Effect of Phenylalanine Acid and Urea on Growth, Yield and Chemical Composition of Gladiolus Plants Grown on a Clayey Soil

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Abstract: A field experiment was carried out on alluvial clay soil at Experimental Farm, Faculty of Agriculture, Menoufia University, Shebin El – Kom, Egypt (30.52°N and 30.99°E) during two successive winter seasons (2013/2014 and 2014/2015) on gladiolus plants (*Gladiolus grandiflorus* L.), rose supreme cv. to evaluate the individual and combined effects of foliar spray of amino acid (phenylalanine) at different rates of 0; 50; 100; 150 and 200 mg/l and soil application of mineral nitrogen fertilization at different levels of 0; 50; 75 and 100 % of recommended dose which were 0; 120; 180 and 240 kg urea/fed, respectively on growth; yield qualitative; chemical composition of gladiolus plants and mineral nitrogen fertilization efficiency. The studied treatments arranged in a split-plot design, with the main plots arranged in a randomized complete block design with three replicates. The results showed that, different applications of mineral nitrogen fertilization (urea) and phenylalanine alone or together had a significant increase in the flowering growth parameters (flowering stem length; flowering stem diameter; number of florets/spike; inflorescence length; fresh and dry weights of inflorescence); corms and cormels production (corms diameter; corms fresh and dry weights; number and dry weight of cormels/plant) as well as chemical composition (chlorophyll a and b; carotenoid; total carbohydrate; N; P; K; Fe; Mn and Zn contents) in both seasons compared with untreated plants. Combined treatment of phenylalanine at 150 mg/l + 180 kg urea/fed gave the highest flowering growth; corms and cormels production. While, combined treatments of urea at 180 kg/fed + each of 100; 150 and 200 mg/l phenylalanine produced the highest concentrations (%) of N; P and K respectively, in the two seasons. On the other hand, the combined treatment of 100 mg/l phenylalanine plus low urea level gave the greatest promoting effect on photosynthetic pigments (chlorophyll, a and b) in the leaves during two seasons.

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Key words: Phenylalanine, Urea, Gladiolus, Growth characters and Chemical composition.

1. Introduction

Gladiolus (*Gladiolus grandiflorus*) is a member of the Family Iridaceae and sub family Ixiodeae. Gladiolus genus involves a variety of about 180 species with more than 10 000 cultivars with wide range of colours **(Sinha and Roy, 2002).** They are attractive cut flowers, which considered one of the most important florist crops in Egypt and all over the world **(Bai *et al.,* 2009).** *Gladiolus grandiflorus* plants are grown for commercial purposes both locally and for export to the foreign markets especially during winter season **(Sheela, 2008)**. They are especially used as landscape plants in home gardens, as specimen for exhibitions and in decoration as a lovely colored cut flower spike with long vase life **(Bose *et al.,* 2003).** Moreover, gladiolus plants are ordinarily used in borders and beds of many gardens **(Rees, 1992).**

Nitrogen is considered the most important essential nutrient in plant nutrition. It is a fertilizer which should be added in a balance and rational way to keep high and stable yield. It is an important component of proteins; enzymes and vitamins in plant. It constitutes a central part of the chlorophyll and essential photosynthetic molecule. The excessive application of mineral fertilizers led to increase in production costs. The non-rational of mineral fertilizers has seriously affected the quality of agricultural products people's health and led to environmental pollution. Therefore a great interest has been directed to apply bioorganic and inorganic fertilizers to maintain a good eco-environment (**Basak, 2006).** Also, nitrogen fertilizers are economically an expensive input. In many instances less than 60 % of the added N is recovered in the (crop + soil), while the remainder are getting lost by processes such as volatilization; leaching; immobilization and denitrification. Thus, it is mandatory to develop fertilizer management practices, that can minimize losses and maximize efficiency of nitrogen use **(Yusron and Phillips, 1997).** Urea is the major nitrogen fertilizer form used in agriculture. Its enormous usage agriculture owes to its high nitrogen content (46%). However, about 20–70% of the applied urea fertilizer is lost somehow in the environment, causing serious pollution and increasing costs. This relatively major loss owes to leaching; decomposition and ammonium volatilization in soil, handling and storage. Nitrogen loss from urea can be minimized by inhibiting urease activity, which in turn delays urea hydrolysis. Similarly, urea fertilizer is subject to NH_3 volatilization through the activity of urease enzyme, which is found in soils everywhere. Many products have been developed to slow urea hydrolysis and other N-transformation processes to synchronize availability of N with the plant needs **(Shaviv and Mikkelsen, 1993)**. Excess nitrogen in fertilizers can be as harmful as inadequate nitrogen. It can lead to excessive vegetative growth in the plant at the expense of flowering and fruiting. Beside these undesirable effects on the crop or plant, excess nitrogen can lead to significant problems in the environment at large **(Baser *et al.*, 2012).**

Amino acids constitute the building blocks of proteins, and they are integral to the chemistry of life. Without amino acids, there would be no enzymes and without enzymes, life as we know it wouldn't exist. So amino acids are used by every living organism on earth, from the simplest microorganism to the most complex plants and animals **(Davies, 1982).** The role played by accumulated amino acids in plants varies from protein synthesis; stress resistance; pollination and fruit formation; equilibrium of soil flora; osmolyte; regulation of ion transport; modulating stomatal opening and detoxification of heavy metals. Amino acids also affect synthesis and activity of some enzymes; gene expression and redox-homeostasis **(Hotta *et al.,* 1997 and Yongin *et al.,* 2003).** Foliar feeding technique is considered a particular way to supply amino acids which results in rapid absorption of it. It is generally considered to be more effective and less costly. During the last decades, foliar feeding of amino acids has become a well adopted procedure to increase yield and to improve the quality of crop products **(Röemheld and El-Fouly, 1999)**. This procedure can also improve nutrient utilization and lower environmental pollution by reducing the amount of fertilizers added to soil **(Abou El-Nour, 2002).**

This study was carried out to evaluate the individual and combined effects of foliar spray of amino acid (phenylalanine) and soil application of mineral nitrogen fertilization (urea) on growth; yield qualitative; chemical composition of gladiolus plants and mineral nitrogen fertilization efficiency.

2. Materials and Methods

A field experiment was carried out on alluvial clay soil at Experimental Farm, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt (30.52°N and 30.99°E) during two successive winter seasons (2013/2014 and 2014/2015) on gladiolus plants (*Gladiolus grandiflorus* L.), rose supreme cv. to evaluate the individual and combined effects of foliar spray of amino acid (phenylalanine) at different rates of 0; 50; 100; 150 and 200 mg/l and soil application of mineral nitrogen fertilization at different levels of 0; 50; 75 and 100 % of recommended dose which were 0; 120; 180 and 240 kg urea/fed, respectively on growth; yield qualitative; chemical composition of gladiolus plants and mineral nitrogen fertilization efficiency.

Before planting of each season, representative surface soil samples (0- 30cm) were taken from the used soil. Soil samples were air- dried, ground, mixed well, sieved through a 2 mm sieve. The samples were then analyzed for determination of some physical and chemical properties, also, the contents of some available macro and micronutrients according to the methods described by **Cottenie *et al.* (1982); Page *et al.* (1982) and Kim (1996).** The obtained data were recorded in Table (1).

Table (1): Some physio-chemical properties and some macro-micronutrients of the experimental soil.

Soil properties	Value
Particle size distribution (%)	
Coarse sand	6.35
Fine sand	14.50
Silt	25.00
Clay	54.15
Texture class	Clayey
Water holding capacity (%)	62.8
Organic matter (%)	1.90
pH (1:2.5 soil: water suspension)	7.55
EC_e in soil paste extract (dSm^{-1})	1.51
CEC (cmol/kg)	37.30
$CaCO_3$ (%)	4.60
Soluble cations (meq/l)	
Ca^{++}	4.48
Mg^{++}	1.81
Na^{+}	6.55
K^{+}	2.27
Soluble anions (meq/l)	
CO_3^{--}	--
HCO_3^{-}	1.44
Cl^{-}	10.30
SO_4^{--}	3.37
Available macronutrients (mg/kg soil)	
N	43.88
P	7.80
K	363.50
Available micronutrients (mg/kg soil)	
Fe	6.82
Mn	3.21
Zn	1.08

Field experiments design

The design of the experiment was a split-plot design, with the main plots arranged in a randomized complete block design with three replicates. The study area was 630 m^2. This area was divided into 60 experimental plots (twenty treatments with three replicates) with 10.5 m^2 (3.5 m length × 3m width) for each plot. Each plot was divided into 6 rows with 50cm wider. The experimental plots were divided into four main groups (15plot/main group) representing application rates of mineral nitrogen fertilizer (urea, 46%N) i.e. 0; 120; 180 and 240 kg urea/fed. The experimental units of each main group were divided into five sub groups (3 plot/sub group) representing application rates of amino acid (phenylalanine) i.e. 0; 50; 100; 150 and 200 mg/l. Before planting, at final soil preparation, all plots were fertilized by ordinary super phosphate (15.5 %P_2O_5) at rate of 200 kg/fed + 50 kg/fed of agricultural sulphur + 25 m^3/fed of compost. The corms of gladiolus plants for this study were imported from Holland with size of (8-10 cm in circumference) and were sown on the first of October in hills and the distance between the hills was 20 cm, during two growing seasons. All plots have received potassium fertilizer in the form of potassium sulphate (48%K_2O) at rate of 150 kg/ fed, which was added in two equal doses as soil application at 30 and 60 days after planting in both seasons. Also, the added amount of urea and phenylalanine were carried out in three equal doses 30 and 60 days after planting and after cutting the flowering stems in both seasons. Urea fertilizer was applied as soil application, while phenylalanine was sprayed early in the morning on the plant foliage until the run off point. Other farming processes were carried out as recommended by the Egyptian Ministry of Agriculture.

Morphological characteristics

Morphological parameters including flowering stem length (cm); flowering stem diameter (cm); number of florets/spike; inflorescence length (cm); fresh and dry weights of inflorescence (g/plant) were recorded after opening the first floret of a spike which were cut and leaving three leaves on each gladiolus plant. After flowering diminished, underground parts were lifted five weeks after cut spikes to determine the following data: corms diameter (cm); corms fresh and dry weights (g); number of cormels/plant and dry weight of cormels/plant (g).

Chemical analysis

Total carbohydrate percentages in the aerial parts of gladiolus plants were determined by using the colorimetric method of **Dubois *et al.* (1956)**. Chlorophyll (a and b) and total carotenoids content (mg/g fresh weight) were estimated in fresh leaves as described by **Witham *et al.* (1971)**. At the end of the two winter seasons, the plants of the replicates of each treatment were harvested separately from each plot and washed with a tap-water and then two times with distilled water, air-dried, oven-dried at 70°C for 48 hrs. The plant samples were grinded separately to a fine powder in a stainless grinder and stored in plastic bags until analysis. Half g portions of each dried plant sample were digested by a concentrated mixture of H_2SO_4 + $HClO_4$ at (5:0.5) ratio according to **Chapman and Pratt (1982).** The percentages of N; P and K were determined as described by **Chapman and Pratt (1982)** and **Cottenie *et al.* (1982).** In addition, the contents (mg kg^{-1}) of some micronutrients (Fe; Mn and Zn) were measured using Perkin Elmer atomic absorption, spectrophotometer model 2830 **(Cottenie *et al.*, 1982)**.

Statistical analysis

The obtained data of growth parameters were exposed to proper statistical analyses of variance (ANOVA) by using Minitab computer program and least significant difference (L.S.D.) were calculated at level of 5% **(Barbara and Brain, 1994)**.

3. Results and Discussion

Effect of amino acid (phenylalanine); mineral nitrogen fertilizer (urea) and their interactions on:

1- Flowering growth parameters

Data of flowering growth parameters of gladiolus plant such as flowering stem length (cm); flowering stem diameter (cm); number of florets/spike; inflorescence length (cm); fresh and dry weights of inflorescence (g/plant) are presented in Table (2). These data showed that, spraying plants with different phenylalanine concentrations increased all previous parameters and reached to the maximum values with the application of phenylalanine at 150 mg/l during two seasons. This effect on growth could be explained by the role of using amino acids at a specific concentration on plants which consider as source of carbon; nitrogen; energy; enzyme; co-enzymes and plant hormones. Our findings on the effect of phenylalanine are matching with the findings of **Gamal El-Din and Abd El-Wahed (2005)** on chamomile plant.

In addition, data in Table (2) showed that, the higher values of the studied flowering growth parameters reached its maximum as a result of using urea at rate of 180 kg/fed. Generally, the enhancement effect of urea fertilization may be due to the positive effects on activation of photosynthesis and metabolic processes of organic compounds which, in turn, encourage the growth and flowering parameters. Such quality improvements by using urea as N fertilizer source are corresponding to those obtained by **Zubair *et al.* (2013)** on gladiolus plants.

Similarly, the studied growth parameters in this study were significantly increased by the combined treatments of urea fertilizer and phenylalanine. The combined treatment of 180 kg urea /fed and 150 mg/l phenylalanine produced the highest values of the tested flowering growth parameters compared with control and other treatments in the first and second seasons. These results are in accordance with those obtained by **Sewedan *et al.* (2012)** they mentioned that, the interaction between diphenylamine and different levels of ammonium nitrate improved the flowering growth parameters of gladiolus plants.

Table (2): Effect of different application rates of urea; phenylalanine and their interactions on flowering growth parameters of gladiolus plants during two growing seasons.

Urea rates (kg/fed) / Phenylalanine acid (mg/l)	First season					Second season				
	0	120	180	240	Mean	0	120	180	240	Mean
Flowering stem length (cm)										
0	63.05	68.92	73.81	69.77	68.88	70.33	73.24	78.35	75.20	74.28
50	68.95	74.12	79.63	77.58	75.07	75.10	78.88	82.15	81.67	79.45
100	72.45	79.55	84.60	81.16	79.44	79.84	83.56	89.29	86.70	84.84
150	80.30	86.39	91.10	89.44	86.80	85.55	92.18	98.61	95.70	93.01
200	77.23	87.69	81.01	80.57	81.62	81.23	83.80	86.06	86.43	84.38
Mean	72.39	79.33	82.03	79.70		78.41	82.33	86.89	85.14	
L.S.D. at 5%	U. = 7.25 Phe. = 6.18 U. x Phe. = 13.81					U. =6.89 Phe. = 6.12 U. x Phe. = 13.64				
Flowering stem diameter (cm)										
0	0.82	0.87	0.89	0.89	0.86	0.87	0.90	0.95	0.96	0.92
50	0.86	0.90	0.92	0.93	0.90	0.92	0.96	1.03	1.00	0.97
100	0.96	1.03	1.12	1.09	1.05	1.01	1.10	1.16	1.09	1.09
150	1.10	1.18	1.25	1.22	1.18	1.15	1.21	1.29	1.27	1.23
200	0.99	1.08	1.11	1.13	1.07	1.07	1.15	1.20	1.21	1.15
Mean	0.94	1.01	1.05	1.05		1.00	1.06	1.12	1.10	
L.S.D. at 5%	U. = NS Phe. = 0.10 U. x Phe. = 0.22					U. = NS Phe. = 0.24 U. x Phe. = 0.53				
Number of florets/spike										
0	10.25	10.94	11.62	11.47	11.07	10.71	11.20	11.72	11.75	11.34
50	11.58	12.07	12.90	12.82	12.34	12.31	12.63	12.85	12.88	12.66
100	12.35	13.11	13.79	13.81	13.26	12.56	12.96	13.49	13.27	13.07
150	13.21	13.89	14.12	13.93	13.78	12.60	13.17	13.87	13.70	13.33
200	12.65	12.91	13.84	13.78	13.29	12.48	12.88	13.54	13.61	13.12
Mean	12.00	12.58	13.25	13.16		12.13	12.56	13.09	13.04	
L.S.D. at 5%	U. = 0.84 Phe. = 0.45 U. x Phe. =1.00					U. = 0.53 Phe. = 0.61 U. x Phe. = 1.36				
Inflorescence length (cm)										
0	31.29	35.44	38.98	37.05	35.69	37.32	39.00	41.65	40.80	39.69
50	36.60	39.40	42.51	40.97	39.87	40.71	42.64	44.29	44.12	42.94
100	38.69	43.18	45.41	41.77	42.26	42.24	44.57	48.99	47.83	45.90
150	44.86	47.13	50.08	49.34	47.85	45.61	46.70	54.18	51.07	49.39
200	39.20	44.37	42.72	42.90	42.29	43.81	45.39	49.00	46.18	46.09
Mean	38.12	41.90	43.94	42.40		41.93	43.66	47.62	46.00	
L.S.D. at 5%	U. =3.12 Phe. = 3.54 U. x Phe. =7.89					U. = 4.23 Phe. = 4.67 U. x Phe. =10.41				
Fresh weight of inflorescence (g/plant)										
0	29.16	31.65	35.28	35.20	32.82	36.90	37.52	39.49	39.60	38.37
50	35.85	36.77	40.56	39.60	38.19	37.80	39.29	43.15	41.00	40.31
100	38.12	40.79	44.95	39.21	40.76	39.05	41.91	45.76	42.65	42.34
150	41.54	43.71	49.90	45.02	45.04	44.20	47.34	53.26	50.10	48.72
200	38.18	42.60	41.44	40.63	40.71	40.88	41.37	48.70	45.75	44.17
Mean	36.57	39.10	42.42	39.93		39.76	41.48	46.07	43.82	
L.S.D. at 5%	U. =3.05 Phe. = 4.22 U. x Phe. = 9.41					U. = 4.16 Phe. = 4.51 U. x Phe. =10.06				
Dry weight of inflorescence (g/plant)										
0	3.17	3.47	3.58	3.62	3.46	3.51	3.89	3.99	3.84	3.80
50	3.78	4.11	4.36	4.41	4.16	3.89	4.29	4.45	4.37	4.25
100	4.50	4.62	4.81	4.45	4.59	4.71	4.88	4.93	4.79	4.82
150	4.71	5.17	5.78	5.39	5.26	4.92	5.35	5.96	5.80	5.50
200	4.67	4.80	4.75	4.60	4.70	4.83	4.97	5.14	5.06	5.00
Mean	4.16	4.43	4.65	4.49		4.37	4.67	4.89	4.77	
L.S.D. at 5%	U. = 0.23 Phe. = 0.34 U. x Phe. = 0.76					U. = 0.41 Phe. = 0.52 U. x Phe. = 1.15				

U.=Urea Phe.=Phenylalanine U. x Phe. =Intraction

Table (3): Effect of different application rates of urea; phenylalanine and their interactions on corms and cormels production of gladiolus plants during two growing seasons.

Urea rates (Kg/fed) / Phenylalanine acid (mg/l)	First season					Second season				
	0	120	180	240	Mean	0	120	180	240	Mean
Diameter of new corms (cm)										
0	3.42	3.79	3.95	3.76	3.73	3.82	4.02	4.19	4.21	4.06
50	3.93	4.18	4.37	4.40	4.22	4.17	4.58	4.93	4.81	4.62
100	4.13	4.41	4.62	4.52	4.42	4.62	5.12	5.36	5.40	5.12
150	4.28	5.08	5.39	5.26	5.00	4.70	5.22	5.67	5.51	5.27
200	4.20	4.85	4.93	4.77	4.68	4.59	5.07	5.19	5.22	5.01
Mean	3.99	4.46	4.65	4.54		4.38	4.80	5.06	5.03	
L.S.D. at 5%	U. =NS Phe. =0.54 U. x Phe.=1.20					U.= 0.21 Phe. = 0.32 U. x Phe. = 0.71				
Fresh weight of new corms (g/plant)										
0	25.38	29.67	34.14	31.29	30.12	30.72	34.58	38.83	38.26	35.59
50	32.18	37.07	39.95	38.82	37.00	36.50	39.16	41.25	38.72	38.90
100	38.20	42.65	42.71	43.43	41.74	42.13	45.33	47.62	47.69	45.69
150	41.21	44.11	48.66	46.83	45.20	46.78	50.92	53.18	51.09	50.49
200	39.47	40.00	43.31	41.26	41.01	44.20	47.84	50.37	48.64	47.76
Mean	35.28	38.70	41.75	40.32		40.06	43.56	46.25	44.88	
L.S.D. at 5%	U. = 3.87 Phe. = 4.52 U. x Phe. =10.08					U. =4.02 Phe. =5.31 U. x Phe.=11.84				
Dry weight of new corms (g/plant)										
0	7.75	8.69	10.88	9.37	9.17	8.95	10.27	11.46	11.15	10.45
50	9.92	10.58	12.54	11.19	11.05	10.82	11.51	12.45	11.62	11.60
100	11.29	13.16	13.02	12.90	12.59	12.59	12.87	13.93	14.00	13.34
150	12.75	14.08	14.96	14.35	14.03	13.13	14.92	15.20	15.06	14.57
200	12.47	12.80	13.01	13.05	12.83	12.91	13.62	14.16	13.95	13.66
Mean	10.83	11.86	12.88	12.17		11.68	12.63	13.44	13.15	
L.S.D. at 5%	U. =0.56 Phe. =1.02 U. x Phe. =2.27					U. =1.08 Phe. = 1.21 U. x Phe. =2.69				
Number of cormels/plant										
0	31.60	33.84	38.49	36.73	35.16	34.55	37.21	40.38	39.14	37.82
50	35.42	37.51	41.66	42.70	39.32	38.90	42.52	45.17	41.65	42.06
100	39.58	43.32	49.28	50.16	45.58	40.89	45.00	51.70	40.12	44.42
150	45.07	48.15	55.22	51.89	50.08	47.63	49.48	53.30	52.56	50.74
200	42.61	46.75	49.11	49.99	47.11	44.19	44.88	49.96	50.20	47.30
Mean	38.85	41.91	46.75	46.29		41.23	43.81	48.10	44.73	
L.S.D. at 5%	U. = 4.09 Phe. =3.57 U. x Phe. =7.96					U. =2.87 Phe. =3.04 U. x Phe. =6.78				
Dry weight of cormels (g/plant)										
0	4.12	4.37	4.82	4.90	4.55	4.52	4.93	5.02	4.81	4.82
50	4.95	5.18	5.50	5.23	5.21	5.20	5.37	5.80	5.84	5.55
100	5.17	5.40	5.72	5.76	5.51	5.61	6.03	6.40	6.29	6.08
150	5.63	6.30	6.92	6.85	6.42	6.11	6.46	7.18	7.01	6.69
200	5.22	5.63	6.12	6.26	5.80	5.81	6.31	6.29	6.17	6.14
Mean	5.01	5.37	5.81	5.80		5.45	5.82	6.13	6.02	
L.S.D. at 5%	U. =0.44 Phe. =0.89 U. x Phe. =1.98					U. =0.56 Phe. =0.84 U. x Phe. =1.87				

U.=Urea Phe.=Phenylalanine U. x Phe. =Intraction

2- Corms and cormels production

Data in Table (3) show that, increasing rates of phenylalanine from 0 to 200 mg/l led to a significant increase in corms and cormels production such as corms diameter (cm); corms fresh and dry weights (g/plant); number of cormels/plant as well as dry weight of cormels (g/plant). The most pronounced effects on these growth criteria were obtained as a result of phenylalanine application at 150 mg/l during the first and second seasons. This regulatory effect of amino acids on the production of corms and cormels could be explicated by the fact that, amino acids can improve the growth and the development through their influence on gibberellin biosynthesis **(Waller and Nowacki, 1978 and Cline and Trought, 2007).** In addition, **Bidwell (1979); Fowden (1973) and Hassanpouraghdam *et al.* (2011)** they reported that, amino acids act as the building blocks of proteins; regulation of metabolism as well as transport and storage of nitrogen. These findings supported by **Khattab *et al.* (2016)** on gladiolus plants and **Mazher *et al.* (2011)** on *codiaeum variegatum.*

Concerning the effect of mineral nitrogen fertilizer (urea) as soil application, data presented in Table (3) showed that, the production of corms and cormels were enhanced by the different application of urea compared with untreated plants in the first and second seasons. while, the highest significant enhancement in these parameters occurred by using the moderate level of urea fertilization (180 kg/fed). Stimulation effects of adding nitrogen on the growth may be attributed to the well-known functions of nitrogen in plant life, as described in the introduction. Our results are comparable to those obtained by **Zubair *et al.* (2013)** on gladiolus plants.

On the other hand, all combined treatments of mineral nitrogen fertilizer (urea) and phenylalanine concentrations resulted in a remarkable increments in these parameters, reached to the maximum at the moderate level of urea (180 kg/fed) with phenylalanine at (150 mg/l) in two seasons. Similar results were premeditation by **Sewedan *et al.* (2012)** on gladiolus plants.

3- Photosynthetic pigments and the content of total carbohydrate in the leaves

Results in Table (4) indicated that, spraying phenylalanine at different concentrations induced gladiolus leaves with intensive photosynthetic pigments i.e. chlorophyll a; b and carotenoids (mg/g of fresh weight) and total carbohydrate (%) in the leaves of gladiolus plants. Spraying phenylalanine at 100 mg/l gave the highest favorable effect on photosynthetic pigments (chlorophyll a; b and carotenoids) while spraying at 200 mg/l gave the greatest promoting effect on total carbohydrate (%) during the two growing seasons compared to the control. These results are in the same line with those obtained by **Khattab *et al.* (2016)** they found that, using different types of amino acids enhancing leaves chlorophyll content and total carbohydrate (%) in gladiolus plants.

Also, increasing addition of urea increased the values of photosynthetic pigments and total carbohydrate percentages in the leaves (Table, 4), where the highest increments in these variables occurred as a result of 240 kg/fed, except the amount of chlorophyll b (mg/g) in the second season and total carbohydrate (%) in the first season which reached its maxima at 180 kg urea/fed compared to the control. These results are in accordance with those obtained by **Ghatas (2016) and Mazhar and Eid (2016)** on gladiolus plants.

Steady increments in all of the above mentioned determinations were obtained by the interaction between treatments of urea and phenylalanine (Table, 4). It could be concluded that, the combined treatment of 120 kg urea/fed with 100 mg phenylalanine /l produced the highest values of chlorophyll a and b, also the greatest values of carotenoids were obtained by using the highest urea level (240 kg/fed) plus 100 mg/l phenylalanine compared to the control. In addition combined treatment of urea at 180 kg/fed with 200 mg/l phenylalanine produced the highest content of total carbohydrate (%) in the two seasons. The aforementioned results of photosynthetic pigments and total carbohydrate are coincidence with those obtained by **Ali and Hassan (2013)** on *Tagetes erecta,* L. plants.

4- Macro and micronutrients

Data in Table (5) demonstrate that, N; P and K concentrations (%) and Fe; Mn and Zn concentrations (mg/kg) in leaves of gladiolus plants. The results revealed that, increments in the contents of macronutrients (N; P and K %) occurred parallel with increasing phenylalanine concentrations in the spraying solutions. Application of phenylalanine at 100; 150 and 200 mg/l caused the highest contents (%) of N; P and K respectively, in the two seasons. While the highest contents (mg/kg) of micronutrients i.e. Fe; Mn and Zn were recorded by application of phenylalanine at 200 mg/l in the two seasons. These results are in agreements with those explored by **Yongin *et al.* (2003)** on various plant species and **Abdel-Mawgoud *et al.* (2011)** on green bean plants.

Also, data tabulated in Table (5) revealed that, the application of urea at different levels increased the contents of macro and micronutrients as (%) and (mg/kg), respectively. Urea application at rate of 180 kg/fed induced the highest increases in the contents of macro and micronutrients, except N value which reached its maximum at 240 kg urea /fed compared to the control. These results are in harmony with those obtained by **Sewedan *et al.* (2012)** on gladiolus plant and **Awaad *et al.* (2016)** on *Lactuca sativa.*

Generally, it could be concluded that, the combined treatments of urea and phenylalanine had a pronounced effect on the contents of macro and micronutrients. Dual application of both urea at 180 kg/fed + each of 100; 150 or 200 mg/l phenylalanine produced the highest contents (%) of N; P and K respectively, in the two seasons. Finally, the maximum contents of micronutrients were obtained from the treatment of 120 kg urea/fed combined with 200 mg phenylalanine/l, except the highest Fe content was found in plant treated with 180 kg urea/fed combined with 200 mg phenylalanine/l. Similar enhancing effects on macronutrients contents were noticed by **Ali and Hassan (2013)** on *Tagetes erecta,* L. plants, they mentioned that, commercial products like algaefol which contains amino acids and macro nutrient increased the values of N; P and K % in the dried leaves.

Table (4): Effect of different application rates of urea; phenylalanine and their interactions on photosynthetic pigments (mg/g) and total carbohydrate (%) in leaves of gladiolus plants during two growing seasons.

Urea rates (kg/fed) / Phenylalanine acid (mg/l)	First season					Second season				
	0	120	180	240	Mean	0	120	180	240	Mean
Chlorophyll A (mg/g)										
0	3.15	3.45	3.62	4.01	3.55	3.47	3.79	3.98	3.93	3.79
50	4.28	4.39	4.58	4.70	4.48	3.90	4.61	4.82	4.91	4.56
100	5.25	5.79	5.42	5.36	5.45	5.13	5.66	5.59	5.61	5.49
150	5.12	5.26	5.30	5.28	5.24	4.88	5.25	5.34	5.40	5.21
200	4.16	4.37	4.25	4.29	4.26	3.85	4.20	4.45	4.36	4.21
Mean	4.39	4.65	4.63	4.72		4.24	4.70	4.83	4.84	
L.S.D. at 5%	U.= NS	Phe.= 0.57		U. x Phe. =1.27		U.= 0.43	Phe.= 0.51		U. xPhe.=1.14	
Chlorophyll B (mg/g)										
0	1.09	1.18	1.36	1.59	1.30	1.33	1.42	1.53	1.75	1.50
50	1.74	1.81	1.86	1.93	1.83	1.69	1.93	2.02	1.87	1.87
100	2.16	2.54	2.40	2.27	2.34	2.49	2.76	2.80	2.50	2.63
150	2.14	2.04	2.31	2.39	2.22	2.31	2.37	2.41	2.27	2.34
200	1.58	1.69	1.83	1.72	1.70	1.72	1.68	1.81	1.90	1.77
Mean	1.74	1.85	1.95	1.98		1.90	2.03	2.11	2.05	
L.S.D. at 5%	U. =NS	Phe. =0.66		U. x Phe. =1.47		U. =NS	Phe. = 0.47		U. x Phe. =1.05	
Carotenoid (mg/g)										
0	2.27	2.35	2.43	2.30	2.33	2.11	2.25	2.50	2.62	2.37
50	2.62	2.69	2.53	2.40	2.56	2.44	2.49	2.60	2.51	2.51
100	3.18	3.32	3.25	3.60	3.33	2.98	3.19	3.40	3.55	3.28
150	3.20	3.19	3.21	3.09	3.17	3.15	3.24	2.90	2.97	3.06
200	2.54	2.77	2.68	2.93	2.73	2.35	2.47	2.62	2.58	2.50
Mean	2.76	2.86	2.82	2.86		2.60	2.72	2.80	2.84	
L.S.D. at 5%	U. = NS	Phe. =0.91		U. x Phe. =NS		U. =NS	Phe. = 0.72		U. x Phe. =NS	
Total carbohydrate (%)										
0	10.1	10.0	10.6	10.7	10.5	10.8	11.0	11.5	11.5	11.2
50	11.7	12.0	12.1	11.9	11.9	11.9	12.4	12.7	13.0	12.5
100	12.3	12.6	12.5	12.7	12.5	12.5	12.8	12.6	12.6	12.6
150	12.5	12.7	13.0	12.8	12.8	12.7	13.1	13.3	13.5	13.2
200	13.3	13.2	13.9	13.5	13.5	13.0	13.4	13.8	13.8	13.5
Mean	12.0	12.2	12.4	12.3		12.2	12.5	12.8	12.9	
L.S.D. at 5%	U.= 0.26	Phe. =0.34		U. x Phe. =0.75		U.= 0.42	Phe. = 0.67		U. x Phe.=1.49	

U.=Urea Phe.=Phenylalanine U. x Phe. =Intraction

Table (5): Effect of different application rates of urea; phenylalanine and their interactions on some macronutrients (%) and some micro-nutrients (mg/kg) of gladiolus plants during two growing seasons.

Urea rates (kg/fed) / Phenylalanine acid (mg/l)	First season					Second season				
	0	120	180	240	Mean	0	120	180	240	Mean
N (%)										
0	2.27	2.41	2.56	2.59	2.45	2.61	2.75	2.83	2.90	2.77
50	2.73	2.94	2.85	3.02	2.88	2.88	3.03	3.12	3.24	3.06
100	2.98	3.24	3.49	3.37	3.27	3.15	3.61	3.87	3.72	3.58
150	2.77	2.81	2.79	2.94	2.82	3.26	3.31	3.40	3.48	3.36
200	2.65	2.69	2.85	2.92	2.77	3.02	3.35	3.27	3.22	3.26
Mean	2.68	2.81	2.90	2.96		2.98	3.21	3.29	3.31	
L.S.D. at 5%	U.= 0.20	Phe. = 0.35		U. x Phe. = 0.78		U.= 0.16	Phe. = 0.42		U. x Phe. = 0.94	
P (%)										
0	0.21	0.23	0.24	0.22	0.22	0.25	0.27	0.27	0.25	0.26
50	0.25	0.27	0.29	0.29	0.27	0.28	0.30	0.32	0.29	0.29
100	0.26	0.28	0.30	0.31	0.28	0.31	0.33	0.34	0.32	0.32
150	0.29	0.33	0.35	0.29	0.31	0.29	0.35	0.37	0.37	0.34
200	0.27	0.27	0.31	0.25	0.27	0.30	0.31	0.29	0.29	0.29
Mean	0.25	0.27	0.29	0.27		0.28	0.31	0.31	0.30	
L.S.D. at 5%	U. =NS	Phe. = NS		U. x Phe. = NS		U. =NS	Phe. =NS U. x Phe. = NS			

K (%)										
0	1.25	1.28	1.31	1.29	1.28	1.33	1.37	1.37	1.38	1.36
50	1.40	1.43	1.41	1.38	1.40	1.36	1.41	1.45	1.40	1.40
100	1.36	1.42	1.42	1.47	1.41	1.41	1.46	1.43	1.41	1.42
150	1.45	1.47	1.50	1.48	1.47	1.44	1.44	1.49	1.46	1.45
200	1.51	1.54	1.54	1.50	1.52	1.48	1.52	1.58	1.53	1.52
Mean	1.39	1.42	1.43	1.42		1.40	1.44	1.46	1.43	
L.S.D. at 5%	U. = NS	Phe. = 0.18	U. x Phe. = NS			U. = NS	Phe. = 0.NS	U. x Phe. =NS		
Fe (mg/kg)										
0	44.10	46.20	48.70	49.00	47.00	39.70	40.90	43.20	45.30	42.27
50	47.60	50.30	52.40	49.80	50.02	44.10	47.50	49.10	50.50	47.80
100	53.90	55.10	52.30	52.60	53.47	51.60	54.90	57.30	56.80	55.15
150	54.90	57.00	58.40	60.20	57.62	58.30	59.10	64.40	59.80	60.40
200	55.80	59.50	62.70	60.40	59.60	57.90	61.50	65.70	65.00	62.52
Mean	51.26	53.62	54.90	54.40		50.32	52.78	55.94	55.48	
L.S.D. at 5%	U. =2.04	Phe. =3.14	U. x Phe. =7.00			U. =2.31	Phe. = 4.29	U. x Phe. =9.57		
Mn (mg/kg)										
0	22.10	24.50	25.70	23.90	24.05	24.80	24.70	26.90	27.50	25.97
50	25.30	27.60	27.50	29.10	27.37	25.10	28.30	29.80	31.50	28.67
100	28.00	29.70	31.40	26.20	28.82	27.20	29.40	30.30	32.70	29.90
150	27.30	29.10	29.80	31.90	29.52	28.40	30.60	27.00	27.50	28.37
200	30.90	33.50	31.20	29.60	31.30	29.60	32.60	32.10	30.50	31.20
Mean	26.72	28.88	29.12	28.14		27.02	29.12	29.22	29.94	
L.S.D. at 5%	U. =1.11	Phe. = 2.14	U. x Phe. =4.77			U.= 2.00	Phe. = 2.64	U. x Phe. =5.89		
Zn (mg/kg)										
0	23.50	24.20	24.30	26.40	24.60	25.10	27.40	28.40	27.50	27.10
50	24.60	26.90	28.70	27.10	26.82	27.20	30.60	29.60	27.80	28.80
100	23.10	27.50	30.60	28.70	27.47	25.70	28.90	30.10	29.00	28.42
150	27.60	29.30	27.10	27.20	27.80	26.50	28.70	28.40	31.10	28.67
200	27.40	31.30	28.80	25.70	28.30	30.80	34.10	32.30	33.20	32.60
Mean	25.24	27.84	27.90	27.02		27.06	29.94	29.76	29.72	
L.S.D. at 5%	U. =1.02	Phe. = 1.51	U. x Phe. =3.36			U.=1.06	Phe. =1.33	U. x Phe. =2.96		

U.=Urea Phe.=Phenylalanine U. x Phe. =Intraction

Conclusion

The combined effect of urea and amino acid (phenylalanine) gave better results in increasing the yield of gladiolus plants. The combination of N-mineral and amino acid can be considered as an integrated nutrient management to improve the soil fertility and gladiolus yield. Using phenylalanine enhanced also the efficiency of mineral nitrogen fertilizer, wherever the high qualitative and quality of gladiolus yield were obtained by application of 75 % of recommended dose of urea fertilizer. So, Application of phenylalanine may be used to rationalize the use of mineral – N fertilization. This has both a good environmental and economic potential

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