basis for genetic modification of plants for improved performance. Biotechnological and genetic engineering based approaches can be used to enhance the naturally occurring plants to detoxify hazardous compounds.

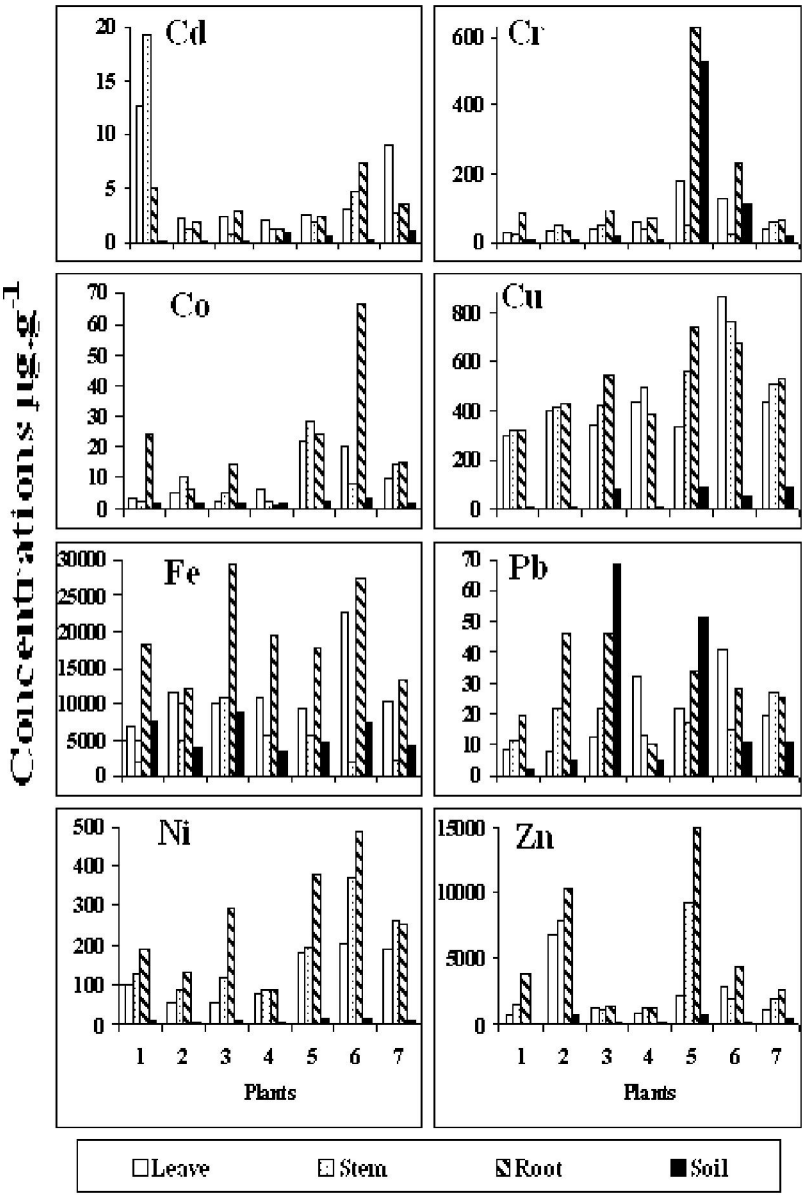


**Figure 1.** Average Cd,Cr,Co,Cu,Fe,Pb,Ni and Zn concentrations (ug.g-1 dry weight) in leaves, stems and roots as well as soil associated with *Calotropis procera* (1) , *Citrullus colocynthis* (2), *Rhazya stricta* (3), *Cassia italic* (4), *Phragmite australis* (5), *Cyperus laevigatus* (6) *and Argemone Mexicana* (7)

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3/28/12

# McGill Exercises versus Conventional Exercises in Chronic Low Back Pain

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**Abstract: Background:** McGill exercises are designed to impose minimal spinal loading while sufficiently challenging the abdominal and spinal muscles. The purpose of this study was to compare between the effects of McGill exercises and conventional exercises on physical function in patients with nonspecific chronic low back pain (LBP). **Setting:** A physical therapy outpatient clinic. **Participants:** Sixty participants with nonspecific chronic LBP completed the program. Pain duration was more than 12 weeks. **Interventions:** The first group (n=30, mean age= 44.7±15.1 years) received Infra-red and conventional exercises (stretching and strengthening exercises). The second group (n=30, mean age=47.2±13.8 years) received Infra-red and McGill exercises. **Materials:** Performance based measures (the fifty-foot preferred speed walk, fifty-foot fast walk, and distance walked in five minutes) were used to measure physical function before and after 6 weeks of treatment. **Results:** The second group showed statistically significant increase in physical function as measured by the fifty-foot preferred speed walk ($F_{1,57}$=6.7, $P$=.01), fifty-foot fast speed walk ($F_{1,57}$=7.4, $P$=0.001), and distance walked in five minutes ($F_{1,57}$=10.4, $P$=0.001). **Conclusion:** McGill exercises increased physical function of patients with nonspecific chronic LBP. In this study, McGill exercises were of value for patients with nonspecific chronic LBP.
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**Key Words:** Low back pain, McGill, Exercises, Function

## 1. Introduction

Low back pain (LBP) is one of the most common costly health problems due to the considerable impact on daily functioning, sickness absence, and work disability.[1,2] The prevalence of LBP and the number of patients seeking care with physical therapy has increased over the last two decades.[3] There are many approaches to treat LBP such as medications, surgery, massage, traction, ultrasound, laser, ergonomics, heat, stretching and strengthening exercises.[4] However, there is no agreement among physicians and physical therapists about the best interventions for LBP.[5]

Various programs of stabilizing exercises have been used in treatment of patients with LBP.[6,7-8] McGill proposed safe stabilizing exercises to enhance spinal stability without imposing high loads on the spine in patients with LBP.[9] These exercises would achieve appropriate levels of activation of all back and abdominal muscles (rectus abdominis, quadratus lumborum, obliques, transversus abdominis, multifidus, and erector spinae), with minimal spinal loading to ensure spinal stability in patients with LBP.[9]

Some commonly used conventional exercises provide substantial compressive loads on the spine that would serve only to ensure the patient would remain a patient.[10] For example, extending the trunk and arms from a prone position resulted in 6000 Newtons of spinal compression thus exceeding the National Institute of Occupational Safety and Health (NIOSH) guidelines.[10] Researchers of the NIOSH conducted a field study recording injury rates with various levels of calculated spine compression (NIOSH, 1981).[11] They set the action limit for compressive loading of the lumbar spine at 3400 Newtons. Repetitive loading above this limit is not recommended and activities are safe as long as the spinal loading is below that number.[9] Therefore, patients with LBP should receive exercises that do not impose spinal loading in excess of 3400 Newtons such as the stabilizing exercises of McGill. There has been little research about using McGill exercises in different patient populations. Patients with LBP shows decreased physical function.[12] Therefore, enhancing physical function is of a high priority in treating patients with LBP. No randomized controlled trial tested the assertion that McGill stabilizing exercises is beneficial in a sample of patients with nonspecific chronic LBP using physical performance tests as outcomes. The purpose of this study was to compare between the effect of McGill exercises and conventional exercises in increasing the physical function of patients with nonspecific LBP.

## 2. Material and Methods

### Design

Participants were randomly assigned to one of two treatment groups: (1) a group that received Infra-red and the conventional exercises or (2) a group that received Infra-red and McGill exercises. The research physical therapist who performed the outcome assessments of participants and data analyses was unaware of group allocation. However, the clinical physical therapist who administered the exercises was aware of group allocation. Participants were not

aware of the theoretical bases of each of the exercise regimens because the study's objective was described to them in the following way: "to compare between two physical therapy programs for the trunk muscles, which may have a role in increasing physical function of patients with LBP.

**Participants**

Sixty seven participants with nonspecific LBP were recruited from a physical therapy clinic. Inclusion criteria included males or females of any race with a history of nonspecific LBP between T12 and the gluteal fold for more than 12 weeks. Exclusion criteria included a history of previous lumbar surgery, spinal stenosis, spondylolisthesis, neurological dysfunction, systemic disease, injection therapy, carcinoma, or pregnancy. All participants received their assigned interventions two times a week for the six week period of the study. They signed a consent form prior to participation in the study.

**Materials**

Three performance-based measures (fifty-foot preferred speed walk, fifty-foot fast speed walk, and distance walked in five minutes) were used to measure physical function in patients with LBP. For the fifty-foot preferred speed walk, the patient walks forward at his/her preferred walking speed for 25 feet and turns around and returns to the starting position.[12-13] For the fifty-foot fast speed walk, **t**he patient walks as fast as possible forward for 25 feet and turns around and returns to the starting position. [12-13] For the distance walked in five minutes**,** the therapist measures the farthest distance the patient can walk within five minutes.[13-15] They have been reported as valid and reliable measuring tools in LBP.[12-13]

**Interventions**

Prior to participating in the study, each participant was randomly assigned to either a control group (Group 1) or a treatment group (Group 2), using a table of random numbers. A physical therapist tested the participants at both the initial and final sessions. Another therapist performed all interventions. Participants of both groups received infra-red for 15 minutes. The conventional exercises included stretching and strengthening exercises for the trunk and the lower limbs. Participants received a series of progressive exercises building up to a maximum of 10-12 exercises by the final visit based on their individual needs. Participants carried out one set of 10 repetitions for each exercise, with a 30-second to one-minute rest between each set during each exercise session. For a home exercises, participants also performed four to six exercises of the conventional exercises on the basis of individual needs. Participants performed two sets of 10 repetitions for each exercise, with a 30-second to one-minute rest between each set, twice per day on the days when they did not come to the clinic.

Participants in the second group received McGill exercises. Each patient was trained to find his/her neutral spinal posture prior to initiating the stabilizing exercises. The McGill program begins with a motion exercise (cat-camel motion exercise). It consists of six-to-eight cycles of spinal flexion and extension in a quadruped position. This is followed by the curl-up exercises, in which the patient flexes one knee while keeping the other straight to minimize loss of the neutral posture. Then, the patient gently raises just the head and shoulders a short distance off the floor. This exercise can be followed by the side-support exercise. The patient is positioned as follows: lying on the side supported on his/her elbow and hip, knees bent to 90º, free hand placed on the opposite shoulder. The patient then raises his/her trunk until the body is supported on the elbow and the knee. If the patient was not able to perform the side support exercise, the patient would assume the side lying position and initiate an isometric contraction of the quadrates lumborum by trying to lift both lower limbs up toward the ceiling. Upon successful performance of the side support exercise, the birddog exercise (opposite arm and leg extension in the quadruped position) was carried out. In the quadruped position, the patient can also perform single leg lifting and/or single arm lifting. However, they performed one set of 10 repetitions for each McGill exercise, with a 30-second to one-minute rest between each set during each exercise session.

For a home program, participants performed four to six McGill exercises. They performed two sets of 10 repetitions for each exercise, with a 30-second to one-minute rest between each set, twice per day on the on the days when they did not come to the clinic. In both groups, the therapist asked the participants to use weekly self-report exercise logs to monitor the home program.

**Data Analysis**

Separate univariate analyses of covariance with the pretest scores as the covariates, were performed to determine whether there is a difference between the two groups on the posttest scores of physical function. A Bonferroni approach was used to maintain the alpha level at $P < 0.05$.

**3. Results**

Sixty seven participants with nonspecific chronic LBP participated in this study. However, in Group 1, three participants missed more than two physical therapy sessions due to scheduling and transportation difficulties In Group 2, four participants missed more than two physical therapy sessions due to scheduling conflicts. Data of 60

participants who completed the study were statistically analyzed.

Group 1 comprised 30 participants (19 females and 11 males) average age 44.7±15.1years, height 67.2 ±11.2 inches, and weight 159.2 ± 23.2 pounds. Group 2 comprised 30 participants (17 females and 13 males), average age 47.2 ±13.8 years, height 70.1 ± 9.4 inches, and weight 165.1 ±22.3 pounds. No adverse events were observed or reported by any participant in either intervention group. The ANCOVA revealed significant differences between the two groups on the fifty-foot preferred speed walk ($F_{1,57}$=6.7, $P$=.01, Table 1), the fifty-foot fast speed walk ($F_{1,57}$=7.4, $P$=0.001, Table 2) and the distance walked in five minutes ($F_{1,57}$=10.4, $P$=0.001, Table 3), in favor of the second group. The second group displayed higher mean post-test scores as measured by the three physical performance measures.

**Table 1**. Analysis of Covariance for the Variable of Fifty-Foot Preferred Speed Walk Using the Pretest as the Covariate

| | Sum of Squares | Df | Mean Square | F |
|---|---|---|---|---|
| Main Effects Group | 77.6 | 1 | 74.6 | 6.7[a] |
| Covariate Pretest | 1133.3 | 1 | 1123.3 | 97.5 |
| Residual | 575.3 | 57 | 10.5 | |
| Total | 10502 | 60 | 198.2 | |

[a]$p$<0.0167

**Table 2.** Analysis of Covariance for the Variable of Fifty-Foot Fast Speed Walk Using the Pretest as the Covariate

| | Sum of Squares | Df | Mean Square | F |
|---|---|---|---|---|
| Main Effects Group | 28.7 | 1 | 27.7 | 7.4[a] |
| Covariate Pretest | 140.1 | 1 | 140.1 | 38.6 |
| Residual | 181.35 | 57 | 3.6 | |
| Total | 5301 | 60 | 100.01 | |

[a]$p$<0.0167

**Table 3.** Analysis of Covariance for the Variable of Distance Walked in Five Minutes Using the Pretest as the Covariate

| | Sum of Squares | Df | Mean Square | F |
|---|---|---|---|---|
| Main Effects Group | 2535.3 | 1 | 2535.3 | 10.40[a] |
| Covariate Pretest | 314375.4 | 1 | 314375.4 | 1468.3 |
| Residual | 10705.2 | 57 | 214.1 | |
| Total | 10448329 | 60 | 197138.3 | |

[a]$p$<0.0167

**4. Discussion**

All of the participants in this report showed increase in physical function in both intervention groups, although the improvements were statistically significantly greater in the McGill group. Some authors designed specific stabilizing exercises that focus on reeducating the motor control system to activate the transversus abdominis and multifidus in patients with LBP.[12] There have been several studies investigating the effects of those exercises in different patient populations with LBP.[14-21] There have been contradictory results of these studies. In this study, participants received another program of stabilizing exercises based on measured, biomechanical factors.[9]

Improvements of participants in the McGill group can be attributed to better training of abdominal and back muscles without imposing high loads. Our results support the previous work done by Callaghan et al.[10] and Axler and McGill.[22] Those authors tested various types of therapeutic exercises and showed that McGill exercises can enhance the muscular work without high spinal loads (<3400 Newtons) in healthy subjects.

There was only one controlled-randomized trial that evaluated the effects of McGill stabilizing exercises in postnatal LBP.[23] The group that received McGill exercises had decreased pain intensity and disability compared with the control group post-treatment postpartum.

In this study, we used performance-based procedures to measure physical function. Simmonds et al. demonstrated that physical performance tests such as the fifty foot walk and distance walked in 5 minutes were reliable, valid, and able to distinguish between patients with LBP and healthy subjects.[12] Physical performance tests are objective standardized tests of physical function and are easy to demonstrate and need no equipment.[24-25] They also help control for errors in judgment, memory, and the ability to answer questions correctly.[26]

Seven participants withdrew from the study. However, the loss of participants to follow up was

associated with difficulties primarily related to scheduling the intervention sessions in both groups. No adverse effects were recorded in any of the patients in either group. Therefore, they did not withdraw due to the interventions.

In this study, self report logs were used to measure adherence of patients. Self report logs often overestimate adherence; however, they are still commonly used methods to assess adherence.[27] It should be pointed out that adherence to home exercise programs has not been adequately reported in many randomized controlled studies.[28]

There is a need to measure long-term outcomes to further substantiate the present study findings. Also, electromyography should be used to assess muscle recruitment during the performance of exercise programs. Future studies should include measuring psychological outcomes. There is also an urgent need to develop a universal classification system for LBP. Based on the results of the statistical analyses and within the limitations of the study, it can be concluded that McGill exercises may increase physical function in patients with nonspecific LBP.

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3/26/12

# Assessment of Urban Geomorphological Hazard in the North-East of Cairo City, Using Remote Sensing and GIS Techniques

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**Abstract:** This study aims to assess the geomorphological hazard of urban area in the north eastern side of Cairo City, based on analyses of remote sensing and GIS. In addition the objective of this study is to develop a GIS-aided urban geomorphological hazard zoning in the north-east of Cairo city. The main landforms of the area were delineated by using remote sensing and land surveying data, applying multi criteria decision analysis to evaluate it. This criterion includes geomorphic factors and sub factors such as: urban site location, urban morphology, Slope gradient, Digital Elevation Models, Gully Density. The research methodology focused on the analysis of those variables factors to indentify urban hazards areas. The results indicate that most of lands with grad (1) which is about 62% are in low risk area, and about of land 26% of the total area is in moderate hazard, and >12% in high hazards of the study area is in high risk hazards.

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**Key words:** GIS, Remote Sensing, Hazard, Urban, Geomorphology

## 1. Introduction

Remote sensing technology has greatly facilitated investigation of land use/cover changes. For example, Sultan et al. (1999) used the Landsat Multispectral Scanner (MSS, 79 m resolution) and TM images to study the urbanization process on the Nile Delta during the 70s and 80s. They found that urban areas increased 58% in 18 years since 1972. Another study, Fahim et al., (1999), found accelerated urban growth at the expense of agricultural land during 1987–1995 as compared to the period 1950–1987. There has long been an urgent need for land use management and policies should be based on an accurate understanding of current land use conditions to ensure future sustainable growth, Hefny, (1983).In addition Diae Chengta, (1996) have been presented a new approach of urban geomorphological, taking an elevation about catastroability of the geomorphological environment in Chongqing City.

This study uses image processing and analysis integrated in a geographic information system (GIS) to assess spatial analysis in north eastern area of Cairo city. Spatial patterns of urban land use and population distribution were compared quantitatively. Such analyses would shed a light on the overall impact of political-economic environment and policy changes on urbanization processes for Cairo, a large city of a developing country, which can be used effectively in geomorphological hazard mapping of urban location.

**The objectives of the study are as follow:**

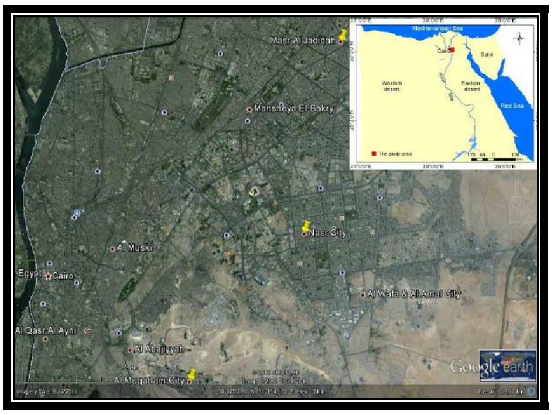
1. To assessment the geomorphological hazard of urban area in the north eastern sector of Cairo City, based on analyses of remote sensing and GIS.
2. Application of GIS methods of urban geomorphology to determine the hazard degree of the buildup in study area.
3. Extracting geomorphological hazard map of the area of study according to physical parameters

**The study area**

Cairo is located in northern Egypt- Lower Egypt- near Delta Apex, 165 kilometers (100 mi) south of the Mediterranean Sea and 120 kilometers (75 mi) west of the Gulf of Suez and Canal. Cairo City Coordinates are 30°3′29″N 31°13′44″E. Figure (1).The study area located in the north -east sector of Cairo City, the major towns of the study area are, Masr Aljididia, Naser City, Manshyit Naser, and Al Mouqattam City which are located in the eastern bank of River Nile.

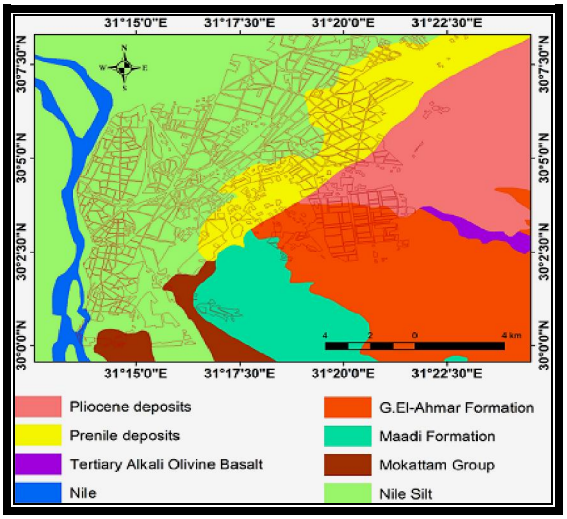
**Geology and Geomorphology features:**

The area belongs to the late Pleistocene which is represented into the Valley by the deposits of the Neonile which lowering its course at a rate of 1m/1000 years Said,R.(1967).and represented in the margin of eastern desert by rock formation belong to Eocene, Pleistocene and Holocene Figure(2).These rocks are the most wide spread covering the eastern part of Cairo City. In general the Eocene rocks are composed of bedded Carbonates and Calistics with varying proportion, alternating with marl and shale .The fractures ,joints and falts and bedding planes are great planes are of great important in the frequencies of rock fall in this area.




**Source:** Google earth2012, Topographic map Scale 1:25000

**Fig (1):** location map of Cairo City Geology and Geomorphology




After :CONOCO 1984,Scale 1:50000

**Fig(2):** Geological formation in the area of study

**The Geomorphology features of area noted as:**

**The structural plateau** in the east of River Nile is underline essentially by limestone rocks belong to Eocene and dissected by a number of faults running mainly in the north west-south east direction, such faults affect the morphology of the surface,the plateau of Mouqattam is the most important physical features in the area of study . **The edges** of this plateau are marked by a variety of slopes some of which are fault determined and consequently developed into steep escarpments. **The narrow piedmont plain** occupies the strip lying between the cultivated land and the foot slopes of the structural plateau. **The flood plain** is related to the River Nile. **Drainage lines** from conspicuous basin which follow mainly plain in the west, the fault lines of weakness, (Armanious, G, M,1990).the main features in the study area represents in Mokattam hill, it is a small limestone plateau lying to the south east of Cairo, rising to about 300 m above sea level, the bedrock of Mokattam plateau is highly fractured, resulting in the collapse of huge blocks from free faces in several sites particularly at the entrances of cave ceiling at the edges of what is called the power plateau at the sides of the road, that leads to the new town (Mokattam town)which has been build atop the upper plateau. The rock fall and rock sliding reflect the negative human interference such as miss use of swage water (sanitation) which absorbed in shale's through fractures causing hazards of mass wasting.

## 2.Materials and Methods

**Input data:**

The collected is based on field work in the north eastern of Cairo City. Over 100 points were located using Global Position System (GPS) receivers and described for the land use/cover conditions. Topographic maps of 1:50,000 scales were collected, scanned, digitized and Land sat thematic mapper (ETM) of 2009 was selected as it covers the study area, DEM of 20 m resolution was interpolated using Top grid module in Arc GIS. Geomorphological map descriptions were classified as table (1).

**Table (1) illustrates the main data set in the study area**

| Class Name | Description |
|---|---|
| Built-up | All types of man-made surfaces, including residential, commercial, industrial, transportation, etc |
| Geological Map | After CONOCO 1984,Scale 1:500000,presented the main formation in the area of study |
| DEM | Digital elevation model is used to describe a digital representation of the terrain surface |
| Slope | Slope map is expressed as a change in elevation over a certain area |
| Gully density | Gully density applied to define the hydrological parameter of the study area to asses hazards |
| Relief degree | The relief degree of land surface is an important reference factor of geomorphologic form classification used to describe and reflect the macroscopic characteristics of topography of the surface in a large area |

**Data processing**

The present work involves to establishment of data base in a digital format for the study area.

The flow chart summarizing the methodology that has been used in the study area as shown in figure (3). The processing of data input are geometrical analysis of urban –build up –area, surface analysis and spatial analysis. GIS application have been carried out to produce map of different factors controlling geomorphogical hazard in order to produce hazard map of the study area.

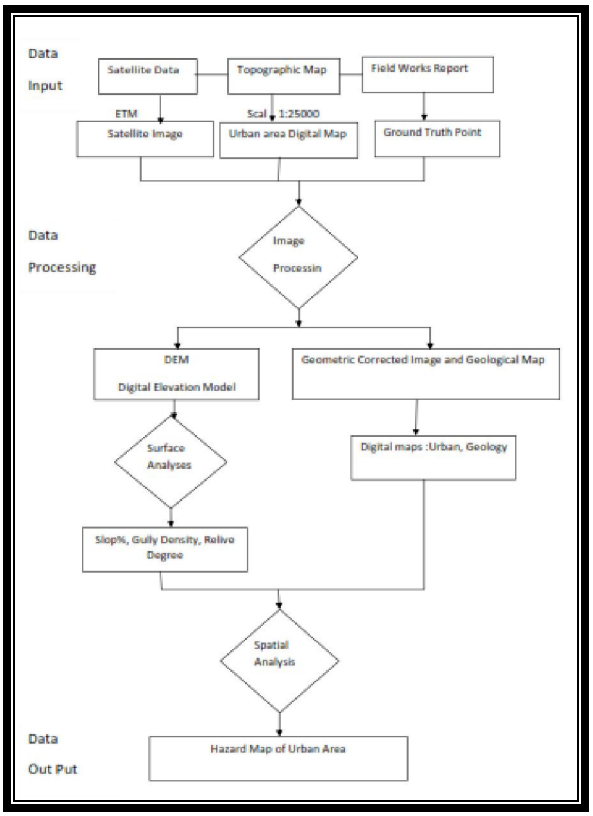
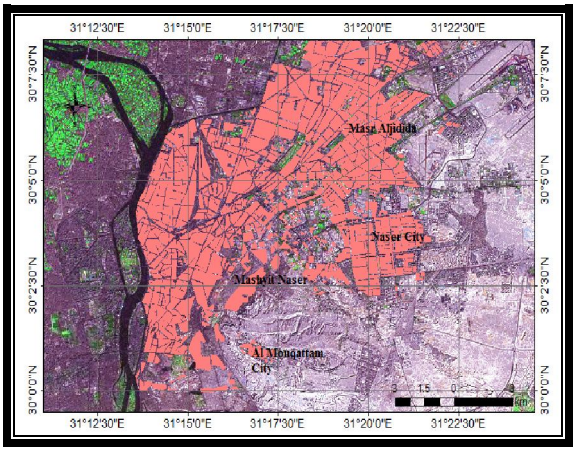



**Fig (3): Model constructed in urban geomorphological hazard map**

## 3. Results and Discussions

**Mapping of geomorphological units and urban location:**

From landsat image ETM, and topographic map Scale 1:50 000 the urban map of the study are presented in figure (4). Each united are characterize by particular spectral characteristics (i.e. color, pattern, and boundary).




**Fig(4): The build up area in the north eastern sector of Cairo City**

**The physical parameter of study area:**

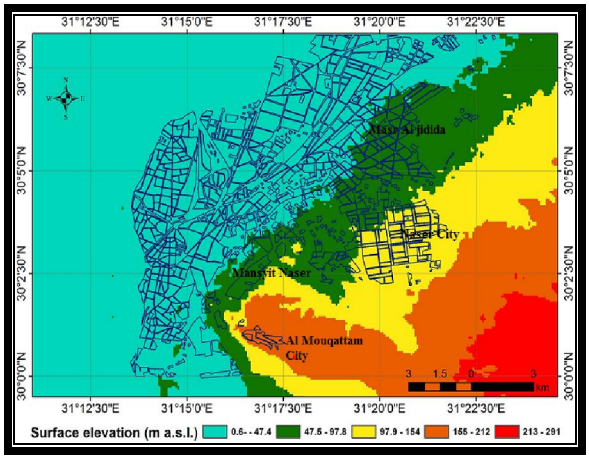
Based on the Land sat ETM images, the physical evaluation index of parameter are presents in table (2).

**Table (2) Evaluating index of parameters**

| Evaluating index | 1 Low | 2 Moderate | 3 High | 4 Very High | 5 Dangerous |
|---|---|---|---|---|---|
| Elevation(m) | <47.4 | 47.5-97.8 | 97.9-154 | 155-212 | 213-291 |
| Slope gradient(%) | <2 | 2-4 | 4-6 | >6 | |
| Gully density Km.Km2 | <1.25 | 1.25-5 | 5-10 | 10-15 | >15 |
| Relief degree(m) | <1 | 1-5 | 5-10 | 10-15 | >15 |

**1. The Digital Elevation Model (DEM)**

The Digital Elevation Model (DEM) ,Masr Al jidida and Manshit Naser City are located between 47.4m and 97.8m,Naser City is located between 79.9m and154m,and Al Mouqattam 155m and 212m. Figure (5)




**Fig(5) Digital Elevation Model of the study area**

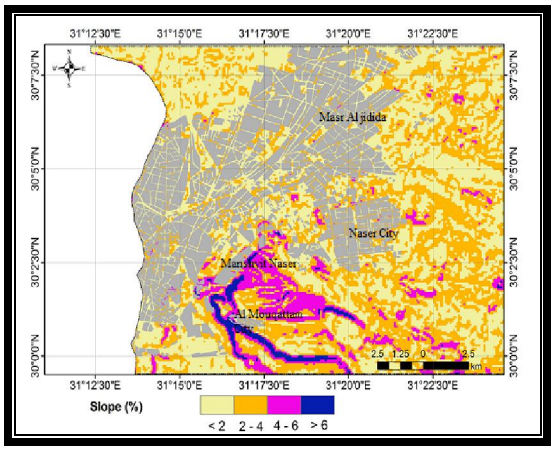
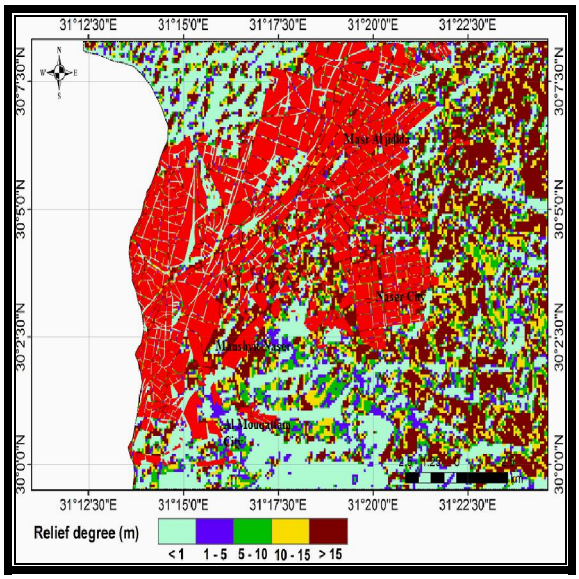


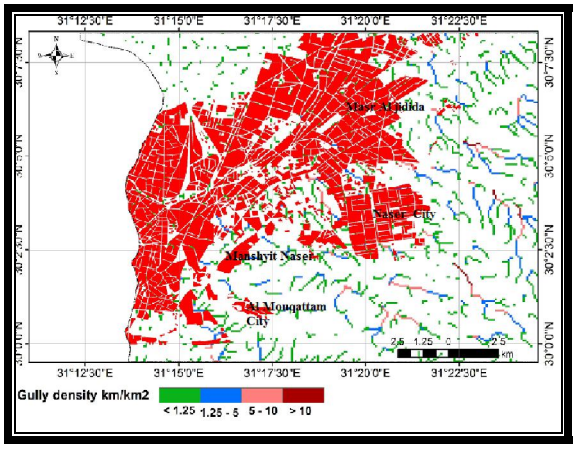

**Fig (6) slope degree in the study area**

The percentage of slope are ranging from 2% and 6%,Masr Al jidida and Manshit Naser , meanwhile Al Mouqattam town is ranging between 4% and 6% of slope figure(6).

The relief degree of land surface of the study area, based on DEM. Based on the extraction, classify the national geomorphologic form slow undulation, low undulation, moderate undulation, and mountainous undulation. According to classification the land surface degree of relief degree is ranging of <1. 25m, 1.25-5m, 5-10m, 10-15m and >15m.The results show that there is a very good correlation between the relief degree of land surface and the urban susceptibility, with the increase of the value of relief degree of land surface, its information content gradually increases up to >15 m.figure(7).the gully density <1.25 figure(8).




**Fig(7) Relief degree in the study area**




**Fig(8) Gully density in the study area**

The result indicates that 62% of urban build up are located in low hazard location, such as Masr Al jidida 25% in moderate hazard Such as Nasr city ,10% in high hazard in Al Mouqattam City, and Mashyiat Naser >1% in very high hazard location there are outside the area of study figure(9).

**Table (3) the percentage of urban geomorphological hazards In the north eastern side of Cairo City.**

| | Low | Moderate | High | Very high | Total |
|---|---|---|---|---|---|
| Number of units | 24158 | 9898 | 4072 | 233 | 38361 |
| Area(km$^2$) | 195.68 | 80.17 | 32.98 | 1.89 | 310.72 |
| Areal percentage(%) | 62.98 | 25.80 | 10.61 | 0.61 | 100.00 |

Note: Unit area = 90 * 90 m

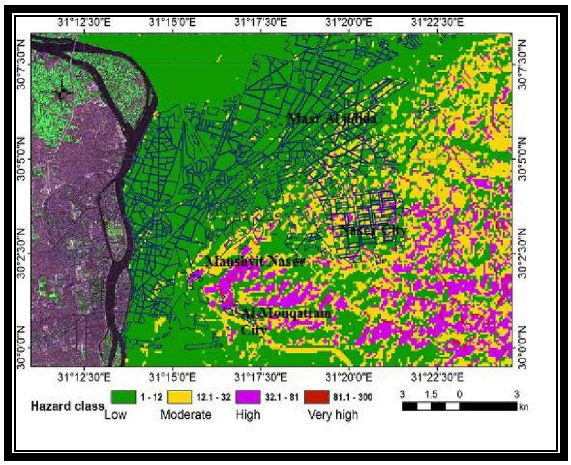



**Fig (9) hazard map degree of urban-build up- area of the study area**

## 4. Conclusion

- The use of satellite data proved to be useful in mapping the geomorphic land types.
- The urban extend are firmly related to geomorphology.
- Combination of field observation and Remote sensing analysis with satellite image, interpretation may provide valuable information about geomorphological sitting.

The study area is characterized by geomorphological features, each combines with urban distribution. The use of combined FCC of several band rendered in different colors for the same area is necessary for the discrimination between land units due to the fact that every feature has a unique spectral response with every band. Multi-band concert of satellite images was confirmed to be useful in discriminating features of similar spectral characteristics.

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3/28/12

# JAK2-V617F Mutation and BCR-ABL Rearrangement in Chronic Myeloproliferative Neoplasms

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**Abstract:** Myeloproliferative neoplasms (MPNs) are a group of clonal hematologic diseases that are thought to arise from a transformation in a hematopoietic stem cell that leads to overproduction of mature, functional blood cells in the bone marrow. Chronic myeloid leukemia (CML) is a myeloproliferative disorder that is defined by its causative molecular lesion, the BCR-ABL fusion gene, while vast numbers of Philadelphia negative MPNs are characterized by the presence of JAK2 V617F mutation. Detection of JAK2 V617F mutation so convincingly establishes the presence of a clonal disorder. The present work aimed to study the expression of JAK2 V617F mutation by real-time PCR in chronic myeloproliferative disorder patients as well as the study the BCR/ABL gene rearrangement by FISH. **Subject and Methods:** The subjects of this study consist of 40 patients of newly diagnosed MPNs and 10 apparently healthy individuals serving as a control group. The patients were subjected to routine laboratory investigation, detection of BCR/ABL fusion gene by FISH technique and detection of JAK2 mutation expression in MPNs by real time PCR. **Results:** BCR/ABL fusion gene was detected in 100% of CML patients, while it was absent in other MPNs. JAK2 mutation was detected in (80%) of polycythemia vera (PV) cases, (60%) of essential thrombocythemia (ET) cases, (70%) of myelofibrosis (MF) cases and it was absent in CML. **Conclusion:** JAK2 V617F mutation is a risk factor for MPNs to develop approving the strong association between the JAK2 mutations and these disorders, which when present, can definitively confirm the diagnsosis. JAK2 mutation testing should be considered as a front-line screening test for suspected MPNs, and its use as a first-intention diagnostic test may spare some patients further investigations.
[Zahra MK; El-Fadaly NH; Aboul-Enein KM; Elgamal BM; Amira Y. Abd El-Naby and Eman A. Amer. **JAK2-V617F Mutation and BCR-ABL Rearrangement in Chronic Myeloproliferative Neoplasms.** Life Sci J 2012;9(2):403-414]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 62

**Key words:** Myeloproliferative neoplasms, JAK2V617F mutation, chronic myeloproliferative disorders

## 1. Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal hematologic diseases that are thought to arise from a transformation in a hematopoietic stem cell that leads to overproduction of mature functional blood cells (red blood cells, platelets and white blood cells) in the bone marrow. The cardinal features of the three main myeloproliferative disorders are an increased red-cell mass in Polycythaemia Vera, a high platelet count in Essential Thrombocythemia, and bone marrow fibrosis in Idiopathic Myelofibrosis. These three disorders share many characteristics, including marrow hypercellularity, a tendency to thrombosis and hemorrhage, and a risk of leukemic transformation on the long term. Chronic myeloid leukemia is a myeloproliferative disorder that is defined by its causative molecular lesion, the *BCR-ABL* fusion gene, which most commonly results from the Philadelphia translocation (Ph) [(1)].

JAK2 "Just another kinase" or "Janus kinase" is a tyrosine kinase involved in cytokine receptor signaling [(2)], it has a critical role in mediating the signaling pathways of thrombopoeitin (TPO), erythropoietin (EPO) and other cytokines involved in haemopoiesis. JAK2 is activated by the binding of these ligands to cytokine receptors [(3)]. The JAK family of tyrosine kinases includes JAKs 1-3 and tyrosine kinase 2 (TYK2). JAKs are expressed equally in all cells with the exception of JAK3, which is found only in hematopoietic cells [(4)]. The most important structural domain of the JAK molecule is the enzymatic kinase domain (JH1), which phosphorylates tyrosine on target proteins. The pseudokinase domain (JH2) has no enzymatic activity and is thought to inhibit the kinase domain, while the FERM domain is important in regulating binding of the JAK proteins to cytokine receptors[(3)].

The acquisition of a mutation in the Janus kinase 2 (JAK2) genes by hematopoietic cells has been described as a genetic, defect underlying myeloproliferative disorders. The mutation leads to constitutive activation of JAK2, a tyrosine kinase involved in cytokine receptor signaling [(2)]. So the impact of the JAK2 V617F mutation on the cytokine signaling pathways suggests that it plays an important role in the pathogenesis of MPNs and represents a

major breakthrough in molecular understanding of the myeloproliferative disorders (MPDs) that may have significant implications for diagnosis and treatment [4].

Janus kinase signaling is activated in hematological malignancies by a number of mechanisms including the down regulation of negative regulators of JAK-STAT pathways, amplification of the JAK2 locus and the involvement of JAK2 in chromosomal translocations

G-T mutation results in phenylalanine being substituted for valine at position 617 (V617F) in the pseudokinase/JH2 domain and results in a protein with increased kinase activity and hyper-responsiveness to cytokine signaling [5].

There is a much higher frequency of JAK2 mutation in PV while it is less frequent in IMF and ET [6]. The recently discovered JAK2 V617F point mutation, found in 50-60% of ET patients, has been reported to be associated with a higher risk of thrombotic events[7]. The mutation has been screened for in a number of other hematological malignancies and was found in some cases of atypical MPD [8], in a subset of patients with MDS perhaps in association with 5q- and rarely in AML unless it is secondary to a previous MPD [9]. No cases have been described in lymphoid malignancies[10] although a distinct mutation, JAK2 L611S, was discovered in one case of pre-B-ALL during a screen for JAK2 mutations using denaturing high-performance liquid chromatography [11]. The discovery and the study of JAK2 V617F mutation represent a major advance in the molecular understanding of MPNs that may have implications for diagnosis and treatment. JAK2 V617F, a somatic point mutation that leads to constitutive JAK2 phosphorylation and kinase activation, has been incorporated into the WHO classification and diagnostic criteria of myeloid neoplasms [12]. The discovery of the JAK2 V617F mutation in the classical myeloproliferative neoplasms (MPNs) essential thrombocytosis, polycythemia vera, and primary myelofibrosis has ushered in a new era of scientific discovery in these diseases, resulting in a molecular classification and an improved understanding of disease pathogenesis[13].

**Aim of work:**

The present work aimed to study the expression of JAK2 V617F mutation by real-time PCR and study the BCR/ABL gene rearrangement by FISH in different MPNs and report their prevalence in Egyptian patients.

## 2. Subjects and Methods

**Subjects:**

The subjects of this study were selected from Hematology/Oncology Unit of Internal Medicine Department, Tanta University and National Cancer Institute (NCI) Cairo University. They were classified into the following groups:

**Group I:** Consists of 10 apparently healthy individuals matched in age and sex with the patients group to serve as a control group.

**Group II:** Consists of 40 patients of newly diagnosed MPNs (10 patients were PV, 10 were ET, 10 were MF and 10 were CML).

**Methods:**

The patients were subjected to the following:

I-Detailed history and thorough clinical examination searching for important signs of prognostic significance mainly pallor, purpura, bleeding, hepatomegaly, splenomegaly, lymphadenopathy and fever.

II-Radiological study mainly abdominal sonar and CT for detection of clinically undetected organomegaly or lymphadenpathy.

III-Laboratory investigation including: Complete blood count, B.M. aspiration and/or biopsy, LDH estimation, cytochemical staining (LAP score), conventional cytogenetic, detection of BCR-ABL rearrangement by FISH and detection of JAK2 mutation by allele specific real Time PCR.

Informed written consent was taken from every patient and control before enrollment in the study. The research was approved by the ethical committee of research of Tanta University.

**Sampling:**

eight ml venous blood was collected from every patient and control under complete aseptic condition and divided: 1 ml put in EDTA vacutainer tube for CBC; 3 ml put in plain vacutainer tube for serum LDH; 2ml in EDTA vacutainer tuber for real time PCR to study JAK2 mutation and 2 ml put in vacutainer tube containing non-preservative heparin for study of BCR-ABL fusion gene by FISH.

**Detection of JAK2 mutation by real time PCR:**

1- DNA was extracted from the venous blood using QIA amp DNA blood minikit from Qiagen (Applied biosystem, step I version).

2- The putative short fragment spanning the mutation site of JAK2 V617F mutation was amplified using real time PCR with optimized primers and probes mix. The primers flanking the mutant region included:

F.primer 5'-AAGCTTTCTCACAAGCATTTGGTTT-3'.

R.primer 5'-AGAAAGGCATTAGAAAGCCTGTAGTT-3'

They were employed together with Taqman probes which were specific for either:

Wild type:

VIC-5'-TCTC-C ACAGACACATAC-3'MGB.

Or mutant JAK2 allele:

FAM-5'-TCCACAGAAACATAC-3'-MGB.

**Cell culture and *in situ* hybridization:**

PB and/or BM specimens were cultured for 48 and 72 hours at 37°C in RPMI medium supplemented with 10% fetal bovine serum without the addition of any mitrogen (unstimulated). Colcemid (0.02ug/ml) was added to the cultures 30 minutes before harvest. After 30 minutes of hypotonic treatment with 0.075M KCl, the cells were fixed with methanol and acetic acid (3:1) and cells were made into slide preparations.

FISH assay: was performed according to manufacture's instructions, hybridization mixture (10ul) was then applied to each slide, which was cover slipped and sealed. Hybridization solution contained (hybridization buffer, purified water, and the specific probe). Specific probe (LSI) BCR-ABL dual colour dual fusion translocation probes (Vysis, Inc, Downers Grove, TL 60515 USA) for detection of t(9;22)(q34,q11) was used. Hybridization was performed for 10 hours at 37°C in a humidified chamber. Post hybridization washes consisted of rinses in 0.4x SSC at 37°C and 2xSSC at room temperature. Finally, nuclei were counter stained with DAPI. Cells were analyzed under a fluorescence microscopes equipped with Quips spectra vision hardware and software.

**Statistical methods:**

Data was analyzed using SPSSwin statistical package version 17 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA). A $p$-value < 0.05 was considered significant and of < 0.001 was considered highly significant.

## 3. Results

The subjects of this study were classified into the flowing groups: **Group I:** Consisted of 10 apparently healthy individual serving as a control group. **Group II:** Consisted of 40 patients of newly diagnosed MPNs which were classified into:

Group A: 10 are PV. Group B: 10 are ET. Group C: 10 are MF. Group D: 10 patients are CML. Table (1) shows insignificant difference between the control individuals and the MPNs patients as regards to age and sex (Table 1).

As regards the laboratory findings in the studied groups, Hb concentration: the comparison revealed statistical significant difference between the two groups ($p$=0.041). WBCs count: the comparison revealed statistical significant difference between the two groups regarding JAK2 mutation ($p$=0.012). Platelets count: the comparison revealed statistical significant difference between the two groups regarding JAK2 mutation ($p$=0.028). LAP Score: the comparison revealed highly statistical significant difference between the two groups regarding ($p$=0.01) (Talbe 2). Serum LDH level: the comparison revealed highly statistical significant difference between the two groups regarding with $p$-value 0.001 (Table 3).

**Table (1):** Distribution of the studied cases according to age and sex

| | | Control N =(10) | CMPNs patients N=(40) | *p*. value |
|---|---|---|---|---|
| **Age** | **Range** | 37-45 | 33-48 | 0.639 |
| | **Mean ±SD** | 41.10±3.24 | 40.42±3.08 | |
| **Sex** | **Male (N%)** | 5(50%) | 26(65%) | 0.995 |
| | **Female (N%)** | 5(50%) | 14(35%) | |

**Table (2):** Important clinical data of the studied cases compared with the control

| Clinical data | | Control N =(10) | CMPNs patients N=(40) | *p*. value |
|---|---|---|---|---|
| **Splenomeglay N (%)** | **Present** | 0(0%) | 32(80%) | **0.001** |
| | **Absent** | 10(100%) | 8(20%) | |
| **DVT N (%)** | **Present** | 0(0%) | 2(5%) | **0.046** |
| | **Absent** | 10(100%) | 38(95%) | |

**Table (3):** Laboratory findings of the studied groups

| Laboratory Data | | Control N =(10) | CMPNs patients N=(40) | *p*. value |
|---|---|---|---|---|
| **HB(g/dl)** | **Range** | 10.50-18.5 | 6.48-20.55 | **0.041** |
| | **Mean ±SD** | 14.63±3.26 | 11.78±4.16 | |
| **TLC(x10$^3$/cmm)** | **Range** | 7.20-13.84 | 3.48-79.45 | **0.012** |
| | **Mean ±SD** | 11.32±4.32 | 23.3±24.2 | |
| **Platelets (x10$^3$/cmm)** | **Range** | 190-620 | 131.2-1124.9 | **0.028** |
| | **Mean ±SD** | 346.5±125.03 | 494.4±334.2 | |
| **LAP score N(%)** | **Decreased** | - | 8 (20%) | **0.01** |
| | **Normal (12-120)** | 8(80%) | 18(45%) | |
| | **Hight(>120)** | 2(20%) | 14(35%) | |
| **Serum LDH** | **Range** | 200-480 | 236-1200 | **0.001** |
| | **Mean + SD** | 337.7±81.16 | 690.1±281.4 | |

Cytogenetic abnormalities (BCR-ABL) gene rearrangement of the studied cases as compared with the controls: BCR-ABL was absent in GI while as regards to GII it was present in 10 out of 40 patients (25%). The comparison revealed highly statistical significant difference between the two groups ($p$=0.007) (Table 4).

**Table (4):** BCR-ABL gene rearrangement of the studied cases as compared with the controls

| Laboratory Data | | Control N =(10) | CMPNs patients N=(40) | *p.* value |
|---|---|---|---|---|
| Cytogenetic abnormalities BCR-ABL | Present | 0(0%) | 10(25%) | 0.007 |
| | Absent | 10(100%) | 30(75) | |

All the ten individuals of the control group (100%) had normal JAK2 expression (wild type) while none of the ten individuals (0%) had JAK2 mutation expression. 19 patients with CMPNs out of 40 (47.5%) had wild JAK2 expression, while 21 patients out of 40 **(**52.5%) had mutant JAK2 expression in whom 20 out of 21 patients (95.2%) were heterozygous and 1 out of 21 patients (4.8%) was homozygous. This revealed a statistical highly significant difference between the two groups regarding JAK2 mutation ($p$=0.001) (Table 5).

**Table (5):** Statistical comparison between CMPNs patients and the control group as regards JAK2 mutation

| | Control N =(10) | CMPNs patients N=(40) | *p.* value |
|---|---|---|---|
| **JAK2 wild type** | 10(100%) | 19(47.5%) | 0.001 |
| **JAK2 mutant** | 0(0%) | 21(52.5%) | |
| **heterozygous** | 0(0%) | 20(95.2%) | |
| **homozygous** | 0(0%) | 1(4.8%) | |

**In PV patients:**

There was no significant difference between the control individuals and the PV patients with mutant JAK2 as regards to age and sex. The main clinical presentations were splenomegaly in 6 patients out of 8 patients (75%) with significant difference in comparison to control group ($p$= 0.025), and there is none of the PV patients with mutant JAK2 showed DVT with no significant difference in comparison to control group ($p$ = 0.059) (Table 6).

*Hb concentration:* there was statistical significant difference between the PV patients having mutant JAK2 expression and the control individuals ($p$=0.009)**.** *WBCs count:* revealed a near significant difference between the PV patients having mutant JAK2 expression and the control individuals ($p$=0.057). *Platelets count*: there was statistical significant difference between the PV patients having mutant JAK2 expression and the control individuals ($p$=0.032). *LAP Score*: there was statistical significant difference between the PV patients having mutant JAK2 expression and the control individuals ($p$=0.029). *Serum LDH levels*: there was insignificant difference between the PV patients having mutant JAK2 expression and the control individuals ($p$=0.075) (Table 6).

**In ET patients:** As regards to age and sex, there was insignificant difference between the control individuals and the ET patients with mutant JAK2. Splenomegaly was present in 5 out of 6 patients (83.3%) with significant difference in comparison to control group ($p$=0.035). DVT was present in 2 out of 6 patients with mutant JAK2 with significant difference in comparison to control group ($p$=0.050) (Table 6).

*Hb concentration*: revealed a near significant difference between ET patients with mutant JAK2 expression and the control individuals ($p$=0.051). *WBCs count*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and the control individuals ($p$=0.856). *Platelets count*: there was statically significant difference between ET patients having mutant JAK2 expression and the control individuals ($p$=0.001). *LAP Score*: there was statistical significant difference between the ET patients having mutant JAK2 expression and the control individuals ($p$=0.011). *Serum LDH levels*: there was statistical significant difference between ET patients having mutant JAK2 expression and the control individuals ($p$=0.001) (Table 6).

**In MF patients:** As regards to age and sex, there was insignificant difference between the control individuals and the MF patients with mutant JAK2. Splenomegaly was present in 6 out of 7 patients (85.7 %) with significant difference as comparison to control group ($p$=0.002). None of the patients with mutant JAK2 showed DVT with no significant difference in comparison to control group ($p$ =0.073) (Table 6).

*Hb*: There was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals ($p$=0.003). *WBCs count*: there was significant difference between the MF patients having mutant JAK2 expression and the control individuals ($p$=0.003). *Platelets count*: there was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals ($p$=0.001). *Serum LDH levels*: there was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals ($p$=0.001). *LAP Score*: there was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals ($p$=0.039) (Table 6).

**Table (6):** Statistical comparison between PV, ET, MF patients with mutant JAK2 and the control group as regards clinical and laboratory data

| | | PV N =(8) | ET N =(6) | MF N =(7) | Control N =(10) |
|---|---|---|---|---|---|
| **Age** | | 37-48 | 34-47 | 36-43 | 37-45 |
| | **M± SD** | 42.3±5.6 | 39.5±6.3 | 37.9±7.1 | 41.6±6.2 |
| | *p* | 0.523 | 0.741 | 0.652 | - |
| **Sex** | **Male** | 5(62.5%) | 3(50%) | 5(71.4%) | 5(50%) |
| | **Female** | 3(37.5%) | 3(50%) | 2(28.6%) | 5(50%) |
| | *p* | 0.253 | 0.636 | 0.963 | - |
| **Splenomegaly** | **Present** | 6(75%) | 5(83.3%) | 6(85.7%) | 0(0%) |
| | **Absent** | 2(25%) | 1(16.7) | 1(14.3%) | 10(100%) |
| | *p* | 0.025 | 0.035 | 0.002 | - |
| **DVT No(%)** | **Present** | 0 | 2(33.3%) | 0 | 0 |
| | **Absent** | 8(100%) | 4(66.7%) | 7(100%) | 10(100%) |
| | *p* | 0.059 | 0.050* | 0.073 | - |
| **HB(g/dl)** | **M± SD** | 17.63 ±1.25 | 11.32 ±1.88 | 8.11 ±1.63 | 13.61 ±3.25 |
| | *p* | 0.009 | 0.051 | 0.003 | - |
| **TLC(x10$^3$/cmm)** | **M± SD** | 12.36 ±1.25 | 11.35 ±2.88 | 5.32 ±1.84 | 10.25 ±2.88 |
| | ***p* Value** | 0.057 | 0.856 | 0.003 | - |
| **Platelets (x10$^3$/cmm)** | **M± SD** | 633.2 ±112.5 | 1147.1 ±369.1 | 197.1 ±65.8 | 513.5 ±71.5 |
| | *p* | 0.032 | 0.001 | 0.001 | - |
| **LAP score N (%)** | **Low** | 0 | 0 | 0 | 0(0%) |
| | **Normal** | 3(37.5%) | 4(66.7%) | 3(42.7%) | 8(80%) |
| | **High** | 5(62.5%) | 2(33.3%) | 4(57.3%) | 2(20%) |
| | **P** | 0.029 | 0.011 | 0.039 | |
| **Serum LDH** | **M± SD** | 320.6 ±84.5 | 698.5 ±156.8 | 946.8 ±195.2 | 369.2 ±47.8 |
| | **P** | 0.075 | 0.001 | 0.001 | - |

**In PV patients:** There was insignificant difference between the PV patients having wild type JAK2 expression and those with mutant JAK2 as regards to age and sex. **In ET patients:** There was insignificant difference between the ET patients having wild type JAK2 expression and those with mutant JAK2 as regards to age and sex. **In MF patients:** There was insignificant difference between the MF patients having wild type JAK2 expression and those with mutant JAK2 as regards to age and sex. **In CML patients:** No one showed JAK2 mutation. 7 males out of 10 patients (70%) and 3 females out of 10 patients (30 %) showed wild type JAK2 expression (Table 7).

**Table (7):** Statistical comparison of mutant and wild type JAK2 expression in PV, ET, MF and CML patients as regards age and sex

| | | PV | | ET | | MF | | CML | |
|---|---|---|---|---|---|---|---|---|---|
| | | mutant JAK2 N=8 | Wild type JAK2 N=2 | mutant JAK2 N=6 | Wild type JAK2 N=4 | mutant JAK2 N=7 | Wild type JAK2 N=3 | mutant JAK2 N=0 | Wild type JAK2 N=10 |
| **Age** | **Range** | 37-48 | 33-45 | 34-47 | 35-46 | 36-43 | 37-43 | - | 33-48 |
| | **Mean ±SD** | 40.39 ±3.21 | 37.5 ±6.25 | 38.9 ±4.2 | 38.6 ±3.58 | 40.2 ±1.52 | 39.22 ±2.96 | - | 40.42 ±3.08 |
| **p. value** | | **0.325** | | **0.536** | | **0.658** | | **-** | |
| **Sex** | **Male (N%)** | 5(62.5) | 1(50) | 3(50) | 3(75) | 5(71.4) | 2(66.7) | - | 7(70) |
| | **Female (N%)** | 3(37.5) | 1(50) | 3(50) | 1(25) | 2(28.6) | 1(33.3) | - | 3(30) |
| **p. value** | | **0.874** | | **0.685** | | **0.741** | | **-** | |

**In PV patients**: as regards splenomegaly, there was no significant difference between patients of PV having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.242); on the other hand there was no PV patients having DVT. **In ET patients:** there was splenomegaly in 5 patients out of 6 ET patients having mutant type JAK2 expression (83.3%), while 2 patients out of 4 ET patients (33.3%) having wild type JAK2 expression. As regards splenomegaly there was no significant difference between patients of ET having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.366). As regards to DVT no significant difference between patients of ET having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.196). **In MF patients:** there was no significant difference between patients of PV having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.147) as regards to splenomegaly and there was no MF patients having DVT. **In CML patients:** There was splenomegaly in 10 patients out of 10 CML patients (100 %) having wild type JAK2 expression and there was no CML patients having DVT (Table 8).

**Table (8):** Statistical comparison of mutant and wild type JAK2 expression in PV, ET, MF and CML patients as regards to clinical data

| Clinical data | PV | | ET | | MF | | CML | |
|---|---|---|---|---|---|---|---|---|
| | mutant JAK2 N=8 | Wild type JAK2 N=2 | mutant JAK2 N=6 | Wild type JAK2 N=4 | mutant JAK2 N=7 | Wild type JAK2 N=3 | mutant JAK2 N=0 | Wild type JAK2 N=10 |
| **Splenomeglay** | 6(75) | 2(100) | 5(83.3) | 2(50) | 6(85.7) | 1(33.3) | - | 10(100) |
| ***p*. value** | **0.242 NS** | | **0.366 NS** | | **0.096 NS** | | **-** | |
| **DVT** | 0(0) | 0(0) | 2(33.3) | 0(0) | 0(0) | 0(0) | - | 0(0) |
| ***p*. value** | **-** | | **0.196** | | **-** | | **-** | |

**In PV patients:** *Hb concentration*: there was no statistical significant difference between the PV

patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression ($p$=0.536). *WBCs count*: there was no statistical significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression ($p$=0.523). *Platelets count*: there was a near significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression ($p$=0.056). *LAP Score*: there was no statistical significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression ($p$=0.361). *Serum LDH levels*: there was no statistical significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression ($p$=0.449) (Table 9).

**In ET patients:** *Hb*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression ($p$=0.741). *WBCs count*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression ($p$=0.321). *Platelets count*: there was highly statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression ($p$=0.002). *LAP Score*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression ($p$=0.523). *Serum LDH levels*: there was statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression ($p$=0.029) (Table 9).

**In MF patients:** *Hb*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression ($p$=0.325). *WBCs count*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression ($p$=0.489). *Platelets count*: there was statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression ($p$=0.014). *LAP Score*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression ($p$=0.365). *Serum LDH levels*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression ($p$=0.637). CML patients: All CML patients had wild type JAK2 expression (Table 9).

**Table (9):** Statistical comparison of mutant and wild type JAK2 expression in PV, ET, MF and CML patients as regards to laboratory Data

| Laboratory Data | | PV | | ET | | MF | | CML | |
|---|---|---|---|---|---|---|---|---|---|
| | | mutant JAK2 N=8 | Wild type JAK2 N=2 | mutant JAK2 N=6 | Wild type JAK2 N=4 | mutant JAK2 N=7 | Wild type JAK2 N=3 | mutant JAK2 N=0 | Wild type JAK2 N=10 |
| HB(g/dl) | Mean ±SD | 17.63 ±1.25 | 18.23 ±2.32 | 11.32 ±1.88 | 10.52 ±2.99 | 8.11 ±1.63 | 7.99 ±2.11 | - | 12.16 ±2.51 |
| *p.* value | | 0.536 | | 0.741 | | 0.325 | | - | |
| TLC ($x10^3$/cm m) | Mean ±SD | 12.36 ±1.25 | 11.41 ±1.58 | 11.35 ±2.88 | 10.47 ±1.88 | 5.32 ±1.84 | 8.74 ±4.11 | - | 59.27 ±22.29 |
| *p.* value | | 0.523 | | 0.321 | | 0.489 | | - | |
| Platelets ($x10^3$/cm m) | Mean ±SD | 633.2 ±112.5 | 365.2 ±86.3 | 1147.1 ±369.1 | 796.1 ±59.6 | 197.1 ±65.8 | 219.8 ±144.1 | - | 209.1 ±154.1 |
| *p.* value | | 0.056 | | 0.002 | | 0.014 | | - | |
| LAP score N(%) | low | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 8(80) |
| | Normal | 3(37.5) | 1(50) | 4(66.7) | 3(75) | 3(42.8) | 2(33.7) | 0(0) | 2(20) |
| | High | 5(62.5) | 1(50) | 2(33.3) | 1(25) | 4(57.1) | 1(33.3) | 0(0) | 0(0) |
| *p.* value | | 0.361 | | 0.523 | | 0.365 | | | |
| Serum LDH | Mean ±SD | 320.6 ±84.5 | 363.2 ±79.9 | 698.5 ±156.8 | 809.8 ±23.1 | 946.8 ±195.2 | 886.7 ±180.3 | - | 886.8 ±256.3 |
| *p.* value | | 0.449 | | 0.029 | | 0.637 | | - | |
| BCR-ABL (%) | Pres. | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 10(100) |
| | Abse. | 8(100) | 2(100) | 6(100) | 4(100) | 7(100) | 3(100) | 0(0) | 0(0) |

**In PV patients:** 2 patients out of 10 patients (20%) had wild type JAK2 expression while 8 patients out of 10 patients (80%) had JAK2 mutation expression in which 7 patients (87.5%) had heterozygous mutation while one patient (12.5%) had homozygous mutation. **In ET patients:** 4 patients out of 10 patients (40%) had wild type JAK2 expression while 6 patients out of 10 patients (60%) had JAK2 mutation expression in which 6 patients (100 %) had heterozygous mutation while no patient (0%) had homozygous mutation. **In MF patients:** 3 patients out of 10 patients (30%) had wild type JAK2 expression while 7 patients out of 10 patients (70%) had JAK2 mutation expression in which 7 patients (100%) had heterozygous mutation while no patient (0%) had homozygous mutation. **In CML patients:** 10 patients out of 10 patients (100%) had wild type JAK2 expression while no patient out of 10 patients (0%) had JAK2 mutation expression (Table 10).

**Table (10):** Statistical comparison between PV, ET, MF and CML patients as regards JAK2 mutation

| | PV N=10 | ET N=10 | MF N=10 | CML N=10 | *P.* value |
|---|---|---|---|---|---|
| JAK2 wild type | 2(20%) | 4(40%) | 3(30%) | 10(100%) | 0.002 |
| JAK2 mutant | 8(80%) | 6(60%) | 7(70%) | 0(0%) | |
| Heterozygous | 7(87.5%) | 6(100%) | 7(100%) | 0(0%) | |
| Homozygous | 1(12.5%) | 0(0%) | 0(0%) | 0(0%) | |

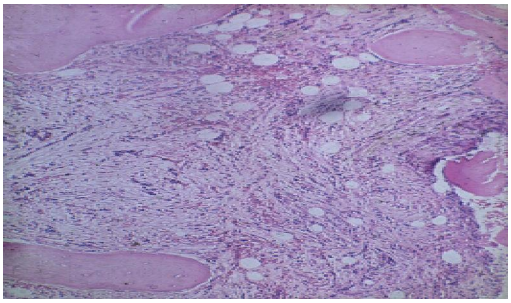


**Fig. (1):** This slide shows a trephine biopsy specimen of idiopathic myelofibrosis patient which has been cut into thin sections and stained with haematoxylin and eosin (H&E). The pink areas are bone. The area between the bone trabiculae should contain a mixture of hematopoietic cells and fat cells. In this specimen the normal bone marrow has been largely replaced by fibrous tissue.

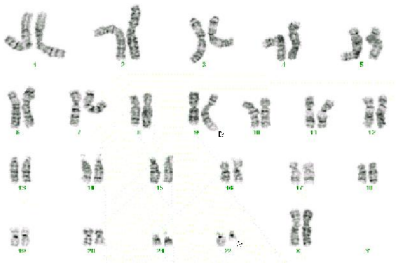


**Fig.(2):** Karyogram of CML patient showing BCR/ABL fusion gene.

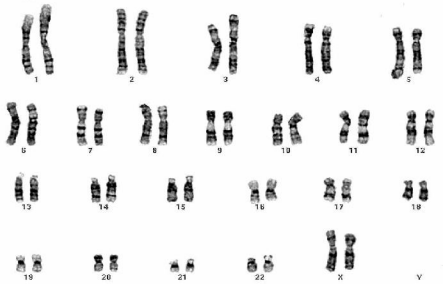


**Fig. (3):** Karyogram of PV female patient showing negative Philadelphia.

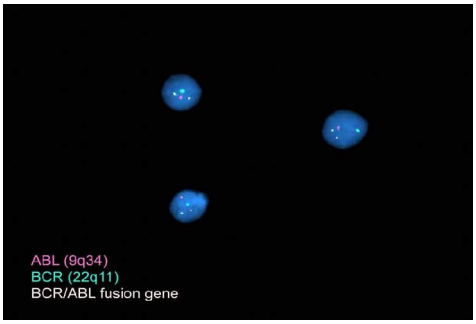



**Fig. (4):** A case of CML showing BCR/ABL fusion gene (Ph chromosome) one orange signal, one green signal and two fusion signals.

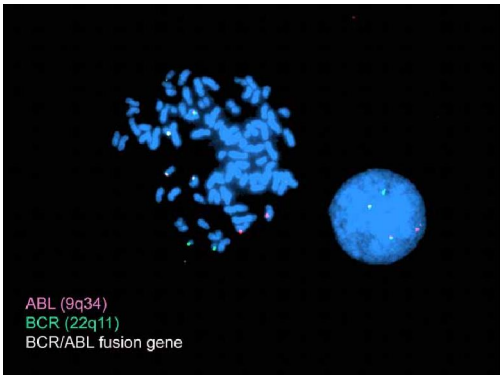



**Fig.(5):** A case of CML in blast crisis showing hyperdiploid metaphase, two normal 9, two normal 22 and two derivative 9, two derivative 22. One interphase BCR/ABL fusion gene (double fusion). A case of double Philadelphia.

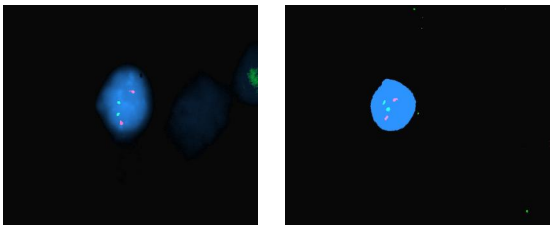
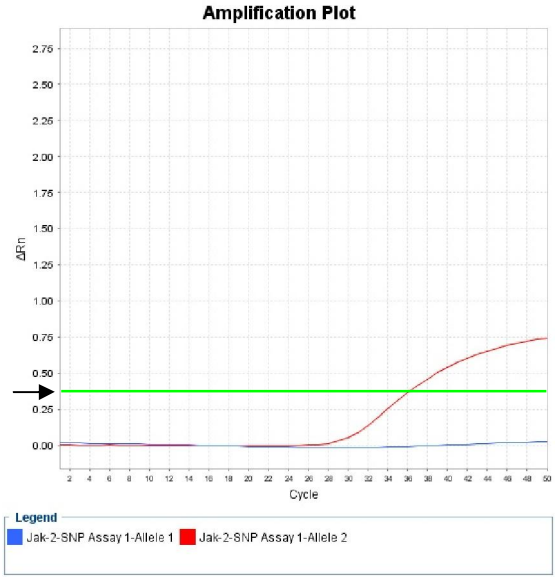
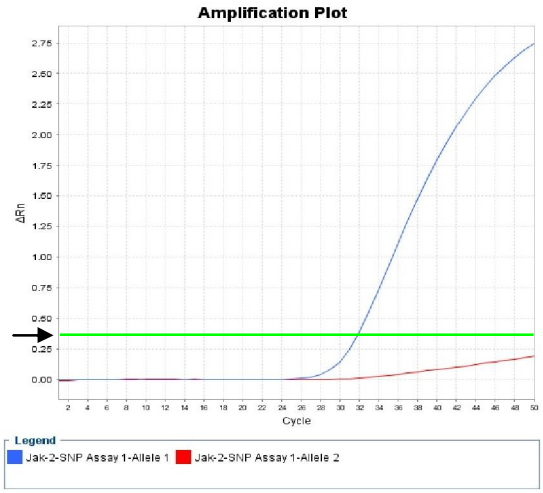


**Fig.(6):** A case of Philadelphia negative PV showing interphase cells with two orange signals and two green signals i.e. two normal chromosome 9 and two normal chromosome 22.



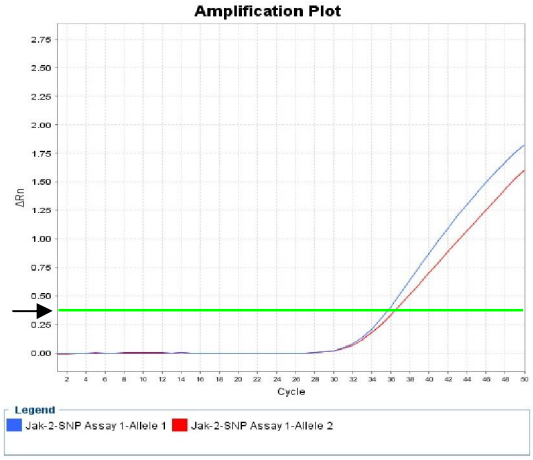

Allele 1 = Mutant JAK2 expression
Allele 2 = Wild type JAK2 expression

**Fig (7):** Print out of step one software PCR analysis show normal gene (wild type) is expressed (above the green line), this patient is negative for JAK2 mutation expression.




Allele 1 = Mutant JAK2 expression
Allele 2 = Wild type JAK2 expression

**Fig (11):** Print out of step one software PCR analysis show normal gene (wild type) is not expressed (below the green line), this patient is positive for JAK2 mutation expression of homozygous type (above the green line).




Allele 1 = Mutant JAK2 expression
Allele 2 = Wild type JAK2 expression

**Fig (8):** Print out of step one software PCR analysis show normal gene (wild type) is expressed (above the green line), this patient is positive for JAK2 mutation expression of heterozygous type (above the green line).

**4. Discussion:**

The JAK family of tyrosine kinases includes JAKs 1-3 and tyrosine kinase (TYK2). JAKs are expressed ubiquitously in all cells, with the exception of JAK3, which is found only in hematopoietic cells[4].

JAK2 V617F is a gain of function mutation that contributes to the expansion of the MPN clone by increasing the tyrosine phosphorylation activity that promotes cytokine activity and include erythrocytosis. The mutation is primarily associated with PV, ET and MF and is not found in CML[10].

Substitution of phenylalanine for valine, both are hydrophobic non polar amino acids, at position 617 of the JAK2 protein, within the JH2 pseudokinase domain[14].

Loss of JAK2 auto inhibition results in constitutive activation of the kinase, analogues to other mutations in MPDs and leukemia that; aberrantly activate tyrosine kinases [14].

Subsequently, 10 other mutations in exon 12 of the JAK2 gene were discovered[15]. These mutations were associated with PV and idiopathic erythrocytosis in patients negative for V617F mutation. Taken together, the prevalence of JAK2 mutations is ~ 99% in PV and ~50% in both ET and MF [16].

A positive JAK2 V617F mutation detection strongly favours MPNs as it is usually negative in normal individuals, secondary erythrocytosis, reactive tbrombocytosis, secondary myelofibrosis, leukaemoid reaction, lymphomas, sarcomas, CML, AML (unless those evolving from MPNs), and MDS (except for those associated with features of MPNs), and it is a sensitive marker detecting MPDs as the underlying etiology of splanchnic venous thrombosis affecting splenic, portal and hepatic veins [3].

***Kiladjian et al.,*** [17] had developed an algorithm in which screening of JAK2 can be the initial test in establishing a diagnosis of MPN. In addition, the sensitivity, standardization and convenience of JAK2 analysis and also many, authors have speculated that patients with the Jak2 mutation, but without' overt MPN may have 'latent' or 'early' MPNs and be at much higher risk of developing overt disease at a later date.

As JAK family members play a crucial role in the immune system; for example, inherited JAK3 deficiency causes severe combined immunodeficiency, and JAK2 plays an important role in cardiovascular signaling systems. Development of inhibitors that inhibit V617F without undesirable side effects may therefore be challenging[18].

The JAK2 mutation also affects response to treatment. Among patients with Essential Thrombocythemia, those with the V617F mutation are more sensitive to Hydroxyurea than are patients without the mutation. The response to therapy can now be directly monitored through quantification of JAK2-positive cells in peripheral blood [19].

The present work aimed to study the expression of JAK2 V617F mutation expression by real-time PCR in chronic myeloproliferative disorder patients and study the BCR/ABL rearrangement by FISH.

This study included 40 patients with newly diagnosed chronic myeloproliferative disorders; 10 cases were polycythaemia Vera (PV), 10 cases were essential thrombocythemia (ET), 10 cases were

myelofibrosis (MF), and 10 cases were chronic myeloid leukemia (CML).

In the present study, none of the ten individuals of the control group (0%) had shown JAK2 mutation expression while in CMPNs patients, 21 patients (52.5%) had JAK2 mutation expression, and this is consistent with ***Kralovics et al.,***[20] who reported in his study that prevalence of JAK2 mutation among examined CMPN patients was (52.5%) of cases, while none of the control group (0%) had shown JAK2 mutation expression.

Twenty MPNs patients out of 21 patients (95.2%) had heterozygous JAK2 mutation, while only one patient (4.8%) had homozygous mutation and this is supported by ***Nelson and Steensma,***[14] who implied that prevalence of heterozygosity may be due to mixed clonality or heterozygosity for the autosomal mutation (encoded on chromosome 9p24, and also this agrees with ***Kralovics et al.,***[20] who reported higher frequency (98%) of the JAK2 V617F heterozygous mutation in CMPDs cases.

In the current study, the frequency of JAK2V617F mutation among PV patients was (80%). This is consistent with ***Jones et al.,***[21] who reported JAK mutation in (81%) of PV patients, ***Zhao et al.,***[22] whose study reported that JAK2 mutation was expressed in (83%) of PV patients, ***James et al.,***[5] whose study reported that JAK2 mutation was expressed in (89%) of PV patients and ***Bock et al.,*** [23] who reported (90%) of JAK2 mutation expression among PV patients.

***Baxter et al.,*** [24] and ***Kreft et al.,*** [25] reported a higher frequency of JAK2 mutation expression among PV patients and this is due to the use of a more sensitive technique i.e. allele-specific PCR methodology.

In PV patients, only one patient (12.5%) expressed homozygous mutation, while 7 patients (87.5%) expressed heterozygous mutation. ***Scott et al.,*** [26] stated that homozygous JAK2 V617F favors the diagnosis of PV over ET. ***Baxter et al.,***[24] stated that (30%) of PV patients expressed homozygous mutation in their granulocytes while approximately (90%) of them expressed the homozygous mutation when the hematopoietic progenitor cells were examined and this difference suggests that V617F homozygosity promotes the development of PV. Our study had shown that only two patients (20 %) didn't express JAK2 mutation and this is consistent with ***Scott et al.,***[27] who explained in his study that the few patients with PV who didn't show the homozygous JAK2 V617F mutation often have a different mutation in the same gene, one of a number of different mutations on exon 12.

In ET patients, our study showed the prevalence of JAK2 mutation among 6 patients (60 %) and this agrees with ***Baxter et al.,***[24] whose study showed (57%) frequency of JAK2, mutation expression, while this disagreed with ***Horn et al.,***[28] who reported higher incidence of JAK2 mutation (74%) among ET patients and ***Levine et al.,***[10] who found JAK2 mutation expression in only 32% of ET patients. Six positive JAK2 mutation ET patients (60%), were heterozygous while none of the ET patients (0%) expressed homozygous mutation. Similarly, ***James et al.,*** [5] reported in their study that the large majority of patients with JAK2 V617F positive ET are heterozygous. Also ***Baxter et al.,***[24] stated that homozygosity is rarely detectable in peripheral blood cells from patients with ET.

In MF patients, 7 patients (70 %) had JAK2 V617F mutation. ***Kralovics & Skoda,***[29] reported that 57% of MF patients had the mutation, while ***Jelinek et al.,***[30] reported that 95% of MF patients had JAK2 mutation. ***Levine el al.,***[10] stated that only 32% of MF patients expressed JAK2 mutation. Our study showed that (100%) of patients with JAK2 V617F positive MF were heterozygous while none of the MF (0%) patients showed homozygous mutation and this disagrees with ***Baxter et al.,***[24] who reported the prevalence of homozygosity in his study and that (85%) of patients with JAK2 V617F positive MF were homozygous, and also ***Vannucchi et al.,***[31] stated that homozygosity seems to be related to an increased risk of transformation in myelofibrosis. However, definitive identification of V617F zygosity in individual cases is complicated by the fact that it is not possible to distinguish between a relatively small homozygous clone and a larger heterozygous clone when mixed populations of cells are analyzed [20].

On comparing positive JAK2 mutation in PV, ET and MF patients with the controls as regards clinical and laboratory data higher hemoglobin level were found in JAK2 positive PV patients with a highly statistical significant difference of a p-value (0.009*) and this is consistent with ***James et al.,***[5], ***Vannucchi et al.,***[31] and ***Villeval et al.,***[32] who stated that V617F positive PV patients had significant higher Hb levels. There was no significant difference between positive JAK2 mutation and the control individuals in TLC with p value (0.057). On the contrary ***James et al.,***[5] reported that JAK2 positive PV patients had lower total leukocytic counts. Also there was statistical significant difference in PV with mutant JAK2 as regards platelets with *p* value (0.032) and LAP score with p value (0.029) while, there was no statistical significant difference as regards serum LDH level with *p* value (0.075).

As for ET positive JAK2 mutation patients, they had higher platelet count compared to the other groups with a highly statistical significance and a p-value 0.001. This agreed with ***Khwaja***,[3] who reported that JAK2 positive ET patients tend to have higher platelet

count than the control individuals. Also there is highly statistical significance difference with a *p*-value 0.001as regards to serum LDH level and LAP score while there was no significance difference as regards Hb with a *p* value (0.051) and TLC with a *p* value (0.856).

As for MF positive JAK2 mutation patients, splenomegaly was prevalent among MF patients (85.7%), and this is consistent with ***Villeval et al.,***[32] who reported in his study that the incidence rate of splenomegaly showed significance as regards the mutation loads. Also the leukocytic count was higher in control individuals with significant difference in comparison to those with JAK2 positive and a *p*-value 0.003, platelet count with p-value 0.001, HB with *p*-value 0.003, serum LDH level with *p*-value 0.001 and LAP score with *p*-value (0.039).

As for CML none of the patients showed JAK2 mutation expression while all of them were Philadelphia positive and this comes in consistency with ***Haferlach et al.,***[33] and ***Jelinek et al.,***[30] who reported absence of JAK2 mutation in Philadelphia positive CML cases in their studies.

There was no statistical significant difference found between the PV, ET, MF and the CML regarding age and sex prevalence. This disagrees with ***Campbell et al.,***[34] who suggested in his study that sex prevalence might influence the phenotypic presentation of JAK2V617F positive disease, as JAK2 positive PV is more frequent in males while JAK2 positive ET is more frequent in females.

There was statistically non significant difference between positive & negative JAK2 mutation CMPNs patients regarding age, sex prevalence, splenomegaly, DVT, Hemoglobin level, Total leukocytic count, LAP score and serum LDH level. While there was significant difference between positive and negative JAK2 mutation CMPNs patients regarding platelet count in ET and BCR-ABL abnormality in CML patients.

On comparing PV, ET, and MF as regards JAK2 mutation expression, PV was the most prevalent in mutation expression (80%) among the three groups of CMPNs, and this approves with ***Baxter et al.,*** [24] who reported that JAK2 is present in hematopoietic cells in the majority of PV patients, and that the difference in JAK2 allele burden was highly significant between the MPNs diseases, and this is also explained by ***Wernig et al.,***[35] who stated that when mutant JAK2 is transfected into animal models, it becomes sufficient to develop a CMPNs mimicking PV which tends to terminate in myelofibrosis, while for the PV negative for the JAK2V617F it was referred to the newly diagnosed JAK2 gene mutation in exon 12.

Results of this study showed that JAK2V617F mutation expression was associated with increased risk of CMPNs including PV, ET and MF and that this mutation is a risk factor for these disorders to develop. In approval with this finding and owing to the strong association between the JAK2 mutations and MPNs, the World Health Organization (WHO) included the JAK2 mutations among their major diagnostic criteria for PV, ET, MF[31].

Also, this comes in consistency with ***Tefferi and Vardiman,***[37] who stated that the most important role of JAK2 mutation testing at present seems to be during the initial evaluation of patients with myeloproliferation. Given the high specificity of the mutation for clonal myeloid disease, JAK2 V617F, when present, can definitively confirm the diagnosis; so JAK2 mutation testing should be considered as a front-line screening test for suspected MPNs, and its use as a first-intention diagnostic test may spare some patients further investigation. However, the differences in the reported rates are likely because of differences in diagnostic precision and assay sensitivity. A direct sequencing technique was used for the detection of the JAK2 V617F mutation in some studies and this has a lower sensitivity than techniques using PCR amplification of the mutant allele, such as allele-specific PCR or an amplification refractory mutation system PCR[34]. Additionally, early studies indicate that the JAK2 allele burden decreases with successful therapy, disappears in some patients, and reappears during relapse. Thus, JAK2 testing appears useful for both diagnosis, management and follow up[38].

**Conclusion**

We concluded from this study that JAK2 V617F mutation plays a fundamental role in the pathogenesis and development of CMPNs, and that its detection is very useful to confirm diagnosis and to provide an early detection assay of the CMPNs, and definitely the presence of JAK2 mutation should be interpreted in conjunction with other laboratory and clinical findings as well as to rule out a false diagnosis of reactive thrombocytosis, myelofibrosis or secondary erythrocytosis. Also it may be of use in the management and the follow up of the patients and as a biological marker for monitoring the response to treatment.

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# A Framework of Quality Indicators System for Evaluating Hyderabad Urban Sustainability

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**Abstract:** Remote sensing technology using satellite access has been increasingly helpful in performing natural resources mapping and management. This includes processes that cannot be done manually or might take many years to complete when you are covering vast areas of land such as satellite imaging, accuracy assessment, image processing, classification, and geometric or radiometric corrections. It is evident that any nation's economic development is largely supported by the richness of its water and land resources. The management capability and mapping tools use to monitor these resources are crucial to raise the economic development of specific regions. Accuracy is a general requirement in managing delicate land and water resources for sustainable development. The remote sensing using satellite based approach in generating data ensures updated cost effective natural resources monitoring and management in Iran. This research will demonstrate the need to maintain remote sensing for mapping and managing natural resources in Iran as well as enhancing and supporting the decision making capabilities of the government regarding the use of its natural resources.
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**Keywords:** Urban Sustainability, Hyderabad, Evaluation, Indicator

## 1. Introduction

Sustainable urban planning should aim at achieving economical, social and environmental equity while improving the lives of the people. For that to happen we need to have a sustainable city form and also provision and suitable management of the services. Sustainable urban development means achieving a balance between the development of the urban areas and protection of the environment with intent to Justice in employment, shelter, basic services, hygiene, social infrastructure and transportation in the urban areas (Bertolini, 2005).

A city is a relatively large, dense, and permanent settlement comprising of socially heterogeneous individuals (Ziari, 2009, Wirth 1938, Pijanowski et al., 2009). In accord with Aristotle, cities are places which include gladness and security for its residents. Plato also explains a city as suitable location for citizens to live in and also the birthplace for civilizations. As a matter of fact, at the time when human beings obtained a relative amount of peace, safety and security in thought and action, urban areas were generated. Eventually, with the passage of time and creation of cities, the human race gradually started thinking about comprehending ideals such as justice, social relations, lawfulness, and prettiness (Broadbent, 1990).

At the same time, in recent years metropolises are confronted with unpleasant situation such as immoderate population and the conditions arising out of it, including pollution, dirt, congested traffic, destruction and plundering of natural resources. In the same manner, Hyderabad is also faced with managerial, environmental, infrastructural, physical, social and economic problems which collectively decline the city's environment quality (Divan, 2001, GOI, 1992, Prasad et al., 2007). As a most evident many of Hyderabad's regions has been considered "critical" for their high polluted air conditions, water pollution, sold waste management, uncontrolled industrial effluents, indigent sanitation and inadequate water supply (Rama Rao, 2004, Ramachandraiah, 2003, Venkateswara Rao et al., 1998).

The urban environment quality is declining day by day with the greatest cities reaching saturation points and unable to handle the increasing pressure on their infrastructure. Rapid urbanization brings with it many difficulties as it places enormous demands on land, water, housing, transport, health, education etc (Gyananath et al., 2001). The city witnessed an increase in population from 0.448 million in 1901 - 1.429 million in 1961, between 1981 and 1991 the population went up to 4.34 million and the growth rate so far is 67.04% (Census of India,

1991). As per the population estimates, Hyderabad is likely to become a metropolis with about 7.5 million populations by 2011(Census of India, 2011). This rising population density will continue to have an impact on the quality and quantity of environment and natural resources.

In this regard, similar studies have been done by scholars and national and international organizations based on determining and evaluating a collection of city environmental quality indicators. Urban sustainability indicators (Mitlin,1992), Indicators of sustainable development (Meadows, 1998), Encyclopedia of Earth (EOE) (Bartelmus, 2008), Quality of Living global city rankings Mercer survey (MHRC, 2007), Sustainability Plan for the City of San Francisco (SCS, 1997) and Urban indicators and the integrative ideals of cities (Holden, 2006) are a number of these studies. In order to select the desired indicators in this study, the whole of indicators used in the aforementioned studies and other similar sources have been compared and their proficiency for evaluating Hyderabad's environmental quality have been analyzed. These studies showed that some of the indicators introduced in them were a lot more general or much more insignificant than the measures of city indicators, lacked measurement criteria and importance coefficients and in some cases even had lack of clarity in concept. Also, in some cases the indicators introduced are not compatible with India's cultural and social conditions or do not have documented statistics in India's official organizations. In the next stages, the attempt was made to alternative indicators that had more clarity, contained measurement standard and to the extent that was possible, had accessible documented information and statistics. On the other side, for the feasibility of the evaluation from the outlook of time and executive expenses, the most extensive and proficient indicators have been chosen from the comparable indicators. At last the model and collection of chosen indicators with the adjusted classification and important coefficients have been used in order to evaluate the quality of Hyderabad's environment. Afterwards, subsequent identifying the problems of Hyderabad's environmental quality, planning solutions for decreasing the inadequacies and enhancing the quality has been presented. It have to be mentioned that evaluating Hyderabad's environmental quality according to the mentioned model demonstrates part of the reality which has been stated in mathematical language and based on the country's official statistics; thus, there is the possibility of differences between the model with its chosen indicators and existing realities.

## 2. Case study of the research

The study area of Hyderabad city and its environs extend from 17.010 -17.050 N and 78.010 - 78.050 E. Hyderabad is a capital of Andhra Pradesh state and the total area of Municipal Corporation of Hyderabad (MCH) is 650 square kilometers and divided into 11 planning zones (GHMC, 2011). The city has 6,809,970 residents and the metropolitan area comprise of 7,749,334 residents making it the fourth most populous city and the sixth-most populous urban agglomeration in India (Urban Agglomerations/Cities having population one lakh (100,000) and above, (Census of India, 2011). The city is located at 550 meters above sea level in the center of the Deccan plateau in the southern part of India. Hyderabad is located in a rocky, sparsely-wooded area surrounded by hills that contain a large number of lakes, ponds, streams, and rivers (HMWSSB. 2010). Hyderabad experiences a minimum temperature of 11.600 C and a maximum of 40.500 C with an average annual rainfall of 73.55 cm (Asadi, 2007). The daily mean maximum temperature varies from a minimum of 14.10 C during the month of December to 38.80 C in the month of May (HUDA, 2003).

**Figure 1:** Showing the location map of the study area

## 3. Material and Methods

The principal research method of this study is based on usage of indicators. An indicator is a marker that is helps us to understand where we are, where we are going and how far we are from the goal or showing a special action, path or state (Rodenburg, 1995). In fact, each indicator is a determiner which explains the cause and effect elements and the actions and outcome of policies (Westfall, 2001). In the present study, in order to obtain indicators suitable for evaluating the quality of Hyderabad's environment, in the first step a comparative analysis of the varied classifications and models of the introduced indicators in different researches has been carried out (Table 1). This has led to the select of the preliminary model and categorization for the evaluation indicators collection. The main structure for the model used in this research has been extracted from the model of estimation of urban environmental quality (Bahrainy and Tabibian, 1999). Then in the

following stages a number of substitute indicators were recognized and chosen during a comparative analysis and placed in the model. Table 1 shows an example of comparing various models of indicators based on their essence, so that in next stage the model and collection was adjusted based on the substitute indicators and obtaining documented and statistical data. The ultimate model shows the selected indicators and their importance coefficients (Fig. 2).

The model comprises of six layers; in the first layer there is the "final indicator" which shows the whole amount of urban environmental quality. The final indicator has a 1000 important coefficient which is reached from the sum of the measure's importance coefficients in the lower layers. Measure's importance coefficient has been arbitrarily considered for each measure.

**Table 1:** Comparative analysis of indicators presented by various studies for evaluating the urban environmental quality of cities

| Bahrainy, Tabibian, 1999 | | Bartelmus, 2008 | | MHRC, 2007 | | Westfall, 2001 | | SCC, 2006 | | Majumder, 2007 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Indicator | Nature | Indicator | Nature | Indicator | Nature | Indicator | Nature | Indicator | Nature | Indicator | Nature |
| Natural environment | Consideration of details, clarity, lucidity. Has capacity to be measured, has importance coefficient | Air quality | Extensive range of indicators; No attention to detail; lack of evaluation criteria; lack of importance coefficient | Sufficient health centers | General; lacking evaluation criteria; lacking importance coefficient ; unclear | Justice | Consideration of details; clear and lucid; has capacity to be measured; lack of importance coefficient | Natural resources | Unclear; general; Ambiguous; Lacking importance coefficient | Air quality | Extensive range of indicators; No attention to detail; lack of importance coefficient |
| Welfare & health | | Biodiversity | | Healthy water, Gas, Telephone, Electricity | | Urban productivity | | Environment quality | | Water quality | |
| Safety & security | | Ozone Destruction | | | | New technology | | | | Traffic jam | |
| Housing | | Agricultural & food | | | | Housing | | | | Noise | |
| Economy & employment | | Economy & economic development | | Suitable climate | | Urban land | | Variety in the ecosystem | | Transport availability | |
| Education | | General Knowledge & education | | | | Health & education | | | | Earthquake | |
| Social environment | | Environmental justice | | Efficient public transport | | Population | | Human needs | | Hill Cutting | |
| | | Water & wastewater | | Low traffic congestion | | City services | | | | Cyclone | |
| | | Energy change & climate | | | | City | | Economy | | Electricity | |
| | | | | | | environment | | | | | |
| Urban facilities | | Public transportation | | | | City transportation | | Globalization | | Gas supply | |
| Energy | | Parks & urban outdoor spaces | | Small amount of natural disasters | | Local government | | | | Sanitation | |
| Transportation | | Solid trash | | | | Urban management | | Natural and man-made disasters | | Educational facilities | |
| Art & cultural heritage | | Dangerous material | | | | | | | | Business facilities | |
| Artificial environment | | Human health | | | | | | | | Slum | |
| | | Risk management | | | | | | | | | |

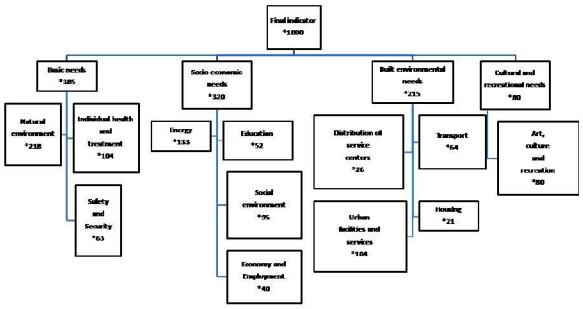


**Fig. 2.** Final model for evaluating Hyderabad's environment quality,
"*" Shows the important coefficient numbers

In the second layer, we have four groups of "main indicators": basic needs, cultural and recreational needs, built environmental needs and socioeconomic needs. In the third layer, twelve main indicators such as energy, safety and security, natural environment and others are placed. In the fourth layer each of the main indicators has been divided into "secondary indicators", such as water pollution, soil resources and so on. In the fifth layer the subdivision of the secondary indicators known as "environmental factors" has been divided into smaller parts, such as human resources, accidents, rescue operations and others. Finally, the sixth layer contains "measures" such as the number of general practitioners, the amount of Sulfur oxides and the average total of rainfall and so on. As it can be seen, measures are the smaller form of the indicators of the higher layers which can be measured. This means that, "measures" are in the lowest layers of the model and "the final indicator" is in the highest layer, in a way that with a mathematical formula in a bottom up order, first the sum of the "measures", then "environment factors" pursued by "secondary indicators", "main indicators", "group of main indicators" and at the end "the final measurement of city environment quality" are calculated. For evaluation, documented, accessible statistics and information from various studies and organizations have been collected. The most important among these resources are the following: central pollution control board (CPCB), state pollution control boards (SPCB), Andhra Pradesh Pollution Control Board (APPCB) and state environment impact assessment authorities.

So that for more clarify the idea of the model and its indicators, following is an example of the method of calculation. For example the "natural environment" indicator is one of the twelve indicators in the third layer which in the fourth layer is divided into the four secondary indicators of "water resources", "soil resources", "air pollutants" and "climate" with importance coefficients of 47, 38, 32, and 14 respectively. The total importance coefficient for the four above-mentioned secondary indicators adds to a 218 importance coefficient for the "natural environment" indicator. In the same manner, for instance 48 as the importance coefficient for the secondary indicator of "air pollutants" itself is the added total of the importance coefficient of five evaluators; SO2, NO2, Pm10, CO, O3 with the importance coefficients of 11, 9, 10, 12 and 9 respectively (Fig. 2). Computations for the amount of the indicators' quality is done in a similar manner of first hierarchically adding the amount of the measures quality in the lowest levels, continuing to the next levels until finally reaching the final quality for the city (in the first layer of the model). At last after calculating the quantitative amount of each of the measures, secondary indicators and the other levels of the model, the amount of the quality of each is determined and evaluated according to Table 2.

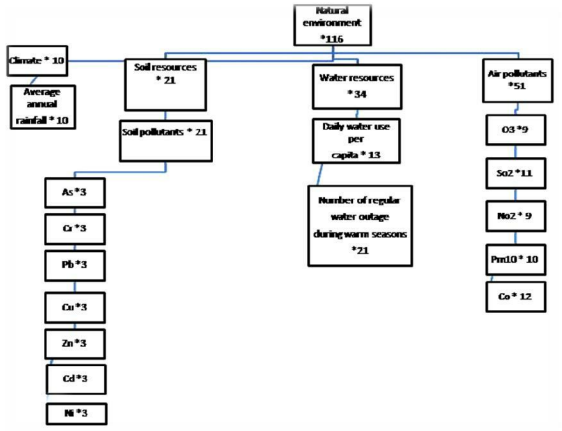


**Fig.3.** Flowchart of "natural environment" indicator,
"*"Shows the important coefficient numbers

**Table 2.** Categorizing of the quality amounts

| Condition | Amounts |
|---|---|
| Best quality (very desirable) | 80% and more |
| Desirable quality | 60-80% |
| Middle ranking quality | 40-60% |
| Low quality | 20-40% |
| No quality (undesirable) | 20% and less |

(Tabibian and Faryadi 2002)

Hyderabad's environmental quality symbolized by the twelve indicators: after carrying out the evaluation in the manner explained in the calculation and research method, Hyderabad's environmental

quality in 2010 has been calculated as demonstrated in Table 3. The resulted amounts show the quality of each main indicator compared to the highest quality considered by the model for that indicator.

**Table 3.** Twelve main indicators of Hyderabad environmental quality

| | |
|---|---|
| Culture, Art, Recreation | 14% |
| Housing | 52% |
| Transportation | 37% |
| Urban facilities | 65% |
| Service centers distribution | 45% |
| Economy and employment | 58% |
| Education | 75% |
| Social environment | 53% |
| Energy | 42% |
| Safety and security | 34.4% |
| Individual health and treatment | 48.6% |
| Natural environment | 65% |

Based on the sum of the twelve main indicators (first model's layer), the scores of the four main indicator groups (model's second layer) were calculated in the following step. The computations in all layers are based in following meanings:

$E_{ij}$: Raw weight of each measure in 4 hierarchical orders:

i=1-5

| | | | | |
|---|---|---|---|---|
| 0 | | | 60 | |
| 0 | | 30 | | 60 |
| 0 | 20 | | 40 | 60 |
| 0 | 15 | 30 | 45 | 60 |

$E_{12}$ = 60: The raw weight of each measure in its best condition which always is 60 in every layer

D: Measure's importance coefficient, which has been arbitrarily considered for each measure

$\Sigma E_{i3} = E_{i2} \times \Sigma D$: Current situation of indicator

i=1-5

$\Sigma E_{13} = E_{12} \times \Sigma D = 60\Sigma$ D: Best situation of indicator

N: Total number of measures in the each main indicator group

N: $n_1+n_2+n_3+.....n_n$

Q = the amount of Quality: $(Current\ situation\ /\ Best\ situation) \times 100$

**Table 4.** "Basic needs" main indicator group (natural environment, individual health and treatment, safety and security):

| | |
|---|---|
| N=15+10+4=35 | Total number of measures in the "basic needs" main indicator group: |
| n1= 14 | Total number of evaluators in the "natural environment" main indicator |
| n2=12 | main indicator Total number of measures in the "individual health and treatment" main indicator |
| n3= 9 | Total number of measures in the "safety and security" main indicator |

Current situation = $\Sigma E^{13}_{N}$ = (9810+1560+945) =12315

Amount of the "basic needs" main indicator group quality in Hyderabad (2010):

Best situation = E12 × Σ D = ΣE13

60 × (218 + 104 + 63) = 23100

*Q*= 7195/ 11580 × 100= 53.3%

Based on Table 2, Hyderabad had a middle quality in 2010 with a score of 53.3% in the "basic needs" main indicator group.

**Table 5.** "Socio-economic needs" main indicator group (energy, social environment, education, economy and employment):

| | |
|---|---|
| N=4+ 2+3+2=34 | Total number of measures in "socio-economic needs" |
| n1= 10 | main indicator group: Total number of measures in "energy" main indicator group |
| n2=11 | Total number of measures in " social environment" main indicator group |
| n3= 7 | Total number of measures in "education" main indicator group |
| n4=6 | Total number of measures in "economy and employment" main indicator group |

Current situation= $\Sigma E^{2}_{i2}$ = (5985+1560+2850+1200) = 11595
Best situation= $E_{12} \times \Sigma D = \Sigma E_{12}$
60 × (133+52+95+40) = 19200
Amount of the "socio-economic needs" main indicator group quality in Hyderabad (2010):
*Q* = 4440 / 6660 × 100= 60.3%
Based on Table 2, Hyderabad had a desirable quality in 2010 with a score of 60.3% in the "socio-economic needs" main indicator group.

**Table 6.** "Built environmental needs" main indicator group (service centers distribution, urban facilities, transportation, and housing):

| | |
|---|---|
| N= 1+5+4+2=11 | Total number of evaluators in "built environmental needs" main indicator group: |
| n1=3 | Total number of evaluators in "service centers distribution" main indicator |
| n2=4 | Total number of evaluator s in "urban facilities" main indicator |
| n3=2 | Total number of evaluator s in "transportation" main indicator |
| n4=2 | Total number of evaluators in "housing" main indicator |

Current situation= $\Sigma E^{13}_{i3}$ = (780+1560+1920+630) = 4890
Best situation= $E_{13} \times \Sigma D$ = 60 × (26+104+64+21) = 12900
Amount of the "man-made needs" main indicator group quality in Hyderabad (2010):
*Q* = 4890 / 12900 × 100= 37.9%
Based on Table 2, Hyderabad had a low quality in 2010 with a score of 37.9% in the "built environmental needs" main indicator group.

**Table 7.** "Cultural and recreational needs" main indicator group (art – culture – recreation):

| | |
|---|---|
| N: n1= 12 | Total number of evaluators in " cultural n1: Total number of evaluators in art – culture – recreation indicator =12 |

Current situation= $\Sigma E^{13}_{i3}$ = (675)
Best situation= $E_{13} \times \Sigma D$ = ΣE13 60 × 80=4800
Amount of the "cultural and recreational needs" main indicator group quality in Hyderabad (2010): *Q* = 675 / 4800 × 100= 14%
Based on Table 2, Hyderabad had an undesirable condition in 2010 with a score of 14% in the "cultural and recreational needs" main indicator group.
Final amount of Hyderabad's environmental quality (2010) in the end, by adding the results of the four groups of main indicators (basic needs, socio-economic needs, man-made needs, cultural and recreational needs) the total score of the final amount is calculated as follows:
n=4 Number of groups of main indicators (basic needs, socio-economic needs, man-made needs, cultural and recreational needs)
Current situation:
$\Sigma E^{4}_{i2}$= (12315+11595+4890+675) = 29475
Best situation
$\Sigma E^{4}_{i2}$= (23100+19200+12900+4800) = 60000
Final amount of quality= Current situation total / Best situation total ×100
$\Sigma E^{4}_{i2}$/ $\Sigma E^{4}_{i2}$× 100= 29475 / 60000 ×100 = 49.1%
Therefore, in 2010 Hyderabad possessed near to half of this model's expected quality with a collective score of 49.1%. This percentage demonstrates Hyderabad's average environmental quality in the studied year (2010) based on the presented model.

## 4. Results and discussion

As it was observed, the final amount of Hyderabad's environmental quality was approximately calculated to be 49.1%. This quantity has been extracted from the scores achieved by the four groups of main indicators (basic needs, socio-economic needs, built environmental needs, cultural and recreational needs). "Basic needs" with a score of 53.3%, "socio-economic needs" with a score of 60.3%, "built environmental needs" with score of 37.9%, and "cultural and recreational needs" with a score of 14% placed Hyderabad in the middle ranking of environmental quality. A general comparison between the evaluation results from the viewpoint of four main indicator groups show that although "basic needs" and "socio-economic" needs have an important role in determining Hyderabad's environmental quality based on their respective importance coefficients of 385 and 320, the "cultural and recreational" main indicator group with a mere importance coefficient of 80 which allocates only 14% of the total importance coefficients, is the most important factor in decreasing Hyderabad's environment quality in 2010. In the main indicator group of "cultural and recreational needs" which incorporates the art, culture and recreation indicator, insufficient exploitable sport areas, museums and theater hall per capita and insignificant library use per capita are the main reasons for the low final quality of this main indicator.

In the "basic needs" main indicators group, the "natural environment" indicator with a score of 65%, the "individual health and treatment" indicator with a score of 48.6%, and the "safety and security" indicator with a score of 34.4% were effective in their group's 53.3% score. In the natural environmental section, the weather desirable

conditions, average rainfall in Hyderabad and providing the residents with drinking water despite insufficient regional water resources are among the effective factors on Hyderabad's desirable situation in this group. It should be added that Hyderabad has an urgent needs of upgrading old sewerage system with laying of proper underground drainage lines and replacement of old water pipelines in the core and outskirt areas. Regarding air pollution's undesirable condition due to the Industries, thermal power plants, Use of coal and fuel wood and also motor vehicles are among the major contributors to air pollution in Hyderabad.

Regarding the "individual health and treatment indicator", the effective factors that helped this group achieve a middle quality were the high percentage of vaccination of children under the age of two, decreasing the amount of patients affected to pulmonary and non-pulmonary tuberculosis to an middle ranking amount, decreasing relative risk of malaria, HIV & AIDS and underweight children of less than 4 years. Finally, presence of specialist doctors and general practitioners, and also the existence of the necessary number of public and private hospital beds (public and private hospitals).

With regard to the "safety and security" indicator, the high stats of in-city car accidents, deception and robberies across the city were reasons for Hyderabad's quality to be 34.4% in this indicator.

Regarding the "socio-economic needs" main indicator group, it can be observed that even with a variety of economic and social problems in metropolises, Hyderabad was able to achieve 60.3% of the model's expected quality in this group. As the scores obtained by the four "main indicators" of this group demonstrate, the "energy" main indicator with a score of 42%, the "social environment" main indicator with a score of 53%, the "education" main indicator with a score of 75%, and the "economy and employment" main indicator with a score of 58% were all effective in this group achieving a 60.3% quality.

In the "energy" indicator, Hyderabad's middle quality was due to insufficient source of energy and also the city's unstable condition from the viewpoint of average electricity outage period. Regarding the "social environment" indicator, Hyderabad's undesirable quality of littering and dumping of garbage, sewage treatment and the city's middle quality for family size caused to its middle ranking place. With regard to the "education" indicator, Hyderabad's desirable quality was due to illiteracy rate (20.4%) and also 100% radio and television coverage across the city and the desirable rate of signing in elementary school. Regarding the "economy and employment" indicator, the city's middle ranking quality was due to unemployment and inflation rate.

In the "built environmental needs" main indicator group, the "public service centers distribution" indicator with a score of 45%, the "urban infrastructures" indicator with a score of 65%, the "transportation" indicator with a score of 37%, and the "housing" indicator with a score of 52% were effective in their group's 56% quality score. Regarding the "public service centers distribution" the average number of vegetable and fruit stands and markets throughout the city has lead to a desirable ranking quality in this indicator. With regard to the "urban infrastructures", Hyderabad achieves a middle quality, the city's low quality in the aspect of wastewater piping, urban drainage networks and canalization, especially in monsoon season. Hyderabad as well achieves middle percentage of amount of phone landlines; recycling house waste and also post office boxes throughout the city on the other hand, have all lead to the achievement of middle quality in this group.

In the "transportation" indicator group, the desire quality of public transportation fleet per capita (number of people per vehicle), small share of bicycles in intercity travelling, and also lack of basic facilities at subway and monorail, the middle ranking percentage of using public transportation for inter-city travelling has lead to an middle ranking quality score in the group.

Achieving a desirable quality score in the housing indicator demonstrates the city's suitable condition in this regard, while at the same time providing residents with housing has always been one of the main problems of the citizens of Hyderabad. It seems that, this inconsistency is related to the type of chosen measures based on the existing data and statistics, most of which emphasize in production of housing (measure of number of families' ratio to housing units) and also buildings conditions from the viewpoint of sustainability and strength. Regarding the slum dwellers, Hyderabad's middle quality is due to health, water sanitation, gender inequality and living condition.

With regard to the "cultural and recreational needs" main indicator group, the main indicator of "art, culture and recreation" was the reason for this group's 14% quality score. Insufficiency of exploitable sport areas per capita, library usage per capita and city parks per capita, as well as the low per capita of museums per 100000 people, are all the main reasons behind Hyderabad's low quality in this indicator.

## 6. Conclusion

The main result for this evaluation was calculating the final amount of Hyderabad's environmental quality in 2010 (the census year based in this research). Based on this evaluation, Hyderabad achieved near half of the best quality expected, that is, 49.1%. Observing this process can signify the city's movement towards a livable and sustainable city. On the other hand, observing these results can make the city's management and planning authorities aware of the city's points of strength and weakness. Finally, it can be asserted that such an insight facilitates major decision-makings regarding the execution of development programs. Accordingly, it seems that creating an integrated urban management approach can have a major role in solving many of Hyderabad's problems and speeding up the process of improving its environment quality. Although obviously Hyderabad's municipality is not capable of solely realizing this integrated management and it requires an all inclusive cooperation on the part of the people and other related sections. Another important result is providing a suitable model for evaluating the city's environment quality based on a collection of environment indicators. The present model is the result of comparing and analyzing similar models and selecting more suitable indicators based on available data and measurable indicators. Therefore, the model presented in this survey is a suited and harmonized model which can be used for evaluating the environmental quality of other areas and cities. This model's dynamism rely on information input and substituting correct data in it which, in a cumulative movement from bottom -up, can explain city environmental quality. Also, the research's results clearly indicate that although in the presented model numerous amounts of factors constituting the urban environmental quality are presented through a limited amount of measures (48) or measurable and more comprehensive indicators, a relatively thorough evaluation of cities' environmental quality is possible to a high extent using of this model. Considering the evaluation results, some planning strategies for improving Hyderabad's environmental quality are presented below. The strategies are classified on the basis of the most important identified problems.

For decreasing high amount of cars and the high traffic it is suggesting utilizing intelligent control and management systems, improving road quality, developing transportation rail lines (urban trains) and more use of public transport in inter-city travelling and increase special bicycle trails throughout the city.

To improve the littering and waste management it is suggests for prohibit littering on the street, devise ways to collect waste from unsanitary and difficult areas such as slums, hotels, restaurants, office complexes and commercial areas, build adequate storage facilities taking into account the population density so as to prevent overflowing of trash cans and color-code waste bins so as to promote segregation of waste at source-green for biodegradable, white for recyclable wastes and black for other wastes. To increase the sport places per capita is suggests for establishing new sport centers throughout the city and its neighborhoods and installing sports equipment in city parks in order to create suitable sport areas.

To decrease the repeated and lengthy period of time occurrences of power outage it is suggests for moving in the direction of privatization and decreasing cities' dependency on the national powerhouse network through establishing new powerhouses around cities.

To increase the library use per capita it is suggest that to increasing libraries' work hours and developing, also improving their services, establishing public and specialist libraries throughout the city and promoting the culture of book reading and using libraries.

To decrease of high air pollution it is suggests for eliminating timeworn vehicles, standardizing new vehicles, improving public transport, using clean technology, improving fuel quality, technical examination of vehicles and traffic management and education. To decrease the high rate of in-city car accidents it is suggesting for improving the content and performance of driving laws, prohibiting the use of mobile phones while driving, driving below the speed limit and standard number of passengers in cars.

To decrease the high rate of inflation it is suggest for using monetary policy strategy (selling partnership papers, decreasing the amount of loan payoffs), utilizing fiscal policy and transition of incumbency activities of the government toward policy making and supervision.

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# Temperature Effect on Corrosion Inhibition of Carbon Steel in Formation Water by Non-ionic Inhibitor and Synergistic Influence of Halide Ions

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**Abstract:** The inhibitive effect of nonionic surfactant namely nonylphenoxy poly (ethyleneoxy) ethanol (NPPE) on carbon steel corrosion in oilfield formation water at temperature range 303-333 K was studied using electrochemical polarization technique.The inhibition efficiency increases with increasing the concentrations of NPPE but decreases with the increase in temperature. Potentiodynamic polarization studies revealed that the NPPE acts as a mixed inhibitor. Adsorption of NPPE on the carbon steel surface in oilfield formation water follows the Langmuir isotherm model. The activation energy and the thermodynamic parameters for the inhibition process were calculated and discussed. The inhibition efficiency of NPPE synergistically increased on addition of halide ions in the order KCl< KBr <KI.
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**Keywords:** Nonionic surfactant; oilfield formation water; corrosion; synergistic inhibition; carbon steel; thermodynamic parameters

## 1. Introduction

The main reason of corrosion problems in the oil industry is attributed to the presence of formation water (oilfield water), which accompanies the oil production. It has been shown that corrosion is related to the water content which contains a various corrosive agents including carbon dioxide, hydrogen sulphide, organic acids and salts such as chlorides and sulfates [1].

Carbon steels are the most commonly used pipeline materials in petroleum production. However, carbon steels are very prone to corrosion in environments containing acid solutions [2]. The corrosion of acid solution on the steel materials causes considerable cost. In order to reduce the corrosion of metal, several techniques have been applied, where among that utilization of organic compounds and more specifically surfactants are gaining high space as corrosion inhibitors. Surfactants are very beneficial reagents and their presence at very low quantity in any medium providing desirable properties to processes in all industries such as, petrochemical, food, paint and coating industry [3].

A fundamental property of surfactants is their ability to form micelles (colloidal sized clusters) in solution. This property is due to the presence of both hydrophobic and hydrophilic groups in each surfactant molecule.Surfactants accumulate in special order at the interfaces and modify the interfaces and thus, control, reduce, or prevent reactions between a substrate and its surroundings, when added to the medium in small quantities. Different surfactant groups have been reported to present corrosion inhibitory potential which depends on the classification of surfactants, the substrate type, inhibitor concentration [4-12], time of immersion in inhibitor solution [13], the type of acid , pH [14], presence of salts [15-23], co-surfactant [24], temperature [25–27] and inhibitor structure [28]. Nonionic surfactants are often used because of their lower critical micelle concentration (CMC), their higher degree of surface-tension reduction, and their relatively constant properties in the presence of salt, which result in better performance and lower concentration requirements [5].

Most acid inhibitors are known for their specificity of action. However, the combination of inhibitors is more likely to provide multiple effects required for effective corrosion inhibition. Synergistic inhibition studies for corrosion inhibitors for metals have been advocated as an effective means of decreasing the amount of inhibitor usage, diversifying the application of the inhibitor and improving the inhibitive force of the inhibitor. Interestingly, addition of halide salts to acid solutions containing any organic compound had been reported to result in a synergistic effect thereby inhibiting iron corrosion [25]. Corrosion inhibition synergism results from increased surface coverage as a result of ion–pair interactions between the organic cation and the anions. Synergistic effect of halide ions on the corrosion inhibition of metals using various substances have been reported by some research groups [29-31].

The synergistic effect was found to increase in the order $I^- > Br^- > Cl^-$. The highest synergistic effect associated with iodide ions has been attributed to their large size and ease of polarizability hence can be chemisorbed onto metal surface [32].

## 2. Experimental

### 2.1. Materials

Tests were performed on carbon steel (CS) of the following composition (wt. %): 0.05% C, 0.28% Mn, 0.023% P, 0.019% S, 0.02% Si, and the remainder Fe. Nonylphenoxy poly (ethyleneoxy) ethanol (NPPOE) was used as inhibitor; it was obtained from commercial source.

### 2.2. Test Solutions

The test solution used was oil field formation water kindly provided by The Gulf of Suez Petroleum Company (GUPCO). A typical chemical composition is given in Table (**1**).The concentration range of NPPOE employed was ($4x10^{-5}$ to $40 \times 10^{-5}$ M). Deionized water was used for the preparation of all reagents. The halides used (KCl, KBr, and KI) were all BDH laboratory supplies chemicals, England .Solutions with 0.01M concentration of KCl, KBr and KI were used for the synergistic studies. The studies were carried out at temperature range of 303–333 K.

### 2.3. Electrochemical measurements

Electrochemical experiments were carried out in the conventional three-electrode cell with a platinum counter electrode (CE) and a saturated calomel electrode (SCE) as the reference electrode. The working electrode (WE) used was in the form of a square CRS embedded in PVC holder using epoxy resin so that the flat surface is the only exposed surface in the electrode, and has an area of 1.0 x 1.0 cm. Before running the experiment the electrode was abraded with emery paper (grade 320–500–800-1200) on test face, rinsed with distilled water, degreased with acetone, and dried with a cold air stream. A computer controlled EG&G PAR 273A Potentiostat/galvanostat (Princeton Applied Research) was used for the electrochemical measurements. Each experiment was repeated at least three times to check the reproducibility. Polarization curves were recorded potentiodynamically, at the scan rate of 1 mV/s, in the range of +250 mV to -250 mV versus OCP potential.

## 3. Results and Discussion

### 3.1. Polarization studies

The inhibition process of the (NPPOE) for the corrosion of carbon steel in oilfield formation water was analyzed by polarization experiments.

Some examples of both the Tafel anodic and cathodic polarization curves for carbon steel in oilfield formation water at different concentrations of the nonionic surfactant (NPPOE) and different temperatures are shown in Figure (1). It is clear that the presence of the inhibitor shifts the corrosion potential to the noble direction, i.e.decreases the corrosion rate. This may be ascribed to adsorption of the inhibitor over the corroded surface. Corrosion parameters such as, the corrosion current density ($I_{corr}$), corrosion potential ($E_{corr}$), anodic and cathodic Tafel slopes ($b_a$ $b_c$ respectively), were calculated from Figure (1) and the values are listed in Table (2).From these data, it is clear that the corrosion current decreases with the increase of the inhibitor concentration. The presence of (NPPOE) surfactant does not remarkably shift the corrosion potential while, both the anodic and cathodic Tafel slopes change with the increase of the inhibitor concentration. Therefore, the nonionic surfactant (NPPOE) can be classified as mixed-type inhibitor in oilfield formation water. These results are in good agreement with the results obtained for other organic compounds in acidic solutions [33-35].

The inhibition efficiency (IE %) and the degree of surface coverage (θ) were calculated according to the following equations [36]:

$$IE\,\% = \left(1 - \frac{I}{I_o}\right) \times 100 \quad (1)$$

$$\theta = 1 - (I / I_o) \quad (2)$$

where $I_o$ and I are the corrosion current density values in the absence and presence of inhibitor, respectively, determined by extrapolation of Tafel lines to the corrosion potential.

Values of the inhibition efficiency were calculated and listed in Table (2), which reveal that the inhibition efficiency (η %) increases with the increment of the inhibitor concentration.

### 3.2. Adsorption considerations

Basic information on the interaction between inhibitors and metal surface can be provided using the adsorption isotherms [37]. The adsorption of an organic adsorbate at metal–solution interface can occur as a result of substitutional adsorption process between organic molecules presented in the aqueous solution ($Org_{(sol)}$), and the water molecules previously adsorbed on the metallic surface ($H_2O_{(ads)}$) [38]:

$$Org_{(sol)} + xH_2O_{(ads)} = Org_{(ads)} + xH_2O_{(sol)} \quad (3)$$

Where $Org_{(sol)}$ and $Org_{(ads)}$ are the organic species in the bulk solution and adsorbed one on the metallic surface, respectively, $H_2O_{(ads)}$ is the water molecule adsorbed on the metallic surface and x is the size ratio representing the number of water molecules replaced by one organic adsorbate. In order to obtain the adsorption isotherm, the degree of surface coverage, θ, for different concentrations of inhibitor in oilfield formation water solutions has been evaluated by the following equation [39]:

$$\theta = I^o_{corr.} - I_{corr.} / I^o_{corr.} \quad (4)$$

The θ values are presented in Table (2). According to the Langmuir's isotherm, the surface coverage (θ) is related to inhibitor concentration (C) by the following equation [39]:

$C_{inh.}/\theta = 1/K_{ads} + C_{inh.}$ (5)

Where $K_{ads}$ is the equilibrium constant of the inhibitor adsorption process and can be calculated from the intercept lines on the $C_{inh.}/\theta$-axis. As seen from Figure (2), the plot of $C_{inh.}/\theta$ versus $C_{inh.}$ yields a straight line with a correlation coefficient more than 0.99, showing that the adsorption of these inhibitors in oilfield formation water is fitted to Langmuir adsorption isotherm.

The data reported in Table (3) reveals that, the adsorptive equilibrium constant (K) decreases with increasing the temperature indicating that, it is easy for inhibitor to adsorb onto the carbon steel surface at relatively lower temperature, but as the temperature increases, the adsorbed inhibitor tends to desorption.These results suggest that, the inhibition of carbon steel in oilfield formation water by the NPPOE is an adsorptive process. This isotherm assumes that the adsorbed molecules occupy only one site and there are no interactions between the adsorbed species [40].

### 3.3. Thermodynamic parameters

Generally, the organic molecules inhibit corrosion by adsorption at the metal-solution interface and the adsorption process depends on the molecule's chemical composition, the temperature and the electrochemical potential at the metal–solution interface [40].

Thermodynamic parameters play an important role in understanding the inhibition mechanism. The standard adsorption heat ($\Delta H^0$) could be calculated according to the Van't Hoff equation [41]:

$\ln K = - \Delta H^o_{ads}/R\,T + \text{constant}$ (6)

Where, R is the gas constant (8.314 J $K^{-1}$ $mol^{-1}$), T the absolute temperature .To obtain the adsorption heat, the regression between ln K and 1/T was dealt with. Clearly, the adsorption heat can be obtained by using the slope of the regression ($- \Delta H^o_{ads}/R$), and the relationship between ln K and 1/T is shown in Figure (3).

Figure (3) indicates that there is a good linear relationship between lnK and 1/T with a correlation coefficient higher than 0.99, meaning that it is safe to utilize the Van't Hoff equation to calculate the adsorption heat. Under the experimental conditions, the adsorption heat can be approximately regarded as the standard adsorption heat ($\Delta H^o_{ads}$).

It is well known that the standard adsorption free energy ($\Delta G^o_{ads}$) is related to the equilibrium constant of adsorption (K), and $\Delta G^o_{ads}$ can be calculated by the following equation (42):

$\Delta G^0_{ads} = - RT \ln (55.5\, K_{ads})$ (7)

Where R is the gas constant (8.314 J $K^{-1}$ $mol^{-1}$), and T is the absolute temperature (K). The constant value of 55.5 is the concentration of water in solution expressed in M. A plot of $\Delta G^o_{ads}$ versus T (Figure 4) gave the heat of adsorption ($\Delta H^o_{ads}$) and the standard adsorption entropy ($\Delta S^o_{ads}$) according to the thermodynamic basic equation:

$\Delta G^o_{ads} = \Delta H^o_{ads} - T\Delta S^o_{ads}$. (8)

Figure (4) clearly shows that the good dependence of $\Delta G^o_{ads}$ on T, indicating the good correlation among thermodynamic parameters. The thermodynamic data obtained for NPPE in oilfield formation water using the adsorption isotherm are depicted in Table (3).

The negative sign of $\Delta G^0_{ads}$ indicates that the inhibitors are spontaneously adsorbed on the metal surface [40, 43]. Generally, the magnitude of $\Delta G^0_{ads}$ around −20 kJ $mol^{-1}$ or less negative, leads to the assumption that an electrostatic interaction exists between the inhibitor and the charged metal surface (i.e. physisorption). Standard free energy of adsorption ($\Delta G^0_{ads}$) around −40 kJ $mol^{-1}$ or more negative indicates that a charge sharing or transferring from organic species to the metal surface occurs to form a coordinate type of bond (i.e. chemisorption) [44].

In the present study, the $\Delta G^0_{ads}$ values obtained for the (NPPOE) in oilfield formation water solution ranges between -33.2 and – 36.8 kJ $mol^{-1}$, which are lower than -40 kJ $mol^{-1}$ but higher than -20 kJ $mol^{-1}$. This indicates that the adsorption is neither typical physisorption nor typical chemisorption but it is complex mixed type. That is the adsorption of inhibitor molecules on the carbon steel surface in the present study involves both physisorption and chemisorption (comprehensive adsorption) but physisorption is the predominant mode of adsorption. This assumption is supported by the data obtained from temperature dependence of inhibition process, reported in Table (2), which shows that the inhibition efficiency of the NPPOE studied decreases with increase in temperature (physisorption) [45]. Thus, we conclude that the adsorption for the inhibitor studied (NPPOE) on the carbon steel in oilfield formation water is complex in nature and predominantly physisorption. Moreover, the calculated $\Delta G^0_{ads}$ values show that an electrostatic interaction exists between the charged molecules and the charged metal surface.

As for the value of $\Delta S^0$ in Table 3, the sign of $\Delta S^0$ is positive, which indicates that the adsorption process is accompanied by an increase in entropy. This could be explained as follows, The adsorption of organic inhibitor molecules from the aqueous solution can be regarded as a quasi-substitution process between the organic compound in the aqueous phase [$Org_{(sol)}$] and water molecules at the

electrode surface [$H_2O_{(ads)}$] [38]. In this situation, the adsorption of organic inhibitor is accompanied by the desorption of water molecules from steel surface. Thus, while the adsorption process for the inhibitor is believed to be exothermic and associated with a decrease in entropy of the solute, the opposite is true for the solvent. The thermodynamic values obtained are the algebraic sum of the adsorption of organic molecules and desorption of water molecules .Therefore, the gain in entropy is attributed to the increase in solvent entropy [46].

### 3.4. Kinetic parameters

The adsorption phenomena have been explained by using thermodynamic parameters, to further elucidate the inhibition properties of the inhibitor, the kinetic model was employed. The thermodynamic functions for dissolution of carbon steel in the absence and in the presence of various concentrations of NPPOE were obtained by applying the Arrhenius equation and the transition state equation [47]:

$$I_{corr.} = A \exp(-E_a^0 / RT) \quad (9)$$

$$I_{corr.} = RT/ Nh \exp(\Delta S^0_a /R) \exp(-\Delta H^0_a /RT) \quad (10)$$

Where $E^0_a$ is the apparent activation energy, A the pre-exponential factor, $\Delta H^0_a$ the apparent enthalpy of activation, $\Delta S^0_a$ the apparent entropy of activation, h the Planck's constant and N the Avogadro number.

Arrhenius plots of Ln $I_{corr.}$ vs. 1/T for the blank and different concentrations of NPPOE are shown in Figure (5). The plots obtained are straight lines and the slope of each straight line gives its activation energy. The negative slope of ($E_a$) indicates the adsorption of the inhibitor on the electrode surface. The regression between ln $I_{corr.}$ and 1/T was calculated and the parameters were calculated and presented in Table (4), it can be seen that apparent activation energy increased with increasing concentration of NPPOE. Also, it is clear that $E_a$ values in the presence of inhibitor are higher than that in the absence of inhibitor indicating higher activation energies for the metal dissolution reaction. Hence, the process is activation controlled. The increase in apparent activation energy with NPPOE concentration thereby indicates a more efficient inhibiting effect that can be attributed to the thickening of the electric double layer and supports the hypothesis that molecules can form micelles on the metal surface.

The increase in apparent activation energy $E_a$ may be interpreted as physical adsorption [48]. Szauer and Brand [49] explained that the increase in activation energy can be attributed to an appreciable decrease in the adsorption of the inhibitor on the carbon steel surface with increase in temperature and a corresponding increase in corrosion rates occurs due to the fact that greater area of metal is exposed to the acidic solution environment.

Figure (6) shows a plot of ln ($I_{corr.}$ /T) vs. 1/T. A straight lines were obtained with a slope equal to ($-\Delta H^o_a$ /R) and intercept equal to (Ln R/Nh + $\Delta S^o_a$/R), from which the values of $\Delta H^o_a$ and $\Delta S^o_a$ were calculated and listed in Table (4). Inspection of these data reveals that the thermodynamic parameters ($\Delta H^o_a$ and $\Delta S^o_a$) of dissolution reaction of carbon steel in oilfield formation water in the presence of NPPOE are higher than in the absence of inhibitor. The positive sign of enthalpies reflect the endothermic nature of steel dissolution process meaning that dissolution of steel is difficult [50].On comparing the values of the entropy of activation $\Delta S^o_a$ given in Table (4**)**, it is clear that entropy of activation increased positively in the presence of NPPOE than in the absence of inhibitor. The increase of $\Delta S^o_a$ reveals that an increase in disordering takes place on going from reactant to the activated complex [51].

### 3.5. Synergistic effect of halide ions

Synergistic inhibition effect of inhibitors takes place when the total action of compounds is higher than the sum of each one individually [52].To elucidate the synergistic influence of halide ions on the corrosion inhibition of carbon steel in oilfield formation water by NPPOE, certain concentration of the inhibitor ($10x10^{-5}$) was studied in the absence and presence of 1 x $10^{-2}$ M KI, KCl and KBr to clarify this phenomenon.The synergistic inhibitive effect brought about by the inhibitor and halide ions are shown in Fig.(7) .The electrochemical parameters of this study are presented in Table (5). It is clear that the addition of halide salts to the inhibitor solution enhanced inhibition and a marked change in the inhibition efficiency occurred from 61.2 to 93 %, 90 % and 87.8 % in presence of KI, KBr and KCl respectively with a noticeable increase in the surface coverage. A shift in $E_{corr.}$ values to more anodic potentials is observed on addition of iodide salts. As $E_{corr.}$ shifted to a more noble direction , $I_{corr.}$ decreased considerably from 18.69 to 3.354, 4.928 and 5.998 $\mu Acm^{-2}$ for KI, KBr and KCl respectively. These values are coinciding with the polarization resistance values which increased considerably with the presence of halide salts; thus the corrosion inhibition effect of NPPOE appeared to be synergistically enhanced by the presence of the halide ions. The synergistic influence may be explained as follows: halide ions are initially chemisorbed on the metal surface and therefore, the surface becomes negatively charged. The positively charged part of the inhibitor molecule tends to become oriented towards the adsorbed anions preferentially by coulombic attraction onto the metal surface where iodide ions

already have been chemisorbed. This behavior suppresses the corrosion rate by stabilizing the adsorbed anion ($I^-$) and the positively charged part of the inhibitor molecule, increasing the surface coverage of the inhibitor and this enhances the efficiency of the inhibition.

The synergistic parameter (S) was calculated using the following equation [53]:

$$S = (1- I_{1+2}) / (1- I`_{1+2}) \quad (11)$$

Where, , $I_1$ is inhibition efficiency of halide, $I_2$ is the inhibition efficiency of the inhibitor, $I_{1+2} = I_1+I_2$ and $I`_{1+2}$ is inhibition efficiency of inhibitor in combination with the anion. The value of (S) parameter was calculated for the case of each halide salt:

$S_{KI} = 1.56$, $S_{KBr} = 1.67$ and $S_{KCl} = 1.88$

The (S) values in the three cases for KI, KBr and KCl respectively are more than unity, indicating clearly that,the enhanced inhibition efficiency is due to synergistic effect of halide and NPPOE (53).

## 4. Conclusions

- NPPOE acts as a good corrosion inhibitor for carbon steel in oilfield formation water.
- The inhibition efficiency of NPPOE increases with increasing the inhibitor concentration but decreases with temperature.
- The adsorption of NPPOE obeys Langmiur adsorption isotherm. The adsorption process is a spontaneous and exothermic process accompanied by an increase of entropy.
- All the values of free energy are negative as well as less than - 40 $KJmol^{-1}$, indicating the spontaneous physical adsorption of the inhibitors on the metal surface.
- Addition of halide salts synergistically increased the inhibition efficiency of NPPOE in the order KCl<KBr< KI.

**Table (1): Chemical analysis of the tested oilfield formation water.**

| Corrosive elements | Concentration | Test method ASTM |
|---|---|---|
| **TDS,** mg/l | 122670 | D-1888 |
| **Sodium as $Na^+$,** µg/g | 36699 | D-3561 |
| **Potassium as $K^+$,** µg/g | 714 | D-3561 |
| **Calcium as $Ca^{2+}$,** µg/g | 3632 | D-511 |
| **Magnesium as $Mg^{2+}$,** µg/g | 4125 | D-511 |
| **Chloride as $Cl^-$,** µg/g | 59981 | D-512 |
| **Sulphate as $SO^{2-}_4$,** µg/g | 120 | D-516 |

**Table (2): Electrochemical polarization parameters for the corrosion of carbon steel in oilfield formation Water containing various concentrations of (NPPOE) at different temperatures 293, 313 and 333K.**

| T (K) | Conc. ($10^{-5}$ M) | R (mpy) | $E_{corr.}$ (mV) | $I_{corr}$ (µ A $cm^{-2}$) | $b_a$ (mV $dec^{-1}$) | $-b_c$ (mV$dec^{-1}$) | $R_p$, (K Ω cm) | IE (%) | θ |
|---|---|---|---|---|---|---|---|---|---|
| 293 | Blank | 15.89 | -694.9 | 34.65 | 158.9 | 234.1 | 1.27 | - | - |
| | 4 | 9.629 | -691.5 | 21.0 | 103.5 | 194.6 | 1.34 | 39.0 | 0.390 |
| | 6 | 8.228 | -685.2 | 17.94 | 116.7 | 176.6 | 1.646 | 48.0 | 0.480 |
| | 8 | 6.610 | -680.8 | 14.41 | 106.8 | 188.1 | 2.041 | 58.4 | 0.584 |
| | 10 | 5.705 | -655.5 | 12.44 | 85.58 | 182.9 | 1.875 | 64.1 | 0.641 |
| | 20 | 3.299 | 546.0 | 7.193 | 81.5 | 155.4 | 2.904 | 79.2 | 0.792 |
| | 40 | 1.643 | -512.3 | 3.583 | 71.16 | 111.0 | 3.667 | 89.7 | 0.897 |
| | | | | | | | | | |
| 313 | Blank | 22.49 | -693.9 | 49.03 | 121.2 | 243.3 | 0.7176 | - | - |
| | 4 | 15.23 | -668 | 33.20 | 124.2 | 204.7 | 0.9452 | 32.3 | 0.323 |
| | 6 | 12.85 | -645.6 | 28.01 | 191.4 | 229.3 | 1.571 | 42.9 | 0.429 |
| | 8 | 10.61 | -675.6 | 23.12 | 105.9 | 187.7 | 1.182 | 52.8 | 0.528 |
| | 10 | 8.572 | -608 | 18.69 | 140.3 | 165.2 | 1.527 | 61.9 | 0.619 |
| | 20 | 6.652 | -588.6 | 15.07 | 96.82 | 212.4 | 1.987 | 70.4 | 0.704 |
| | 40 | 3.593 | -512.6 | 7.833 | 66.68 | 204.3 | 2.540 | 84.0 | 0.840 |
| | | | | | | | | | |
| 333 | Blank | 29.06 | -707.3 | 63.35 | 134.6 | 484.5 | 0.6223 | - | - |
| | 4 | 20.22 | -699.1 | 44.08 | 115.2 | 353.9 | 0.8110 | 30.4 | 0.304 |
| | 6 | 18.50 | -693 | 40.34 | 120.3 | 241.5 | 0.9248 | 36.3 | 0.363 |
| | 8 | 15.82 | -658 | 34.48 | 117.3 | 245.6 | 0.9774 | 45.7 | 0.457 |
| | 10 | 12.75 | -699.3 | 27.81 | 122.4 | 214.5 | 1.171 | 56.1 | 0.561 |
| | 20 | 10.21 | -679.6 | 22.27 | 112.5 | 204.1 | 1.433 | 64.9 | 0.649 |
| | 40 | 5.973 | -634.2 | 13.02 | 98.64 | 195.9 | 1.794 | 79.4 | 0.794 |

**Table (3): Thermodynamic parameters for the adsorption of (NPPOE) inhibitor in oilfield formation water on the carbon steel at different temperatures.**

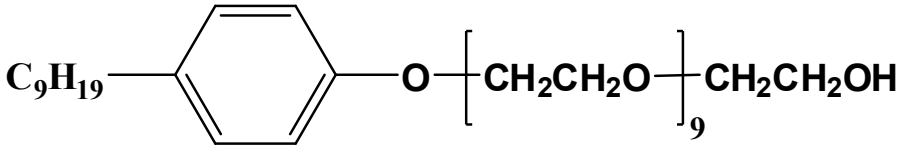
| Temp. (K) | $K_{ads}$ ( $M^{-1}$ ) | slope | $R^2$ | $\Delta G^o_{ads}$ (kJ $mol^{-1}$) | $\Delta H^o_{ads}$ (kJ $mol^{-1}$) | $\Delta S^o_{ads}$ ( J $mol^{-1}K^{-1}$) |
|---|---|---|---|---|---|---|
| 293 | 15760 | 0.9554 | 0.9995 | -33.2 | -11.5 | 95 |
| 313 | 13020 | 1.002 | 0.9962 | - 35.0 | -11.5 | 95 |
| 333 | 10885 | 1.035 | 0.9949 | - 36.8 | -11.5 | 95 |

**Table (4): The values of activation parameters $E_a$, $\Delta H^0_a$ and $\Delta S^0_a$ for carbon steel in formation water in the absence and presence of different concentrations of NPPE.**

| Concentration ( M ) X $10^{-5}$ | $E_a$ ( kJ $mol^{-1}$ ) | $\Delta H^0_a$ ( kJ $mol^{-1}$ ) | $\Delta S^0_a$ (J $mol^{-1}K^{-1}$) |
|---|---|---|---|
| Blank | 12.5 | 10.39 | -186.3 |
| 4 | 14.5 | 12.47 | -182.6 |
| 6 | 16.6 | 14.55 | -177.9 |
| 8 | 17.8 | 16.63 | -172.7 |
| 10 | 16.6 | 14.55 | -187.6 |
| 20 | 22.9 | 20.79 | -164.1 |
| 40 | 27.0 | 24.94 | -155.8 |

**Table (5): Electrochemical polarization parameters for the carbon steel in oilfield formation water containing NPPOE at concentration 10x$10^{-5}$ M in the absence and presence of 1 x $10^{-2}$ M halide salt at 40 °C.**

| Inh.Conc. (M) | R (Mpy) | $E_{corr,}$ (mV) | $I_{corr}$ (μAcm$^{-2}$) | $b_a$ (mVdec$^{-1}$) | $-b_c$ (mVdec$^{-1}$) | $R_p$ (KΩcm$^{-2}$) | IE (%) | θ |
|---|---|---|---|---|---|---|---|---|
| Blank | 22.49 | -693.9 | 49.03 | 121.2 | 243.3 | 0.7176 | - | - |
| 10x$10^{-5}$ | 8.572 | -608 | 18.69 | 140.3 | 165.2 | 1.527 | 61.2 | 0.612 |
| + KI | 1.538 | -495.9 | 3.354 | 73.63 | 120.5 | 4.815 | 93 | 0.930 |
| + KBr | 2.60 | -515.9 | 4.928 | 98.54 | 120.9 | 4.107 | 90 | 0.900 |
| + KCl | 2.751 | -539.2 | 5.998 | 90.21 | 137.9 | 3.188 | 87.8 | 0.878 |




**Nonylphenoxy poly (ethyleneoxy) ethanol (NPPOE)**

**Scheme (1)**

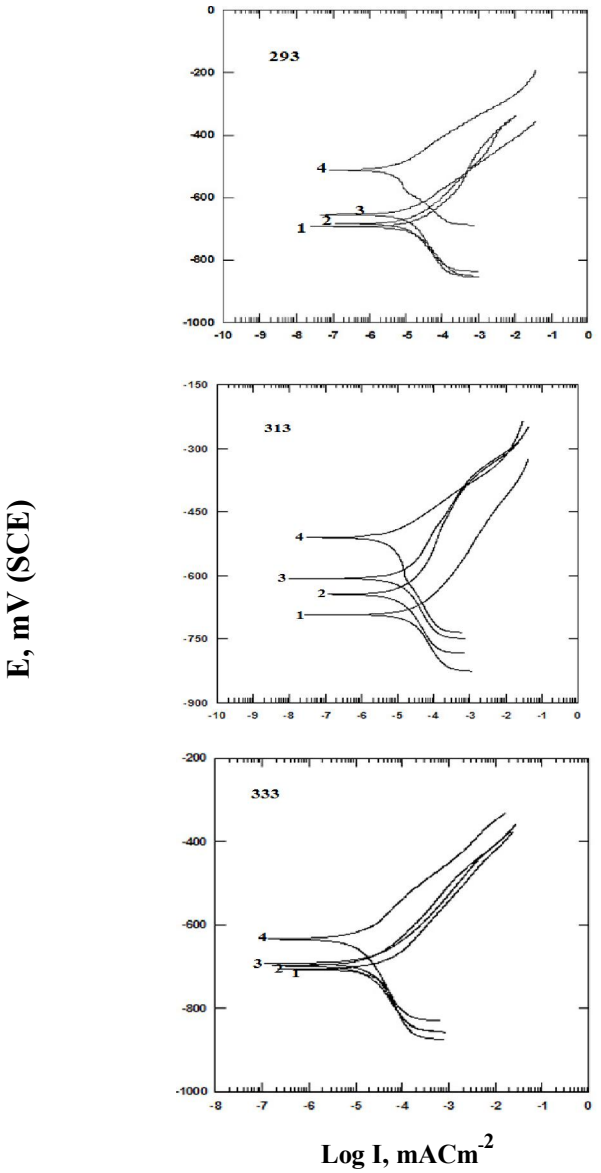



**Fig. (1)- Potentiodynamic polarization curves of carbon steel in oilfield formation water with different concentrations range of NPPOE at different temperatures: (1) blank, (2) $4x10^{-5}$, (3) $10x10^{-5}$ and (4) $40x10^{-5}$ M.**

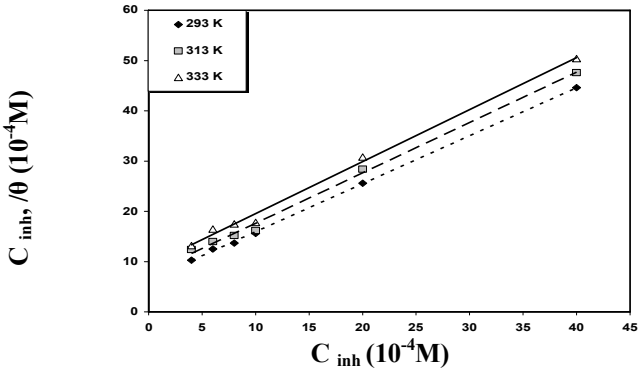



**Fig. (2)- Curves fitting of the corrosion data for carbon steel in oilfield formation waters in the presence of NPPOE according to Langmuir adsorption isotherm at different temperatures.**

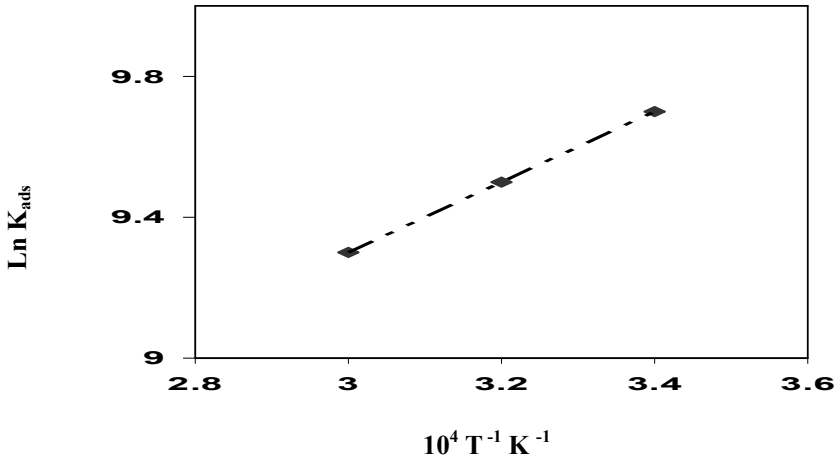
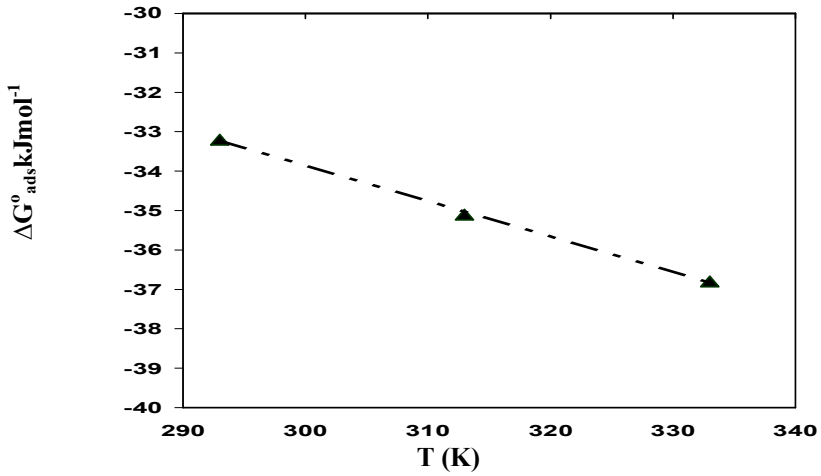



**Fig. (3)-Vant"Hoff plot for the carbon steel/NPPOE/oilfield formation waters.**




**Fig( 4). Variation of $\Delta G^0_{ads}$ versus T on carbon steel in oilfield formation water containing NPPOE inhibitor**

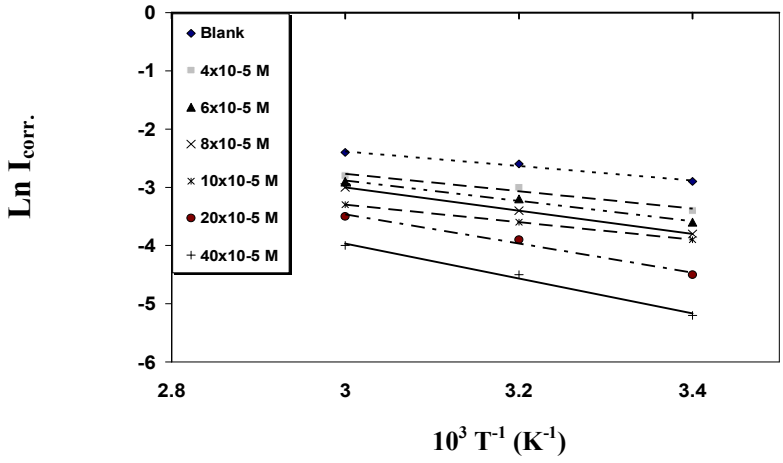



**Fig. (5)-Arrhenius plots related to the corrosion rate of carbon steel in oilfield formation waters in absence and presence of different concentrations of NPPOE at various temperatures.**

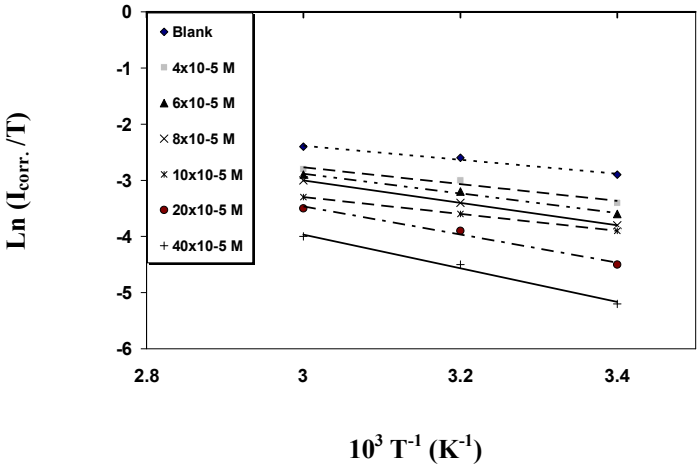



**Fig. (6)-Transition state plot of (Ln $I_{corr.}$/ T) versus 1/T at different concentrations of NPPOE inhibitor in oilfield formation water.**

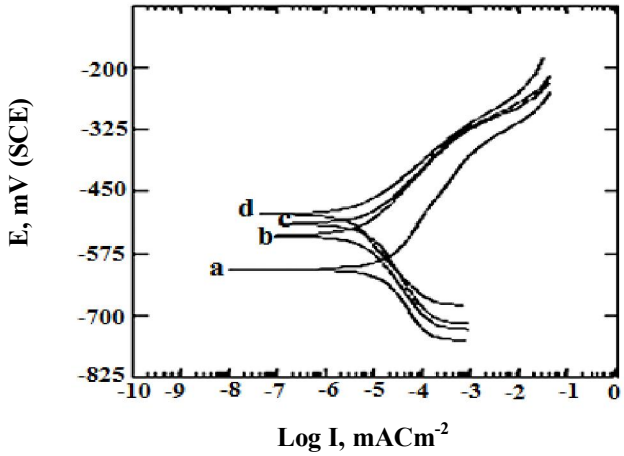



**Fig.(7)-Potentiodynamic polarization curves for carbon steel in oilfield formation water inhibited with NPPOE at concentration $10x10^{-5}$ M in absence and presence of halide salts at concentration $1x10^{-4}$ M at 40 °C, (a) blank, (b) KCl, (c) KBr and (d) KI.**

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# The Effect of Cognitive Behavioral Therapy Program on Insight and Nonadherence to Medication among Psychotic Patients in Psychiatric Hospital at Assiut Governorate

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**Abstract:** Cognitive–behavioral therapy for psychotic patients refers to a structured and time–limited approach to treat a variety of psychiatric disorders. The main goals are: to treat symptoms, to increase insight level and to reduce distress. **Aims of the study:** This study aimed to assess insight among psychotic patients, to determine the relationship between insight and nonadhering to medications and to study the effects of cognitive–behavioral therapy on the insight of psychotic patients. The study was carried out in the Psychiatric Mental Health Hospital, Ministry of Health at Assiut Governorate.. The study sample comprised 20 schizophrenic patients attending to psychiatric unit within a period of three months from October to December 2011, both sexes and agrees to participate in the study, aged from 18- 55 years for three months. Three tools were used for data collection, namely: Structured Questionnaire data sheet, Schedule for the Assessment of Insight (SAI- E) and Drug Attitude Inventory Scale (DAL- 30). **Results:** The main results yielded by the study proved that, 50% (10) of the studied group were single and 40 %(8) were married, the majority of studied group were illiterate, the highest percentage of insight was improved after application of cognitive – behavioral program (60%), and the highest percentage of adherence to medication were improved after application of program. **Conclusion:** cognitive – behavior therapy were effective in improvement level of insight and adherence to medication among psychotic patients. **Recommendation**: the study recommended to continually follow- up on the results of the study repeatedly reviewed the things of patients and the program should be simplified by using simpler language so as to be use to be with patient's with little education The number of the studied group most be increased.

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**Key Words**: Cognitive Behavioral Therapy, Insight, Nonadherence to Medication, Psychotic Patients.

## 1. Introduction

Insight is the awareness of self and acceptance of mental illness and the acceptance of need for treatment. Absence of insight is often described as a defense mechanism protecting the patient from the devastating realization of his or her illness [(1).]

**Baier and Urrage** [(2)] define insight as patient's recognition and understanding of his conditions. While **Sims,** [(3)] describe insight as a profoundly significant human capacity for mental " seeing with the mind's eye " and glimpsing what's going on below the surface as well as in the minds of other people. **Ghami** [(4)] defined lack of insight as the inability to recognize that one possesses a mental illness of that one is experiencing psychopathological symptoms and lack of awareness of need for treatment and lack of recognition of the social consequences.

One of the main problems in the treatment of psychotic patients is their lack of insight and awareness [(5).] Insight is a complex and construct that has various dimensions that are not strongly correlated such as insights into illness, symptoms and for treatment [(6).] Insight on the part of both patients and care taker or family members is thought of as an important factor influencing adherence to medication and treatment of psychotic patients [(7)].

Poor compliance or adherence is an important health care problem, which can result in reduced efficacy or failure of the recommended intervention with detrimental effects on the patient's health [(8, 9)]. Points out that noncompliance usually refer to patient's failure to follow health interventions as recommended by the health care provider. It also refers to the provider's failure to act according to practice guidelines or standards of care.

**Uribe** [(10)] reported that non compliant patients were more likely to be young, unmarried, and have longer disease duration and greater disease activity as assessed by the physician [(11)] showed that about 1.3 million adults with disabilities did not take their medications as prescribed because of cost and that more than half reported health problems as a result .

A number of factors that contribute to non adherence in chronic illness have been identified to improve adherence and better health outcomes for patients' .**Pinikahana** [(12)] explore the complexity of compliance in schizophrenia into factors such as illness factors as insight, symptoms, duration of illness, substance abuse, adverse effects of medication. Psychological factors such as health beliefs and social support. Treatment factors including the patient – physician relationship.

Cognitive – behavioral therapy for psychotic patients refers to a structured and time – limited approach to treat a variety of psychiatric disorders. The main goals are: to treat symptoms, to increase insight level and to reduce distress [13]. So, mental health nurses play an important role for guided information and knowledge for patients about the nature of mental illness, the severity of their illness and the goals of medication in treatment to be insightful and improving their medication adherence.

**Hypothesis:**

Insight level of psychotic patients who received cognitive behavioral therapy program increase than before the participation of the study.

**Aim of the study:**

To assess insight among psychotic patients, to determine the relationship between insight and nonadhering to medications, and to study the effects of cognitive–behavioral therapy on the insight of psychotic patients.

**2. Subjects and Methods:**

**Research Design:**

The design followed for this study is a Quesi Experimental study design.

**Setting:**

The study was conducted at the Psychiatric Mental Health Hospital, Ministry of Health at Assiut Governorate. The hospital is serving Assiut City and all Upper Egypt governorates.

**Subjects:**

Subjects of the study comprised 20 schizophrenic patients attending to psychiatric unit within a Period of three months from October to December 2011 both sexes and agree to participate in the study, aged from 18- 55 years for three months.

**Inclusion criteria:**

1. Patients was no danger to themselves or others (violence , suicide )
2. They were able to communicate.

**Exclusion criteria:**

Patients with mental retardation and drug addiction.

**Tools of the study:**

Three tools were used for data collection:

**1. Structured Questionnaire data sheet:**

This questionnaire schedule developed by the researchers, to assess demographic characteristics of the subjects; e.g., age, sex, residence, occupation, level of education, marital status, and diagnosis.

**2. Drug Attitude Inventory Scale (DAL- 30):**

This scale developed by **Hogen *et al.*** [14]. This scale will be used to measure subjective response to medication in an effort to obtain a more complete understanding of factors influencing medication compliance in psychiatric patients. The scale has 15 items that will be scored as true and 15 items that will be scored as false in the case of a fully compliant (positive subjective response). A correct answer to these items will be scored as plus one. An incorrect answer will be scored as minus one. The final score is the sum of the total of pluses and minus scores. A positive total score mean a positive subjective response (adhering). A negative total score means a negative subjective response (none adhering). This scale translated into Arabic and tested for validity and reliability by **Khalil and El – Hosany** [15].

**3. Schedule for the Assessment of Insight (SAI- E):**

This scale developed by **Kemp and David,** [16]. This scale consists of 3 items scored on a Likert scale of 0 (no insight) to 4 (full insight). The SAI assesses insight into three separate dimensions of insight: treatment compliance composed items no. 1, 2,3,4,5 and6 (0 to 2), recognition of illness composed of items no. 7 and 8 (0to 4), and relabeling of psychotic phenomena of item no. 9 (0 to 4). the total score measured by summed of three scored dimensions ,the patient has no insight when the total score ranged from 0 to 12 grades , while the patient have full or good insight when the total score ranged from 13 to 24 grades .

**Cognitive – Behavior Therapy Program**

Developed by the researcher to test the effectiveness of cognitive behavioral therapy program among psychotic patients. About five different methods of cognitive behavioral therapy as identifying automatic ideas, self monitoring, imagination, distraction technique, and idea's termination technique distributed on "4" sessions "2" sessions per week, each session ranged from 30 to 45 minutes.

**The Procedure:**

The investigator will interview the psychotic patients at Psychiatric & Mental Health Hospital at Assiut Governorate. All ethical considerations will be clarified to each patient before explanation of the nature of the study. The investigator will ask the patient about their sociodemographic data by using the first questionnaire to determine the sociodemographic classes for these patients.

The second step applied tool number 2 (**DAL- 30**) will be applied for each patient to measure subjective response to medication among those patients.

The third step applied tool number3 (**SAI- E**) will be applied for the same patients to assess the patient's insight. Then the cognitive – behavioral therapy program will be applied for each patient. Implementation of cognitive – behavioral therapy sessions includes: an orientation meeting was held with

patients to explain the aim of the cognitive – behavioral therapy sessions, the cognitive – behavioral therapy sessions were held for two days / week for a period of three weeks , every session was from 30 to 45 minutes, cognitive – behavior therapy sessions included different types of activities :

1- identifying automatic ideas
2- self monitoring
3- imagination
4- distraction technique
5- idea's termination technique

The program was applied for psychotic patients (schizophrenic) in Assiut Governorate, Psychiatric Mental Health Hospital, Ministry of Health, each patient interviewed individually. Firstly pre- test should be applied for each patient before applied the program and then immediately post- program implementation was evaluated to test their improvement of insight and adherence to medication. Data were collected in the period from October to December 2011

**Methods of data collection:**

1) Permission was obtained from the dean of the faculty of nursing –Assiut University directed to the director of the Psychiatric Mental Health Hospital, Ministry of Health, at Assiut Governorate.
2) The aim of the study was explained to patients before starting data collection. Patients were informed about what was done for them.
3) Each patient has been interviewed once on an individual basis at psychiatric unit.
4) Consent (verbal agreement) was taken from the patients who were reassured about the confidentiality of the obtained information to avoid misunderstanding and providing privacy for them.
5) The data were collected by the researchers during the period of three months from the first of October to the end of December 2011.
6) The patient was interviewed for about 30 – 45 minutes at one time.

**Statistical analysis**

The data were computerized and verified using the SPSS (statistic among package for social science) version 16 to perform tabulation and statistical analysis. Qualitative variables were described in frequency and percentages, statistical significance was considered at p – value <0.05.

**3. Results:**

Results of the present study showed that:

In the present study, found that equal number between study sample according to age <35, ≥35 and the range of Mean ±SD was 33.45±8.51 (17- 49 years). Equal number between male & female as regarding to sex. Regarding to marital status 50% (10) of the studied group were single and 40 %( 8) were married, while small number of them were divorced & widow (10%).

According to level of education the majority of studied group were illiterate, primary, preparatory and secondary education, (20%, 30%, 20%, 20%) respectively .As regard occupation about 25% of the studied group were not work, While 30% of them were worker & housewife. Nearly ¾ of the studied group were living in rural area, while 30% of them were living in urban area **(Table 1).**

Regarding to the level of insight among the studied group, the highest percentage of insight was improved after application of cognitive – behavioral program (60%). **(Table 2).**

**Table (3)** shows adherence and non – adherence to medication among the studied group, the highest percentage of adherence to medication were improved after application of program.

Regarding to the relation between insight and adherence to medication among the studied group, there were statistically significant differences between insight and adherence to medication were improved after implementation of cognitive – behavioral program **(Table 4)**

In relation between demographic characteristics of the studied group and insight , the highest mean were more in males, single, who were had basic education and lived in rural areas**(Table 5)**

In relation between sociodemographic data of the studied group and patient's attitude toward medication, there was no statistical significant difference between sociodemographic data and medication, regarding to age, marital status, level of education, and urban group **(Table 6)**

**Table (7)** shows the correlation between insight and patient's attitude toward medication, reported that there was no correlation between insight and medication before or after program.

**4. Discussion:**

The value of insight as a predictor of clinical outcome in patients with psychotic disorders has recently drawn increased attention, those who believe that insight influences treatment outcome and also improve insight through the use of psycho-educational program [(17).] Non-adherence is strongly associated with an increased risk of relapse [(18).] Many patients with psychosis are unaware of their disorder and symptoms. Moreover, insight is a clinical modulator of compliance with treatment and a good indicator of prognosis [(19)].

**Table (1): General characteristics of the studied group (no.20)**

| Items | No. (n= 20) | % |
|---|---|---|
| **Age: (years)** | | |
| < 35 | 10 | 50.0 |
| ≥ 35 | 10 | 50.0 |
| Mean ± SD (Range) | 33.45 ± 8.51 (17 – 49) | |
| **Sex:** | | |
| Male | 10 | 50.0 |
| Female | 10 | 50.0 |
| **Marital status:** | | |
| Single | 10 | 50.0 |
| Married | 8 | 40.0 |
| Divorced | 1 | 5.0 |
| Widow | 1 | 5.0 |
| **Level of education:** | | |
| Illiterate | 4 | 20.0 |
| Read and write | 1 | 5.0 |
| Primary | 6 | 30.0 |
| Preparatory | 4 | 20.0 |
| Secondary | 4 | 20.0 |
| University | 1 | 5.0 |
| **Occupation:** | | |
| Not work | 5 | 25.0 |
| Worker | 6 | 30.0 |
| Farmer | 3 | 15.0 |
| Housewife | 6 | 30.0 |
| **Residence:** | | |
| Urban | 6 | 30.0 |
| Rural | 14 | 70.0 |

**Table (2): level of Insight before and after implementation of cognitive behavioral therapy program among the studied group (no.20)**

| Items | Insight | | | |
|---|---|---|---|---|
| | Before (n= 20) | | After (n= 20) | |
| | No. | % | No. | % |
| **Poor** | 12 | 60.0 | 8 | 40.0 |
| **Good** | 8 | 40.0 | 12 | 60.0 |
| **P-value** | 0.206 | | | |

Chi-square test

**Table (3): Adherence to medication before and after implementation of cognitive behavioral therapy program among the studied group (no.20)**

| Items | Medication | | | |
|---|---|---|---|---|
| | Before (n= 20) | | After (n= 20) | |
| | No. | % | No. | % |
| **Non-adherence** | 8 | 40.0 | 3 | 15.0 |
| **Adherence** | 12 | 60.0 | 17 | 85.0 |
| **P-value** | 0.077 | | | |

Chi-square test

**Table (4): Relation between insight and adherence to medication before and after implementation of cognitive behavioral therapy program among the studied group**

| Items | Before (n= 20) | After (n= 20) | *P*-value |
|---|---|---|---|
| **Insight:** | | | |
| Mean ± SD | 10.40 ± 5.90 | 12.80 ± 5.61 | **0.010*** |
| Range | 0 – 19 | 2 – 20 | |
| **Medication:** | | | |
| Mean ± SD | 3.65 ± 9.51 | 9.15 ± 8.57 | **0.000*** |
| Range | -15 – 20 | -5 – 22 | |

Wilcoxon Signed Ranks Test *

Statistical significant difference (***P*** < 0.05)

**Table (5): Relation between demographic characteristics and insight among the studied group**

| Items | Mean ± SD | Range | *P*-value |
|---|---|---|---|
| **Age: (years)** | | | |
| < 35 | 10.40 ± 6.50 | 0 – 19 | 0.909 |
| ≥ 35 | 10.40 ± 5.58 | 3 – 18 | |
| **Sex:** | | | |
| Male | 12.30 ± 6.40 | 0 – 19 | 0.130 |
| Female | 8.50 ± 4.91 | 3 – 17 | |
| **Marital status:** | | | |
| Single | 11.30 ± 5.74 | 0 – 19 | 0.472 |
| Married | 9.50 ± 6.22 | 3 – 18 | |
| **Level of education:** | | | |
| Illiterate/ read & write | 7.60 ± 5.73 | 3 – 17 | 0.401• |
| Basic education | 12.10 ± 4.63 | 3 – 18 | |
| Secondary or higher | 9.80 ± 8.17 | 0 – 19 | |
| **Residence:** | | | |
| Urban | 8.33 ± 7.31 | 0 – 17 | 0.301 |
| Rural | 11.29 ± 5.24 | 3 – 19 | |

Mann-Whitney Test •Kruskal-Wallis Test

**Table (6): Relation between demographic characteristics and adherence to medication among the studied group**

| Items | Mean ± SD | Range | *P*-value |
|---|---|---|---|
| **Age: (years)** | | | |
| < 35 | 1.90 ± 9.94 | -15 – 14 | 0.382 |
| ≥ 35 | 5.40 ± 9.24 | -6 – 20 | |
| **Sex:** | | | |
| Male | 4.10 ± 10.75 | -15 – 16 | 0.790 |
| Female | 3.20 ± 8.65 | -6 – 20 | |
| **Marital status:** | | | |
| Single | 1.70 ± 9.45 | -15 – 16 | 0.518 |
| Married | 5.60 ± 9.65 | -6 – 20 | |
| **Level of education:** | | | |
| Illiterate/ read & write | 6.00 ± 10.95 | -6 – 20 | 0.965 |
| Basic education | 3.20 ± 8.07 | -6 – 16 | |
| Secondary or higher | 2.20 ± 12.38 | -15 – 14 | |
| **Residence:** | | | |
| Urban | 5.67 ± 8.71 | -4 – 14 | 0.561 |
| Rural | 2.79 ± 10.02 | -15 – 20 | |

Mann-Whitney Test •Kruskal-Wallis Test

**Table (7): Correlation between insight and adherence to medication before and after implementation of cognitive behavioral therapy program among the studied group**

| Items | Insight | | | |
|---|---|---|---|---|
| | Before | | After | |
| | *r*-value | *P*-value | *r*-value | *P*-value |
| **Medication** | 0.008 | 0.972 | - 0.119 | 0.617 |

According to demographic characteristics of the studied groups, it was found that most of the studied sample was 33.45± 8.51(17- 49 years) years. 50% of the present study was single, and 45%of them had basic education. These findings may be related to stigma of psychotic patients feeling not accepted from society, or schizophrenic patient unapplied to carry out responsibility of family or may be due to delusion & hallucination and lack of emotion. It was consistent with **Williams and Collins** [20] who reported that the Mean ±SD of the participants were 41.1±8.4 years. This may be related to schizophrenic disorder appear among adulthood and early years of development and the study contain 58 people 25 of them with a diagnoses of schizophrenia, and 33 people with a diagnosis of bipolar disorder, 31 of them were men and 27of them were women, and the most of the subjects were single or never to married (67%) and unemployed. In the study of **Jaime** [21] found that patients mean age was 45 years with arrange between 25 and 63 and the majority of patients was males (60%). **Fred,** [22] stated that non – adherent patients were more likely to be young, unmarried and lived in rural areas. Also in the study of **Uribe,** [10] reported that non- compliant patients were more likely to be young and unmarried.

The present study showed that effectiveness of cognitive- behavioral therapy on the awareness of the illness of patients who entered the program as being higher than pre- program levels, which supports the hypothesis and consistent with the study of **Garety and Kuipers** [23] who reported that during participated six schizophrenic patients in the program at Jitavj Khonkaen Rajanakarindra Hospital, the program emphasizing the improvement of patients insight. Also, in the results of **Turkington and Turner,** [24] that studied and evaluated the result of cognitive – behavioral therapy in an experimental group of schizophrenic patients, found that patients who received the brief cognitive – behavioral therapy showed increased insight. These results agree with the present study that the level of insight was improved after application of cognitive – behavioral therapy.

**Pinikahana *et al.,*** [12] explore the complexity of compliance in schizophrenic patients , they review socio-demographic characteristics of these patients , including age , gender , socioeconomic status, illness factors such as insight , symptoms , duration of illness ..e.t.c .the authors conclude that these factors provide important information to guide the caregiver (physician and mental health nurse) in facilitating patient compliance. The present study found that there was no statistical significant difference between sociodemographic data and attitude of patients of studied group toward medication. The present study was supported by **Dooulout *et al.,*** [25] who found that the association between measures of medication adherence was not modified after adjustment of demographic characteristics (age, gender, educational level, occupational status and marital status). That was related to stigma of mental illness and psychotropic medication or lack of education and importance of medication and poor insight. Also in the study of **McPherson *et al.,*** [26] during discuss non-compliance with medical follow – up after pediatric intensive care, found that no socioeconomic or demographic risk factors were identified for non-compliant patients.

The present study reported that there was no correlation between insight and adherence of medication

before and after program. This may be related to small sample or low level of education or stigma a round psychotic disorder and medication or attitude of nursing staff and lack of social support system. This finding consistent with the study of **Cheng,** [18] who found that there was no significant correlation was found between insight and medication adherence cross- sectionally or prospectively among the subjects with schizophrenia. While **Lincoln *et al.*,** [27] who reported that fifteen cross - sectional studies fulfilled there selection criteria, ninety of them found there was an association between insight and adherence to medication and the majority of the studies speak fore a clear association of insight and treatment adherence.

**Conclusion**:

Based upon the study results, it is concluded that the majority of the studied sample were, single, had basic education and lived in rural areas and the cognitive – behavioral therapy were effective in improving patient's insight and adherence to medication.

**Recommendation:**

In the light of the study findings, it is recommended to:

1- Continually follow- up on the results of the study repeatedly reviewed the things of patients.
2- During application of program , many patients were difficult understand some activities (procedures) as identifying automatic ideas and imagination , so the program should be simplified by using simpler language so as to be use to be with patient's with little education.
3- Increase the period of time during application of program.
4- Applied of the study with large number of participant

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# Experimental Infection of Tenacibaculosis and a Trial for Treatment by Plant Extract Carvacrol in Surge Wrasses Fish (*Thalassoma Purpureum*)

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**Abstract:** The experimental infection of surge wrasses fish by *Tenacibaculum maritimum* was successfully conducted through immersion bath for 18hrs in $1.5x10^6$ suspension, the infected fish exhibited skin ulcers, stomatitis, tail rot, signs of respiratory distress as gasping and accumulation at air source site in association with 60% mortality. Carvacrol is a major compound of oregano and thyme and has antimicrobial activity against wide range of microorganismes. The *in vitro* susceptibility assay proved strong effect of carvacrol on *T. maritimum.* 100ppm of both carvacrol and its precursor cymene for 14 days as food additives controlled the tenacibaculosis in surge wrasses fish and no clinical signs or mortality could be recorded in the treated fish. 50ppm of them prevented the disease clinical signs and reduced mortality to 10%.

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**Key words**: Tenacibaculosis, *T. maritimum,* treatment, Surge wrasses fish, carvacrol, cymene.

## 1. Introduction

Surge wrasses fish (*Thalassoma purpureum*) is a valuable coral reef living fish in the red sea and used as food and ornamental fish. Marine Tenacibaculosis is a serious bacterial disease affecting a great variety of marine fish especially cultured species **(Toranzo *et al.*, 2005)**. It caused massive mortalities and severe economic losses in marine fish cultures worldwide including Japan, Scotland, Spain, France and North America (**Wakabayashi *et al.*, 1986; Bernardet *et al.*, 1990; Alsina and Blanch 1993; Bernardet *et al.*,1994; Ostland *et al.*,1999**). It is caused by *Tenacibaculum maritimum* (formerly *Flexibacter maritimus*) and this pathogen directly and primarily attacks skin, mouth, fins and tail of fish, causing severe necrotic and ulcerative lesions on the body surface (**Toranzo *et al.*, 2005**).

Tenacibaculosis has been reported among wide range of marine fish species including sole, *Solea solea* (L.) (**Bernardet *et al.*, 1990**); Senegalese sole (*Solea senegalensis*) (**Cepeda and Santos, 2002**); Japanese flounder (*Paralichthys olivaceous*) (**Baxa *et al.*, 1986**); turbot (*Psetta maxima*) (**Avendaño-Herrera *et al.*, 2004a**). Also, it is recorded in Atlantic salmon (*Salmo salar* L.,), Rainbow trout (*Oncorhynchus mykiss*), Striped trumpeter (*Latris lineata*), Greenback flounder (*Rhombosolea tapirina*) (**Soltani *et al.*, 1996; Handlinger *et al.*, 1997**), sea bream and sea bass (**Toranzo *et al.*, 2005)** and Picasso tigger fish (*rhinecanthus assasi*) and Black damsel fish (*Neoglyphieodon meles*) (**Abd El-Galil and Hasheim, 2012**).

Up to now, most treatments proposed for the tenacibaculosis outbreaks are based on the administration of antibiotics through feed. Oxytetracycline, amoxycillin, trimethoprim and enrofloxacin are an effective antimicrobial therapy against *T. maritimum*, (**Soltani *et al.*, 1995; Avendaño-Herrera *et al.*, 2008**). The uses of antibiotics in aquaculture may introduce potential hazards to public health and to the environment by the emergence of drug-resistant *T. maritimum* within the population and further diminished the effect of chemotherapy (**Tsoumas *et al.*, 1989; Avendaño-Herrera *et al.*, 2008**). Furthermore, the normal microbial flora in the digestive tract, which is beneficial to fish are also killed or inhibited by oral antibiotic chemotherapy (**Gerald and Jane, 1966; Sugita *et al.*, 1990**).

Plant extract carvacrol had strong antimicrobial activities against both gram-positive and gram-negative bacteria and was generally recognized as safe by the FDA (**Davidson and Saxton, 2011**). Carvacrol had antimicrobial activity against wide range of fish pathogenic bacteria such as *Bacillus cereus* (**UItee *et al.*, 2000**) *Salmonella typhimurium* (**Kim *et al.*, 2006** and **Tohamy, 2006**) *Staphylococcus* and *E. coli* (**Gholam and Mohammad, 2007**) *Streptococcus* (**Botelho *et al.*, 2007**), *A. hydrophila* (**Zheng *et al.*, 2009**) and *E. tarda* (**Rattanachaikunsopon and Phumkhachorn, 2010**). Carvacrol, but not cymene was able to inhibit many bacterial strains and the synergistic effect between carvacrol and cymene against drug resistant bacterial strains was reported **UItee *et al.* (2000)**and **Rattanachaikunsopon and Phumkhachorn (2010).**

This study was designed to investigate the susceptibility of Surge wrasses fish to tenacibaculosis

and the efficacy of the plant extract carvacrol in combination with its precursor cymene to control the disease.

## 2. Materials and Methods

### Fish

One hundreds of apparently healthy Surge wrasses fish were collected from the red sea coral reef and transported alive to the indoor aquaria of The National Institute of Oceanography and Fisheries (NIOF) at Hurghada, the fish were acclimated and accustomed to the commercial fish ration containing 25% protein (ZooControl Company, Ismailia desert road, Egypt) for two weeks in indoor aquaria and used in this experiments**.**

### *Tenacibaculum maritimum* inoculum

*T. maritimum* strain was obtained from previous work (**Abd El-Galil and Hashiem, 2012**) and cultivated on plates of Flexibacter maritimus medium (FMM) (**Pazos *et al.,* 1996**), Pure colonies of the *T. maritimum* isolates were picked up and the strain was passed in small group of bird wrasses fish for reactivation, and reisolated and identified again then used for other studies.

### Experimental infection

Twenty of the acclimated Surge wrasses fish were subdivided into 2 equal groups each of 10 fish and each group was reared in a separate aquarium. The fish of the first group were experimentally infected by *T. maritimum* suspension containing $1.5x10^6$ cell $mL^{-1}$ in a bath immersion for 18 hrs (**Avendaño-Herrera *et al.,* 2006a**). The second group was submitted to the same procedure without bacteria and used as control. Each fish group was preserved separately at water temperature 24±2°C and observed for 14days, the clinical signs and numbers of dead fish were recorded.

### Determination of median lethal dose (LD50)

Fifty of the acclimated Surge wrasses fish were subdivided into five groups each of 10 fish and overnight cultures of *T. maritimum* were adjusted to densities $1.5\times10^6$, $1.5\times10^5$, $1.5\times10^4$ and $1.5\times10^3$. The 1st, 2nd, 3rd and 4th fish groups were subjected to 18hrs immersion bath in the previous dilution respectively and the 5th group was used as control. The five fish groups were closely observed for 2 weeks. Mortalities and clinical signs were recorded daily and the internal organs (Livers and kidneys) were aseptically streaked on FMM for *T. maritimum* reisolation.

**Table (1):** Determination of median lethal dose ($LD_{50}$) of ***T. maritinum***

| Fish groups | No. of fish | Dose/fish | Route of injection |
|---|---|---|---|
| Group 1 | 10 | $1.5\times 10^6$ | Immersion bath for 18hrs |
| Group 2 | 10 | $1.5\times 10^5$ | |
| Group 3 | 10 | $1.5\times 10^4$ | |
| Group 4 | 10 | $1.5\times 10^3$ | |
| Group 4 (control) | 10 | ----- | |

### Susceptibility assay

Sensitivity of *T. maritimum* to combination of carvacrol and cymene was evaluated by agar diffusion susceptibility test on FMM plates, which was prepared using seawater as diluents (Pazos *et al*., 1996). 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10ppm of both carvacrol and cymene (mixed solution) were tested. Bacterial suspension turbidity was prepared, equalized and matched with the MacFarland 0.5 standard, this suspension then spread over a FMM plates and left for minutes to dry then by sterile glass pipette four wells (3mm Θ) were made in each plate. 100µL of a single mixed solution dilution was added to a single well and each plate had control well (had sterile saline). The plates were incubated at 25°C for 72hrs and the diameter of inhibition zones (area at which no growth was visible) were read at right angles by measuring to the nearest millimeter.

### Medicated fish diets

The fish diets were prepared according to attanachaikunsopon and Phumkhachorn (2010).

Diet-1:The commercial fish ration was supplemented with 100 ppm of both carvacrol and cymene.

Diet–2:The commercial fish ration was supplemented with50ppm of both carvacrol and cymene.

Diet-3: The commercial fish ration was used as control diet and prepared by the same process without carvacrol and cymene additives.

### Treatment trial

Thirty acclimated Surge wrasses fish were subdivided into 3 equal groups each of 10 fish and each group was reared in a separate aquarium. The three groups were fasted for 24hrs and experimentally infected with *T. maritimum* suspension containing $1.5x10^5$ cell $mL^{-1}$ in a bath immersion for 18 hrs (Avendaño-Herrera, *et al.,* 2006a). 12hrs later, the fish of 1st group were fed on diet-1, the 2nd group were fed on diet-2 and the 3rd group were fed on diet-3 (control). Each fish group was preserved in glass aquarium at water temperature 24±2°C, fed at feeding rate 3% of its body weight daily. During the observation period

(14days), the clinical signs and numbers of dead fish were recorded.

## 3. Results

### Experimental infection

The experimentally infected Surge wrasses fish showed lesions similar to those of naturally infected fish such as off food, lethargy, skin hemorrhagic ulcers (Photos 1&2), tail rot (Photo -3), ulcerated mouth (stomatitis) (Photo -4), in addition to respiratory distress as gasping and accumulation at the air source. By the end of observation time (14 days) the mortality of the experimentally infected fish reached 60% comparing to zero % mortality in the control group. *T. maritimum* could be reisolated from the experimentally infected fish.

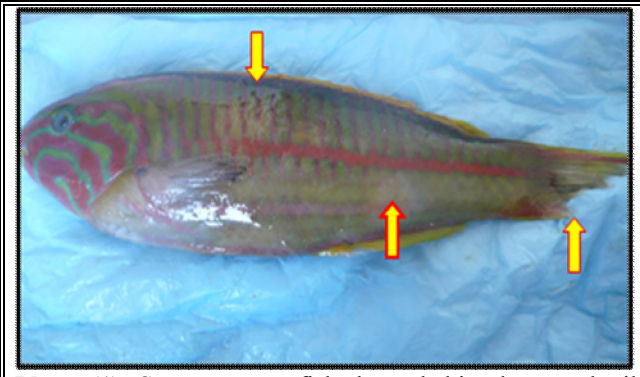

**Photo-(1):** Surge wrasses fish showed skin ulcers and tail rot

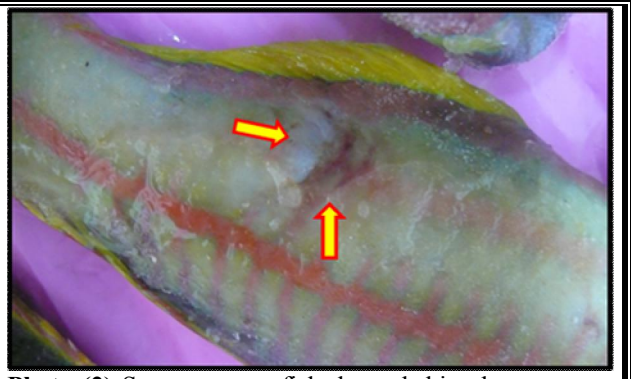

**Photo-(2):**Surge wrasses fish showed skin ulcer

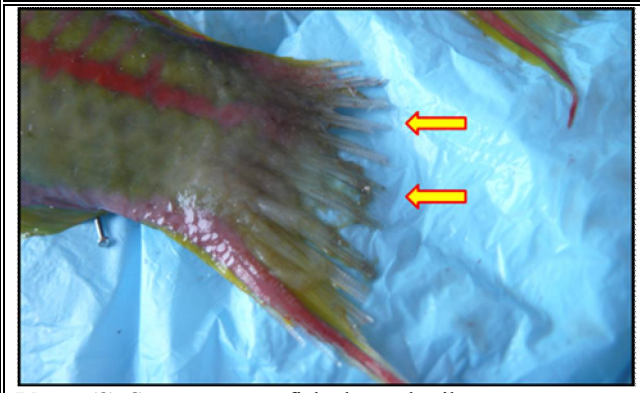

**Photo (3):**Surge wrasses fish showed tail rot

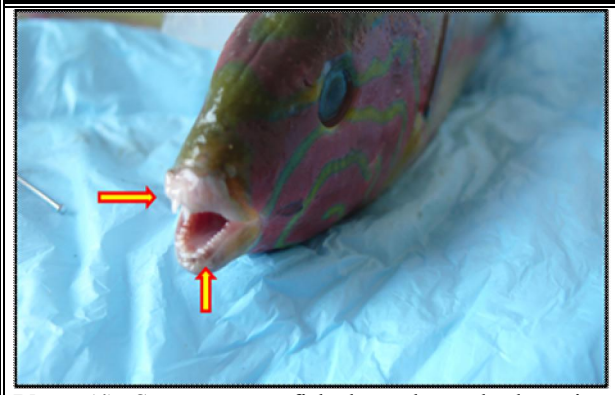

**Photo (4):** Surge wrasses fish showed mouth ulceration (stomatitis)

### Median lethal dose ($LD_{50}$)

The mortality of the experimentally infected Surge wrasses fish was reported for 2 weeks after immersion bath infection with different concentrations of *T. maritinum*. The fish death occurred during the 1st week of the experiment. The $LD_{50}$ of *T. maritinum* for Surge wrasses fish was $1.5 \times 10^5$ $CFUmL^{-1}$. (Table 2).

**Table (2):** Showed the median lethal dose ($LD_{50}$) of *T. maritinum* of Surge wrasses fish

| Fish group (10 fish for each) | Bath conc.$ml^{-1}$ | No. of dead fish / day | | | | | | | | | | Total number of dead Fish | Mortality rate % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 to 14 | | |
| Group 1 | $1.5 \times 10^6$ | 2 | 2 | 1 | - | - | 1 | - | - | - | - | 6 | 60 |
| Group 2 | $1.5 \times 10^5$ | 2 | 1 | 1 | - | 1 | - | - | - | - | - | 5 | 50 |
| Group 3 | $1.5 \times 10^4$ | 1 | 1 | - | - | - | 1 | - | - | - | - | 3 | 30 |
| Group 3 | $1.5 \times 10^3$ | 1 | 1 | - | - | - | - | - | - | - | - | 2 | 20 |
| Control group | No | - | - | - | - | - | - | - | - | - | - | - | - |

**Susceptibility assay**

The results of the agar diffusion test obtained for different carvacrol and cymene concentrations against *T. maritimum* strain demonstrated that there were some variations in the zones size produced by different concentration. Among the tenth concentrations evaluated the largest inhibition zones diameter were detected for the 10, 9, 8, 7, 6 and 5 ppm and they were 31, 30, 29, 29, 28 and 28 mm respectively and the other concentrations (4, 3, 2 and 1 ppm) gave narrower inhibition zones (20, 13, 7, 4 mm, respectively) comparing with 0 mm inhibition zone around the control well (Table - 3).

**Table (3):** The susceptibility of *T. maritimum* to different concentrations of carvacrol and cymene combination

| Carvacrol and cymene concentration (ppm) | Inhibition zone (mm) |
|---|---|
| 0 (control) | 0 |
| 1 | 4 |
| 2 | 7 |
| 3 | 13 |
| 4 | 20 |
| 5 | 28 |
| 6 | 28 |
| 7 | 29 |
| 8 | 29 |
| 9 | 30 |
| 10 | 31 |

**Treatment trial**

No clinical signs and mortality could be reported among the fish of the 1st group and the fish were active with good appetite and appearance. No clinical signs could be observed on the fish of the 2nd group but 10% mortality was recorded between them. On the other hand, off food, lethargy, hemorrhagic ulcers on the skin, ulcerated mouth, tail rot and 50% mortality were reported among the fish of the 3rd group (control).

**4. Discussion**

This study reported the susceptibility of Surge wrasses fish to experimental infection with the marine pathogen *T. maritimum* which was isolated in previous study from the Picasso tigger fish (*Rhinecanthus assasi*) and Black damsel fish (*Neoglyphieodon meles*) of red sea at Hurghada, Egypt (**Abd El-Galil and Hasheim, 2012**). The infected fish showed the classical clinical signs of tenacibaculosis such as off food, leathergic, skin hemorrhagic ulcers, ulcerated mouth (stomatitis) and tail rot, in addition to respiratory distress in the form gasping and accumulation at the air source site and these signs were associated with 60% comparing with no clinical signs and 0 % mortality in the control group. Similar lesions were noticed by **Baxa *et al.* (1986); Santos *et al.* (1999); Suzuki *et al.* (2001); Toranzo *et al.* (2005); López, *et al.* (2009); Abd El-Galil and Hashiem (2012)** in many different marine fish species**.**

The susceptibility of *T. maritimum* to combination of the plant extracts carvacrol and its precursor cymene was investigated in the laboratory by using agar diffusion test. The most effective concentrations were 10, 9, 8, 7, 6 and 5 ppm which reported the largest inhibition zone diameter documenting the antibacterial effects of carvacrol and cymene mixture solution on the *T. maritimum* and these results were confirmed by **Davidson and Saxton (2011)** who stated that carvacrol had strong antimicrobial activities against gram positive and gram-negative bacteria, **Zheng *et al.* (2009)** who pointed out the susceptibility of *A. hydrophila* in channel catfish to the plant extract carvacrol and **Rattanachaikunsopon** and **Phumkhachorn (2010)** who reported the susceptibility *E. tarda* in *O. niloticus* to carvacrol.

The treatment trial of tenacibaculosis in Surge wrasses fish was achieved by using the plant extracts carvacrol in combination with its precursor cymene as fish food additives for 14 days **(Rattanachaikunsopon and Phumkhachorn, 2010)**. Equal amounts (100ppm) of both carvacrol and cymene completely controlled the disease where no clinical signs or mortality could be reported between the treated fish, in addition to, the treated fish were alert (react well to the stimuli) and had good appetite and appearance. 50ppm of them reduced the mortality to 10% and no clinical signs in the treated fish could be noticed. On the other hand, typical clinical signs and 50% mortality were detected in the control infected fish group. These findings cleared out the efficacy of carvacrol and its precursor cymene as tenacibaculosis treatment and these finding were confirmed by Ultee ***et al.* (1998); Zheng *et al.* (2009; Rattanachaikunsopon and Phumkhachorn (2010);** who reported antimicrobial activity of carvacrol against *A. hydrophila, B. citrus* and *E. tarda* and the synergistic action between carvacrol and cymene.

In conclusion, the marine surge wrasses fish is susceptible to tenacibaculosis. The carvacrol in combination with its precursor cymene is effective treatment for tenacibaculosis in surge wrasses fish at 100ppm of each for 14 days as food additives**.** Further studies are required for the treatment duration and dose reduction.

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# Efficacy of Ginger Extract (*Zingiber Officinale* ) and Gamma Irradiation for Quality and Shelf-Stability of Processed Frozen Beef Sausage

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**Abstract:** The present work deal with improving safety of sausages besides introducing trials for decreasing the microbial load without affecting on sensory properties. Survey local processed sausages samples from eleven local markets proved high contamination with microbes as *Escherichia coli(19.71%), Listeria monocytogene (18.82%), Salmonella* (16.47%), Lactobacilli *(14.11%)* and *Staphylococcus aureus* besides total molds (17.94% ). Sausages beef was prepared with recommended raw materials containing fresh ginger extract (GEX) at two concentration (0.5%, 1.0%) besides using γ-irradiation of at 3.0 kGy and 5.0kGy to study the efficiency of these treatment on the microbiological, chemical and sensory characters during frozen storage (90 days). Using irradiation and GEX (1.0%) treatments were sufficient to keep samples even 90 days with safe levels of microbes but not eliminated completely.The values of Thiobarbituric Acid Reactive Substance (TBARS) were less than 2 at zero time but started increased gradually during storage. After two months, most of treatments increased 2 values of TBARS except Ginger extract (1.0%), which was the best treatments even end of storage (90 days of frozen storage). A linear relationship resulted between storage period and TBARS of treated samples with high significant values of coefficient ($R^2$). Irradiation and untreated samples contained high values more than 2 at end of storage. According ,these data GEX (1.0%) was the best treatment to keep samples with good quality rancidity free even 90 days during frozen storage, whereas γ-irradiation increased rancidity values of TBARS rapidly comparing with control samples during frozen storage. Furthermore, sensory properties were more affected with TBARS changes, which were in parallel with the results of sensory evaluation, especially at end of storage. The obtained results showed that it is possible to produce safe and high-quality fresh sausage using natural antioxidants source as GEX 1.0% to improve the quality and stability of frozen sausages .

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**Key words:** ginger, Sausages, Radiation, sensory properties, frozen storage, Microbiological character.

## 1. Introduction

Foodborne pathogens have been estimated to cause >6 million illnesses and approximately 9000 deaths each year (Mead *et al.,* 1999). Bacterial pathogens contribute in more than 60% of the foodborne illnesses that lead to hospitalization and account for nearly two-thirds of the estimated number of foodborne pathogen-related deaths especially through beef or beef products. *Salmonella spp., Listeria spp., Campylobacter spp Escherichia coli* caused various foodborne illness-related hospitalizations and deaths (Mead *et al.,* 1999).Recently, there has been an increase in consumer awareness regarding the use of chemical additives in food and food products (Tiwari *et al*., 2009). This has resulted in an increase in research on natural additives, such as using plant and animal derivatives (Ennajar *et al.,* 2009).

Contamination of meat or meat products with pathogenic microbes are still a major problem in the World, even in well-developed countries (Anonymous, 2002, Pohlman, 2006).The development of new antibiotics and plant based antimicrobial compounds are effective against the resistant organisms. Ginger a common substance found increasingly in the diets of the global population, have known antibacterial effects and are commonly used together in teas. It has strong antibacterial and antifungal properties. *In vitro* studies have shown that active constituents of ginger inhibit multiplication of colon bacteria. It inhibits the growth of *Escherichia coli, Proteus sp, Staphylococci, Streptococci and Salmonella* (Gugnani and Ezenwanze, 1985). The ginger extract has antimicrobial action at levels equivalent to 2000 mg/ml of the spice. Ginger inhibits *Apergillus,* a fungus known for production of aflatoxin, a carcinogen (Nanir and Kadu, 1987; Meena, 1992). Fresh ginger juice showed inhibitory action against *A.niger, S.cerevisiae, Mycoderma SPP. and L. acidophilus* at 4, 10, 12 and 14% respectively at ambient temperatures (Meena,1992). Many studies have implicated *Staphylococcus aureus and*

*Streptococcus pyogene* as leading causative agents of both community and hospital acquire infections (Amita *et al.,* 2003).

Irradiation of food became more easily and application on a commercial scale on more than 40 countries for decontamination purposes especially to control pathogens, spoilage microorganisms, and pests without compromising the nutritional and sensory properties of foods refrigerated or frozen uncooked meat, meat byproducts, and certain other meat food products to reduce concentrations of foodborne pathogens and to extend shelf life (U.S. Department of Agriculture, Food Safety and Inspection Service, 1999). Such treatments may lead to the development of off-odors and can affect flavor. But low-dose, can solve that problem ,US Food and Drug Administration (FDA) permitted irradiation up to 4.5 kGy for refrigerated and 7.0 kGy for frozen red meats ,irradiation of processed meats has not yet been approved (U.S. FDA, 1997; Molins *et al.,* 2001).

The purpose of this study was to evaluate alternative natural preservatives in producing natural sausages, as ginger rhizome extracts comparing using recommended low doses of γ- irradiation to reduce the effect of fat oxidation, off-flavor to get high quality of sausages for storage frozen long time with high quality .Besides, the evaluation of the consumer acceptance, evaluation the quality ,quantity microbe load to avoid the microbial contaminated pathogen which are present extremely in located samples.

## 2. Material and Methods

**A-Survey samples from local markets**:

**Sampling :**

Eleven ready samples of sausage were purchased from local stores in Egypt produced from different companies. Sausage samples were chosen randomly and within validity date and stored at -7°C until use for analysis.

**B-Preparation of beef sausage:**

**1-Meat source:**

Frozen beef lean trim (local markets). Samples were thawed at 4 to 5°c for 4 hours, and then visible bone and connective tissue were removed. Samples were cut separately into small pieces before processing into value added products.

**2-Spices mixture:**

Spices were obtained from local markets from Giza, Egypt. Each spice was powdered in the laboratory in an electric mill. Spices mixture was prepared according to El-Dashlouty (1978) as shown in Table 1. As previously reported by Moawad and Hameida (2002). Replacement of lean trim by 20% organs in beef sausage was in this study, such percentage achieved the best chemical, physical, functional and sensory properties. Beef lean trim (as seen in table 1) were minced twice with 10% water as ice flakes, aiming to keep the mixture smooth as well as to minimize temperature rise and microbial growth during shopping. The other ingredient in Table 1 were then added and mixed together, then meat mixture was ground for 10 minutes using a meat grinder. The obtained emulation was than stuffed into previously cleaned and prepared natural mutton casings. All sausages were packed in polyethylene bags, placed in cooler 4 to 5°c for 6 hours then part of sausage was examined (zero time analysis), while the rest of samples were frozen at -20°c for different time intervals up to 90 days before analysis. The total fat in tested samples were 16 %.

**Table (1)Constituents of beef sausages and spices mixture**

| Ingredient | g/kg | Percent(%) |
|---|---|---|
| Beef lean | 680 | 68 |
| Beef fat | 150 | 15 |
| Ice | 100 | 10 |
| Sodium chloride | 18 | 1.8 |
| Skimmed milk | 43 | 4.3 |
| Powdered rusk | 0.4 | 0.04 |
| Sodium tripolyphosphate | 11 | 1.1 |
| Fresh garlic | 0.3 | 0.03 |
| Sodium glutamate | 1.0 | 0.1 |
| Ascorbic acid | 1.0 | 0.10 |
| Powdered spices mixture | 9.3 | 0.93* |
| Total | 1000 | 100 |

* Powdered spices mixture{fennel(59.76%),coriander(27.99%),cubeb(3.42%),black pepper (3.42%),clove(3.42%),laurel leaves(1.99%)}.

**C- Preparation of ginger extract:**

Ginger was obtained from retail spice seller in Saudi Arabia Kingdom (KSA). The taxonomic identification was performed; the outer covering was peeled off. 20 g of sample was kept in closed containers after being chopped into small pieces. For the preparation of extract, the method as reported by Mohsen and Ammar (2009) was used for this purpose. Ginger rhizomes were minced to a size of 1 mm. ,then extracted at a relation 10:1 using water: Extraction was approved out using a shaking incubator at room temperature for 24 hours, followed by filtration through Whatman No.1 filter paper. The residues were re-extracted in the same method and the two filtrates were combined. The extract was concentrated using a rotary evaporator (BUCHI-Rota vapor R-205 Switzerland) at 55ºC to near dryness (Mohsen and Ammar, 2009). The final extract contained %25 TS .Two concentrations were used as 5% and 10 % by volume respectively.

**D-Microbiological analysis**

25g of each sample (2 replicates) were homogenized in 225 ml of sterile peptone saline (1 g of peptone and 9 g of NaCl per liter water). After shaking, the suspension was serially diluted in triplicate (1:10) in peptone saline, and 1 ml dilutions were inoculated on MacConkey Agar (MCA) to obtain the *E. coli* count, Baird-Parker Agar (BPA) for the determination of *Staphylococcus aureus*, Brilliant Green Agar (BGA) for the determination of *Salmonella typhimurium*, Columbia Agar Base (CAB) for the determination of *Listeria monocytogenes* and finally Potato Dextrose Agar (PDA) for the determination of total moulds and for LAB. Plates were incubated for 48 hrs at 37°C for pathogenic bacteria. and for 5 days at 25°C, for moulds.. Colonies growth was calculated. Selected and Processed sausage samples were tested for microbiological examinations according to ICMSF (1996). Samples were examined for total fungal count, *Staphylococcus aureus, E coli, Listeria monocytogenes and Salmonella spp*. count (CFU/g.), according to American Public Health Association (APHA, 1992).

**E- Preliminary general chemical analysis:**

Proximate analysis of sausage were measured for untreated samples by the methods of AOAC (1995), results were expressed as moisture %, protein %, fat % and ash % contents. Feder Value was calculated as moisture/ protein ratio, according to Pearson (1981). Whereas, all tested samples were analysis for lipid oxidation was assed by TBA methods of Vyncke (1975). Thiobarbituric acid reactive substances (TBARS) Values were expressed as mg MA/kg sample.

**D-Sensory evaluation:**

For sensory analysis, panelists were recruited based on interest and availability .All of the twenty panelists at NRC had experience in sensory testing. Group sessions were held to orient the panelists and determine the terms to include on the ballot for sensory testing of cooked sausage. A complete-block design was used for panel sessions and samples were presented in a random order independently determined for each panelist. For data analysis, categories were assigned values from one to nine (none = one, extreme = 9). Data was subjected to analysis of variance, with treatment and panelist as the main effects. When main effects were significant at $P<0.05$, treatment means were compared by using Duncan test and treated samples were labeled with alphabetic letters .Treated and non treated beef burger samples were evaluated for organoleptic properties by a ten qualified different member sensory panel for the following attributes: aroma, texture, colour, taste and overall according to the method of Wattsg *et al.* (1989).

**F- Irradiation process and storage conditions:**

The irradiation process was carried out at National Centre for Radiation Research &Technology (NCRRT). Some prepared sausage samples were irradiated with γ-rays with different doses 3.0 and 5.0 k Gy. The irradiation process were performed at cold temperature (3-5 $^0$C) by using $Co^{60}$ γ-source with dose rate of ~ 3.52 kGy.h $^{-1}$. The irradiation source had been calibrated by the National Physical Laboratory (NPL, Teddington, UK) using the dichromate dosimetry system. All the treated samples were store at -18$^0$C even end of storage in three replicates. At intervals periods, samples were used directly from frozen storage .

**G-Statistical analyses:**

All data are expressed as mean values ± standard deviation (S.D).Statistical differences between experimental groups were assessed by analysis of variance (ANOVA), using the COSTAT software package (Cohort Software, CA, USA). The main values were compared with LSD test ($P < 0.05$).

**3. Results and Discussion**

**Survey the natural contamination levels in local produced sausages**:

The microbiological analysis of eleven collected fresh samples randomly from local markets (store -7$^0$C) during validity period in Egypt proved high load of contaminated pathogenic bacteria and moulds as in Table (2) and fig.(1). The major types of microorganisms were *Escherichia coli (19.71%), Listeria monocytogene (18.82%), Salmonella* (16.47%), Lactobacilli *(14.11%)* and *Staphylococcus aureus*

(12.94%) besides the total molds 17.94%. Same findings were obtained by Farber *et al*. (1988) and Eisel *et al.* (1997).

*Escherichia coli* occupied the first one with high percentages ,whereas, the values of contamination was( 6.7 Log cfu/g±3.90). Its often use as hygiene indicators of foods of animal origin. There is a highly recognized food pathogen that causes gastro-intestinal diseases in humans, its presence on processed food may give a better indication than coliforms of inadequate treatment or post-process contamination from the environment, and may help to indicate the extent of faecal contamination (Nel *et al.*, 2004, Crowley *et al.,* 2005, MacDiarmid & Cook, 2009). Nel *et al*. (2004) has stated that the maximum limit of *E. coli* in meat and meat products should not be more than 10 cfu/g as proposed by the National Department of Health (DoH) of South Africa (Mathenjwa, 2010).

The second one was *Listeria monocytogene (18.82%)*, presence with average (6.4Log cfu ±1.96). Also, the presence of *Listeria monocytogenes* is recognized as a human pathogen, which is a gastrointestinal food infection that leads to bacteremia and meningitis in humans (Gombas, *et al.,* 2003, Madigan *et al.,* 2003).This organism has been detected in a variety of ready-to-eat food products (Huffman, 2002, Gombas *et al.*, 2003, Madigan *et al.*, 2003). The levels of this organism that has been detected in food is not clear, but it has been suggested that levels of > $10^3$ cfu/g *L. monocytogenes* may result in listeriosis (Gombas *et al.*, 2003).

The third percentage was occupied by *Salmonella*(16.47%),in average present (log cfu 3.3/g±1.05). The presence of *Staphylococci*- in average values (1.7 log/g ±4.4) - in local markets in sausages are good alarm for food-poisoning outbreaks due to produce harmful enterotoxins as proved by many workers (Shale *et al.*, 2005). Same author showed that a maximum count of $10^2$ cfu/g in meat is acceptable in South

Africa. Also, The amount of *Staph. aureus* required for production of toxin is $10^5$ – $10^8$ cfu/g (Farber *et al.,* 1988; Nel *et al*., 2004; Shale *et al*., 2005).

Also, the total mold occupied high percentage (17.94%) for contamination of sausages .But ,usually Lactic acid bacteria (LAB) occupied 18.82%, as starter in culture mainly for fermented sausages, due to its abilities to lower the pH of the product and produce bacteriocin (Kim, 2006). Bacteriocins are antimicrobial peptides produced by lactic acid bacteria. Nisin and pediocin are well known bacteriocins. Nisin is produced by *Lactococcus lactis* and pediocin is produced by *Pediococcus acidilactici*, which have been shown to be effective against *L. monocytogenes* and other Gram-positive pathogens on meat surfaces (Siragusa, *et al.,* 1999 ).

According to the United States Department of Agriculture (USDA, 1999), sausage makers should ensure that their products are not contaminated by pathogens such as *Listeria*, *E. coli* O157, *Salmonella*, *Trichinae* and *Staphylococcus* enterotoxin.

*Escherichia coli* is a highly recognized food pathogen that causes gastro-intestinal diseases in humans, especially *E. coli* O157:H7, which is frequently detected in the intestinal tracts and hide of cattle and pigs. This pathogen is also associated with ground beef products and other bovine products. The consumption of food and water contaminated with faecal matter of animals sometimes result in infections caused by *E. coli* strains (Li *et al.,* 2006). Aerobic colony counts range from 1.5 x $10^3$ – 2.1 x $10^8$ cfu/g for fresh sausage and for frozen sausage from 1.4 x $10^3$ – 3.1 x $10^7$ cfu/g (Farber *et al.,* 1988).

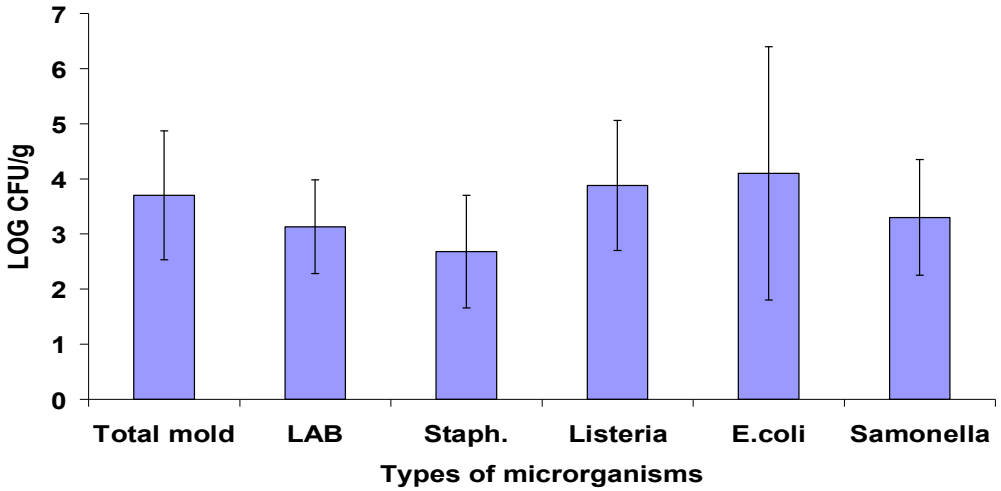


**Fig.(1):** Mean population of eleven collected samples from Egyptian local Markets (log cfu/g.)

**Effect of ginger extract and γ- irradiation on the microbiological load during frozen storage:**

As shown, in Figs (2-5), the obtained results of this study demonstrated that the microbial quality of sausages were more affected by used treatments mainly with ginger 1% and irradiation doses. In the same time ,low concentration of ginger (0.5%) and irradiation (3.0,5.0 kGy) reduced all the pathogenic microbe load even two and three months of frozen storage .But, irradiated sausage at 5.0 kGy samples extend free from most microbes even three months. Only , Staph ,was present due to low irradiation dose for decontamination. Also, after two month re-generation phenomenon raised for some microbes ,its observed and extend to the third month on frozen storage. In the same ,time ,after three months only ginger extract at 1.0 % was sufficient to prevent the growth of the microbes .These differences were significantly as shown in Fig(5), Irradiation doses prevented completely the microbes even three months as happened by 3.0 kGy which decreased only 3.0 Log cycle even end of frozen storage. Whereas, the ginger extract (1.0%) has the same effects like irradiation after two and three months in decreasing the 3.0 Log cycles or more of all micro organisms. Irradiation doses inactivation were more affective as ginger extract (1.0%), to reduced most the pathogenic and molds by more than 3.5 log CFU/g.

The re-generation phenomenon of growth some microbes in most treatments started again as observed after 2-3 months during frozen storage. These results may be due to low doses of irradiation which activate the spores or injured cells of microbes to reclaim or repair the injured DNA-cells as showed by some workers, but this trend was limited or not harmful to cause spoilage. (Sweetie *et al.,* 2005, 2006). Besides the permeability of packaging materials for water and air which activate the re-growth after two or three months..The obtained results by irradiation doses are similar found by workers (Pallas & Hamdy, 1976, Mattimore & Battista, 1996, Sweetie *et al.,* 2005, 2006). Who showed that low doses better to avoid the off-flavor of fat content.

Ginger extracts have antibacterial effects against both gram positive and gram negative bacteria such as Clostridium, Listeria, Enterococcus, and Staphylococcus species, but some of this effect is destroyed by heating as cooking. (Mascolo *et al.,* 1989, Chen *et al.,* 1985, James *et al.,* 1999). The antibacterial, antifungal properties of ginger extract were reported by workers who showed that due to presence of sesqui-caryophellene and limonene (Belantine *et al.,* 2006; Martínez *et al.,* 2007, El-Baroty *et al.,* 2010).

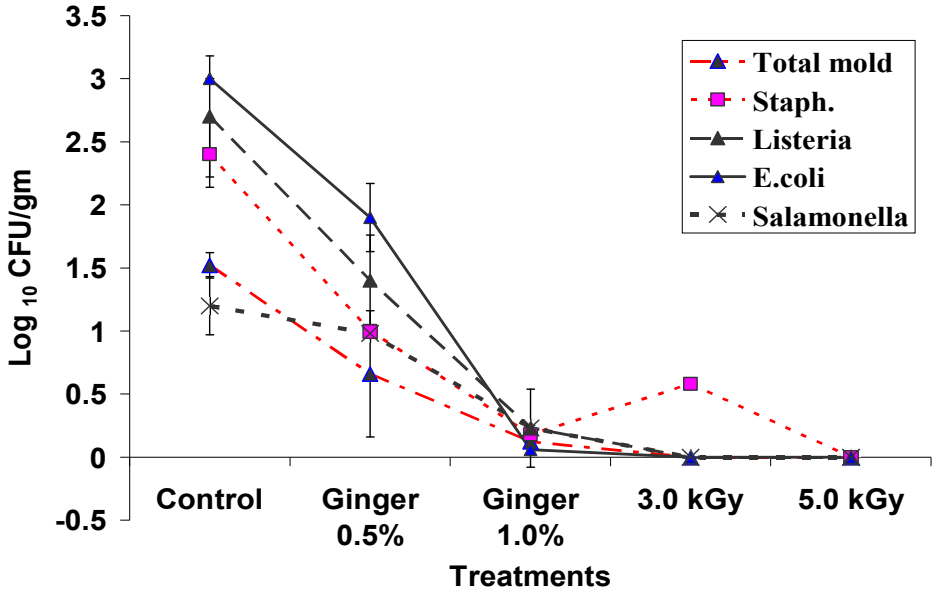


**Fig.(2):** Effect of ginger extract and gamma irradiation on load of microorganisms at processed sausage at zero time log CFU/g

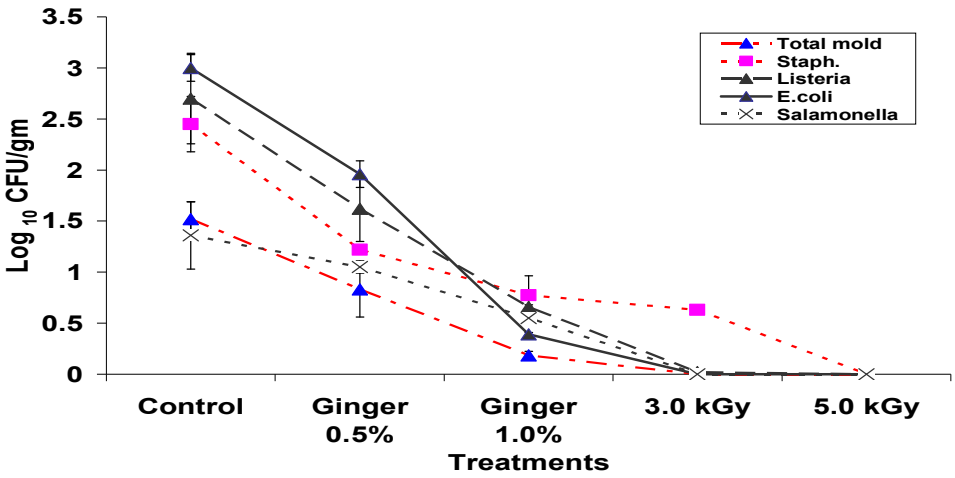


**Fig.(3):** Effect of ginger extract and gamma irradiation on load of microorganisms at processed sausage after one month of storage frozen.(log CFU/g)

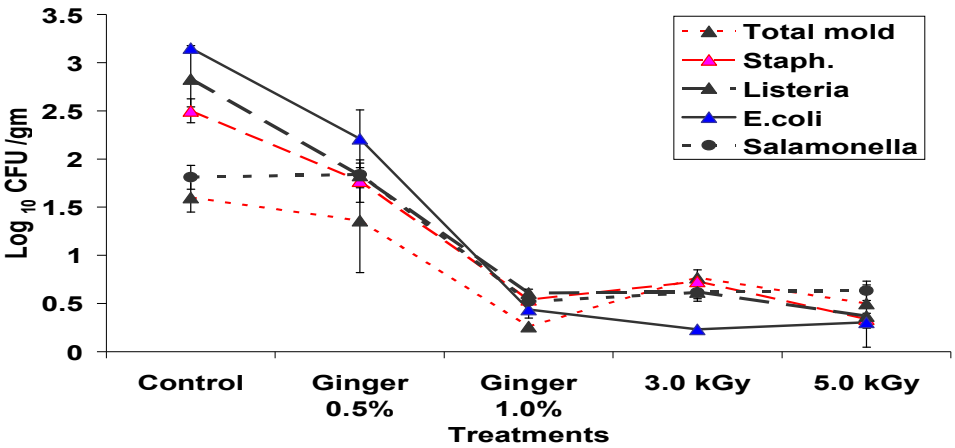


**Fig.(4):** Effect of ginger extract and gamma irradiation on load of microorganisms at processed sausage after two months of frozen storage(log CFU/g)

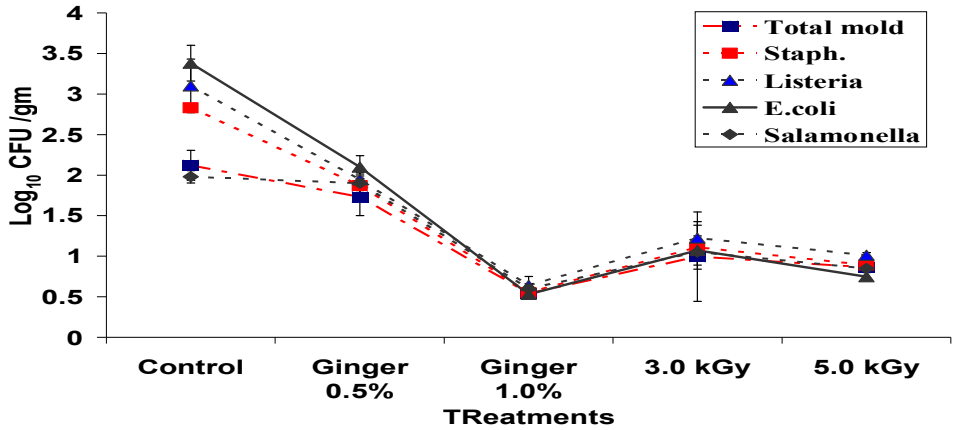


**Fig.(5):** Effect of ginger extract and gamma irradiation on load of microorganisms of processed sausage stored three month on freezing

**Lipid stability**

Lipid oxidation is one of the main parameters that affect the quality of meat and meat products. Lipid oxidation results in the development of unacceptable organoleptic characteristics such as rancid flavour, colour, texture and odour deterioration. Products produced from the oxidation reactions may also pose health risks (carcinogenic, low absorption of fat soluble vitamin), whereas microbial growth causes spoilage and foodborne diseases (Georgantelis *et al*., 2007). The primary determination of fat in tested samples showed presence low fat content (16- 17 %). Controlling both lipid oxidation and preventing microbial growth will have an increase in shelf-life. The use of natural preservatives or additives in food products can provide beneficial effects to consumers and also to the food industry.

The results of the lipid stability of sausages treated with different treatments are presented in Figure (6). The values of Thiobarbituric Acid Reactive Substance (TBARS) were less than 2 at zero time but started increased gradually during storage .After two months, most of treatments increased 2 values of TBARS except Ginger extract (1.0%),which was the best treatments even end of storage (90 days of frozen storage ). To prevent the rancidity in samples. Recent studies in meat such as beef, however, indicate that TBARS values of 2 or greater are considered to be rancid (Suman *et al.,* 2010).

A linear relationship resulted between storage period and TBARS of treated samples with high significant values of coefficient ($R^2$) ,as shown in Fig.(5).The rate of rancidity can calculate per every treatments as (MDA mg /kg fresh weight).These values can descending order as 0.37,0.36,0.35,0.34,,0.29 for 5.0 kGy,3.0 kGy ,control, 0.5% GE and 1.0% GE respectively. According ,these data ginger extract (1.0%)was the best treatment to keep samples with good quality rancidity free even 90 days during frozen storage ,whereas irradiation increased rancidity values of TBARS rapidly comparing with control samples during frozen storage.

When comparing the treatments stored at -18°C for the period of 90 days, the ginger extract (1.0%) treatment maintained the TBARS values from day 1 – 90 less or near 2. These effects of ginger extract (1.0%) may be are the suitable concentration which contain the effective levels of antioxidants and phenols to prevent rancidity besides its antibacterial agent. Whereas, less concentration (0.5%) failed to do same effects, due to lack of that affective levels of antioxidant properties. Ginger contain active phenolic mainly sesquiterpene hydrocarbons, including β-sesquiphellandrene, aryophyllene ,zingiberene, *α*-farnesene, and *ar*-curcumin besides its effect on significant inhibitory activity against selected strains of bacteria and pathogenic fungi. (El-Baroty *et al.,* 2010). These properties of ginger extract prevent the rancidity of fatty content during long storage period. Georgentelis *et al.* (2007) also observed similar trends whereby the fresh pork sausage preserved with rosemary had lower oxidation products of 0.16 mg malonaldehyde (MDA)/kg meat to that of chitosan of 0.37 MDA/kg meat treatment after 20 days storage at 4°C. Same results were obtained on rosemary (Rižnar *et al.*, 2006, Mirshekar *et al.*, 2009).

Increasing values of TBARS of irradiated samples was clear as in Fig (6). These results show irradiation due to presence of water content (58%) and low content of lipids (16-17%) in spite of using freezing storage for 90 days. But these trend of rancidity by irradiation usually done, these effect via lipid oxidation in animal muscles, were observed with increasing doses in irradiated lamb liver as proved by Sweetie *et al.,* 2006. Also, at cold storage, same trend was observed by workers (Sommers *et al*., 2001, Shams El din, 1949, Emam, 1990). In addition, close relationship was observed between oxidative state and sensory during cold storage by (Shults *et al.,* 1977 and Piccinni *et al.,* 1986).

Concerning increasing TBARS values in control samples, may be due to further oxidation of MDA to other organic products of lipid oxidation (alcohol & acids) which are not determined by the reaction with TBA (Soultos *et al.,* 2008). Another possible reason may be due to the decomposition of MDA by bacteria such as pseudomonad's and *Enterobacteriaceae*, which posses the ability to selectively attack and utilize carbonyl compounds, including MDA (Soultos *et al.,* 2008). Another possible reason may be due to the decomposition of MDA by bacteria such as pseudomonad's and *Enterobacteriaceae*, which posses the ability to selectively attack and utilize carbonyl compounds, including MDA (Soultos *et al.,* 2008). Other factors such as temperature have an effect on the oxidation rate of meat and meat products. For example, during the cooking process there is a significant increase in the TBA values because the cooking method disrupts the muscle membrane system, thereby exposing the lipid component to oxygen and/or other reaction catalysts such as iron (Kamil *et al.,* 2002).

The role of ginger extract to keep sausages (1.0%) during long storage at frozen conditions due to presence high content of antioxidants as phenols or like which have been high antioxidant and antibacterial activity properties which prevent oxidation of hemoglobin.

In sausage preserved with ginger extract(1.0%) stored for a period of 90 days at -18 °C, the ginger extract (1.0%) showed lower TBARS values when compared to those irradiated or with ginger extract (0.5%). Ginger extract (1.0%) extract was significantly

the best treatment; its observed to reduce or maintain the TBARS in samples even towards the end of storage (90 days).

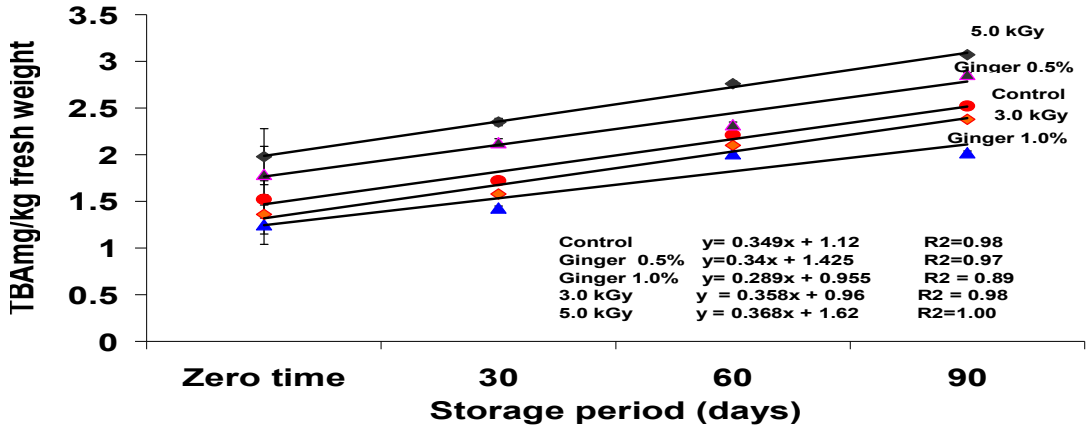


**Fig.(6):Changes of TBA (MAmg /kg. fresh weight basis) of treated sausage during frozen storage.**

**Effect of treatments on sensory of sausage:**

According to the means given by the panelists of cooked samples either at zero time or end of storage as shown in figs 6-10,the addition of ginger extract especially at 1.0 % concentration promoted stability most of sensory properties like fresh samples of sausage at zero time even after storage frozen three months .These effects of roles of ginger extract may be due to presence of antioxidants and phenols which prevent lipids oxidation consequently then keeping redness color , flavor, taste and texture like fresh samples.

These results as evidenced by statistical difference in relation to the control treatment at zero time or third month of freezing. The treatments with addition of ginger extract (1.0 %) always had the highest notes regarding change of color (Fig 6).

The redness of meat is an important aspect which consumers use to purchase meat and meat products (Boles *et al.,* 1998). This has major economic consequences that cause an annual loss of 1 billion USD to the meat industry (Smith *et al.,* 2000). Reclaiming profit via improved colour stability relies on the proper application of the fundamental principles of myoglobin chemistry, including two often overlooked factors: oxygen consumption and NADH regeneration as they impact metmyoglobin reduction The redness colour originates when meat myoglobin is exposed to oxygen resulting in the formation of red myoglobin.

Ginger extract (GE) was the best color especially at 1.0 % keep the original color after treatment directly. Whereas all the other treatments were like control at zero time in color significantly as shown in Fig.(6). At end of storage low levels of ginger (0.5%) keep the color as control but irradiation decreased these values dramatically. As shown in Fig (6), the highest value of color was significantly recorded with ginger extract (1.0%) these results may be due to high content of antioxidants and phenols which prevent the oxidation of hemoglobin. These results are highly significantly for keeping redness of sausages naturally on frozen (-18$^0$C) for 90 days. The explanation of ginger extract roles to improve color, depend on NADH regenerating besides releasing radicals as antioxidants consequently prevent oxygenated process of myoglobin or darkening tissues as showed by previous workers. (Hunter and Mancini, 2009). Whereas, irradiation activated the oxygenating and darkening process then decreased color ranks for consumers. Most of the past studies were in limited period for cold storage, as observed in modified packaged fresh pork sausage, using rosemary with ascorbic acid whereby the redness colour of the product was maintained for 12 days (Martínez *et al.,* 2007). Also, the redness of 1% chitosan preserved beef patties packaged in an oxygen permeable film (PVC) and stored at refrigerated temperatures has been shown to have greater redness than that of control packages in the same material at days 3 – 5 (Suman *et al.,* 2010).

Our findings, results are more significant, economically for keeping stability of color during long storage at frozen even three months with same red original color of hemoglobin of meat products naturally without any harmful additives or chemicals comparing irradiation treatments.

The results of cooked sausage texture as present in Fig.(7), showed no significant differences were obtained between treatments and control samples at zero time. Whereas, GE (1%) recorded the highest rank after three months at frozen storage. Also, GE (0.5%) was the second one. Less significantly ranks were recorded for irradiated samples as control samples,

irradiated samples results were near control samples. These data were significantly recorded as shown in Figs.(7). Decreasing texture ranks by irradiation may be due to activation enzymes at low applied doses during irradiation process. The solubility of the collagen in intact beef sausages muscle was increased by irradiation. The solubilisation of collagen was considered to be the result of an indirect action of radicals formed in water. (Bailey and Rhodes, 1964). Whereas, same changes in collagen were in slow rate in control samples .

Aroma and taste properties are related to volatile oil products due to lipid oxidation and rancidity products. Besides roles of microbial growth causes spoilage and off flavour (Rižnar *et al.*, 2006; Georgantelis *et al.*, 2007). Controlling both lipid oxidation and preventing microbial growth will have an increase in shelf-life of sausage as proved by GE especially at 1% which was the best treatment. The results of aroma and, taste showed the priority of GE especially (1.0%). Whereas, irradiated and control recorded lower values either at zero time or end of storage. Over all acceptance of sausage proved the preferability of GE (1.0%) then 0.5% whereas the other treatments recorded less significant ranks for all accepted samples especially irradiated samples.

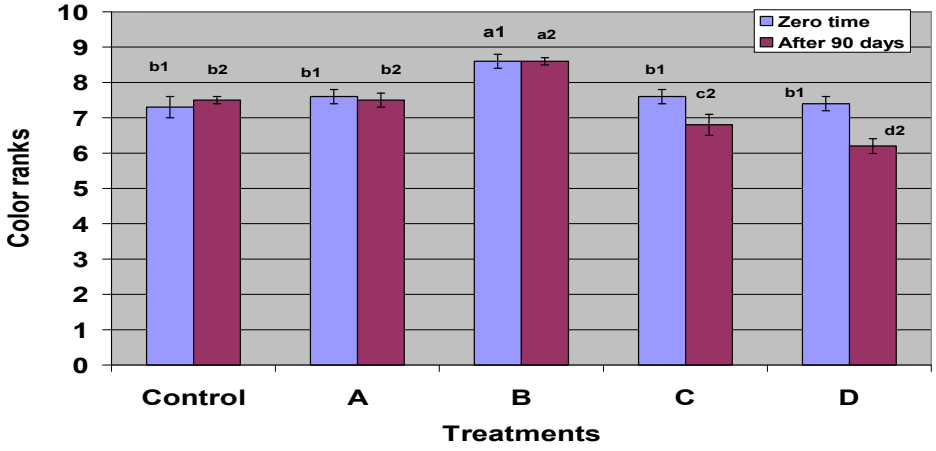


**Figure (7):** Effect of ginger and irradiation at zero time and end of storage on the color of sausages stored at –18°C. Results with different superscripts are significantly different. Error bars represent standard deviations (A,B=0.5%,1% ginger extract, D=3.0 kGy, 5.0 kGy). $LSD_{0.05}$ = 0.3 at zero time, 0.43 after 90 days of storage.

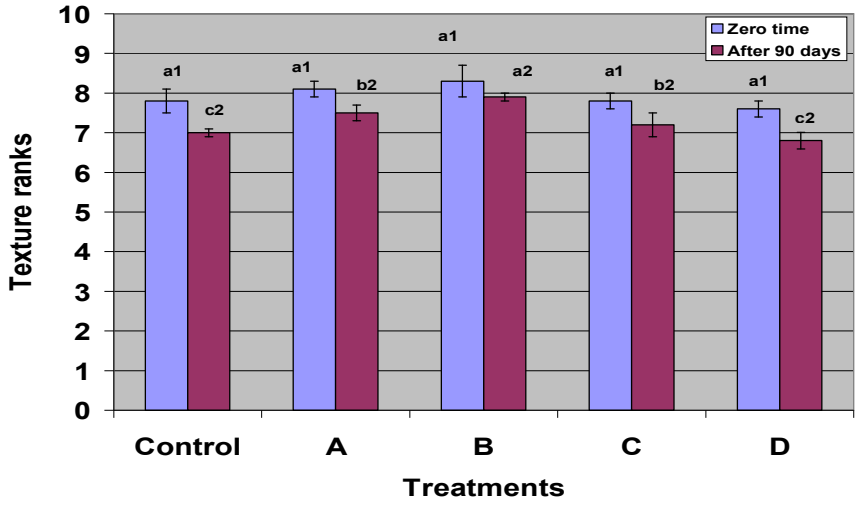


**Figure (8):** Effect of ginger and irradiation at zero time and end of storage on the texture of sausages stored at –18°C. Results with different superscripts are significantly different. Error bars represent standard deviations (A,B=0.5%, 1 % ginger extract, C, D=3.0 kGy, 5.0 kGy). $LSD_{0.05}$=not significant at zero time, 0.06 after 90 days of storage.

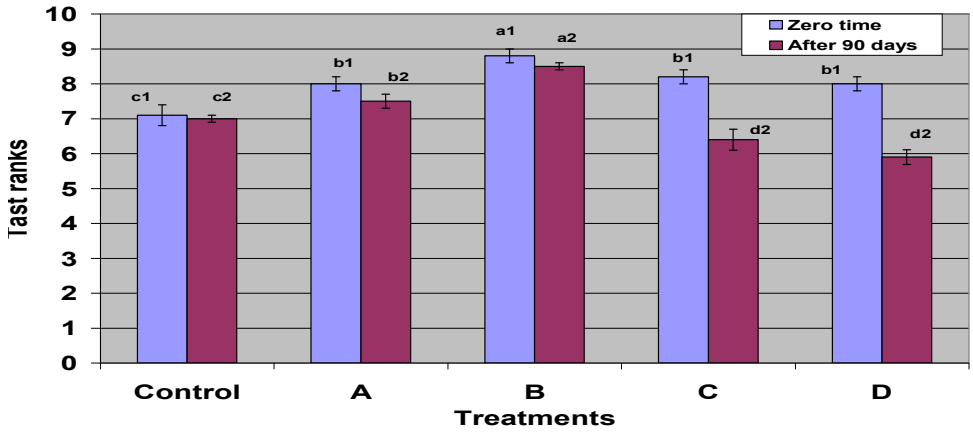


**Fig. (9):** Effect of ginger and irradiation at zero time and end of storage on the taste of sausages stored at –18°C. Results with different superscripts are significantly different. Error bars represent standard deviations (A,B=0.5%,1% ginger extract, D=3.0 kGy, 5.0 kGy). LSD $_{0.05}$=1.09 at zero time, 0.52 after 90days of storage.

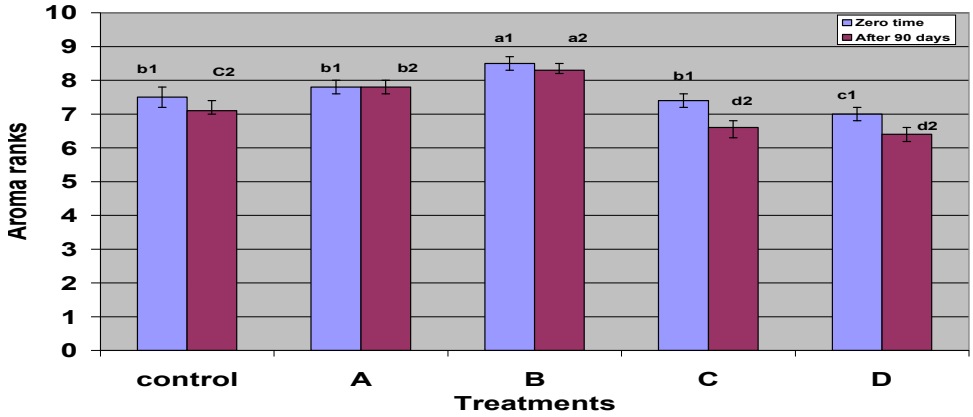


**Fig. (10):** Effect of ginger and irradiation at zero time and end of storage on the Aroma of sausages stored at –18°C. Results with different superscripts are significantly different. Error bars represent standard deviations (A,B=0.5%,1% ginger extract, D=3.0 kGy,5.0 kGy). LSD $_{0.05}$=0.02 at zero time, 0.01 after 90 days of storage.

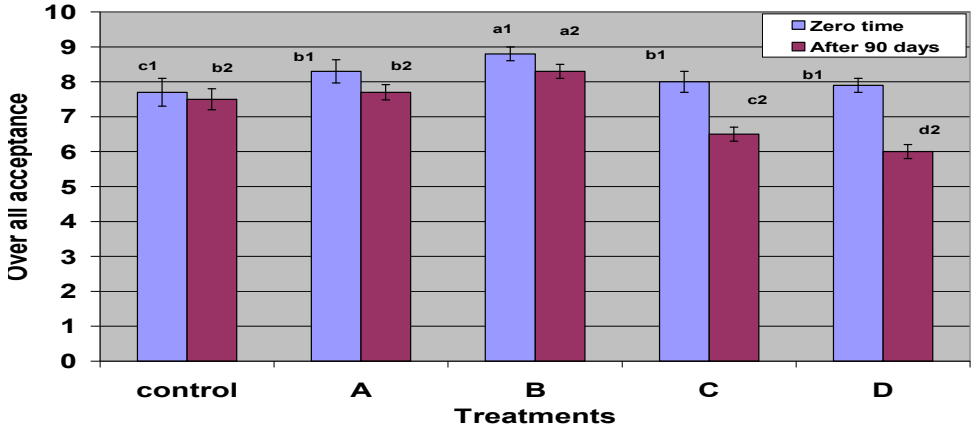


**Fig. (11):** Effect of ginger and irradiation at zero time and end of storage on over all Acceptance of sausages stored at –18°C. Results with different superscripts are significantly different. Error bars represent standard deviations (A,B=0.5%, 1% ginger extract, D=3.0 kGy, 5.0 kGy). LSD $_{0.05}$=0.02 at zero time, 0.01 after 90 days of storage.

**4. Conclusion**

According to our results, the addition of ginger extract as natural additives improved the quality and storage stability of sausages. Ginger extract results were satisfactory effect in protecting against lipid oxidation in processed, cooked and frozen beef sausages. Besides were more affective as antimicrobial agent comparing with irradiation treatments. In the same time, the treatment with ginger extract more effective than irradiation in maintaining the oxidative stability of samples. As for sensory acceptance, the addition of ginger extracts was effective in maintaining the sensory properties of the sausages even after 90 days of storage at -18$^0$C .

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