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Effect of Some Preservative Solutions on Vase Life of Gladiolus Cut Flowers

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ABSTRACT: Keeping quality and length of vase life are important factors for evaluation of cut flowers quality, for both domestic and export markets. These investigations proposed to determine the effectiveness of some preservative solutions as glutamic acid (100, 200 and 300 mg/l), salicylic acid (200,400 and 600 mg/l), calcium chloride (1000, 2000 and 3000 mg/l) and aluminum sulphate (100, 200 and 300 mg/l) on quality parameters of (*Gladiolus grandiflorus* L. cv. 'Rose Supreme') flowers. Results showed that all treatments significantly increased the vase life, fresh weight, water uptake and chlorophyll content with decreasing number of bacteria and proline accumulation compared to control. The highest increase in fresh weight, water uptake and chlorophyll index was obtained by glutamic acid (300 mg/l), salicylic acid (400 mg/l), calcium chloride (3000 mg/l) and aluminum sulphate (300 mg/l). Whilst, glutamic acid (300 mg/l) and salicylic acid (400) resulted in the maximum vase life in both experiments, in addition, glutamic acid (200 mg/l) and aluminum sulphate (200 and 300 mg/l) in second experiment. Moreover, glutamic acid (300 mg/l) recorded the most effect on decreasing number of bacteria and proline accumulation in both experiments, furthermore salicylic (400 and 600 mg/l), calcium chloride (3000 mg/l) and aluminum sulphate (100, 200 and 300 mg/l) on number of bacteria and salicylic acid (200 and 400 mg/l), calcium chloride (3000 mg/l) and aluminum sulphate (100, 200 and 300 mg/l) on proline accumulation in first experiment compared to control.

Key words: Gladiolus, vase life, preservative solutions, cut flowers

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) is an ornamental plant native to South Africa. It belongs to family Iridaceae, having approximately one hundred and fifty known species. This plant is commercially used for cut flowers and occasionally used for landscape purpose. Gladiolus is one of the few plants which produce pleasant cut flowers with long spikes. It is cultivated in most of the tropical and subtropical countries of the world (Adil *et al*., 2013). Gladiolus flowers are considered main exportable ornamental plants in Egypt, and the flower can be available year-around, the foreign markets demand Egyptian Gladiolus with higher quality (Abo-Leila and Eid, 2011). In addition, there is high demand of Gladiolus in the world as cut flower. In USA, 60 million Gladiolus spikes were sold in the market having worth of 16 million dollars which is 4.5% of total produced cut flowers in 2011 (USDA, 2012). Gladiolus is a popular cut flower in the world, but its longevity is very short. The vase life of individual florets is 4 to 6 days. Life of cut flowers is mainly affected by two main factors, namely ethylene which accelerates the senescence of many flowers and by microorganisms which cause vascular blockage and thus reduces the vase life of cut flowers (Mohammadi *et al.,* 2014). Several chemicals solutions were used as pulsing or preservative solutions for increasing the longevity of cut flowers. Those chemicals are very expensive and most harmful preservative for human causing irritating to skin, eyes and respiratory tract as well as using natural products did not have large attentions as safe materials in vase

solutions (Mohamed, 2015). The maintenance of vase life is an important quality attribute in these economically significant cut flowers. A suitable method for vase life extension, which easy to use, natural, safe and inexpensive compounds is always crucial in this respect for large-scale applications (Soleimany-Fard *et al.,* 2013). Glutamine, a multifaceted amino acid used as an energy substrate for most cells. Glutamine plays an important role in the nitrogen and carbon skeleton exchange among different tissues, where this amino acid fulfils many different physiological functions (Zamani *et al*., 2011). Salicylic acid (SA) is considered to be a potent plant hormone because of its diverse regulatory roles in plant metabolism. SA has been found to play a key role in the regulation of plant growth, development and in responses to environmental stresses. Further, its role is evident in ion uptake and transport, photosynthetic rate, stomatal conductance and transpiration (Tehranifar *et al.,* 2013). Calcium chloride is widely used as preservative and firming agent in the fruits and vegetables industry for whole and fresh-cut commodities (Martin-Diana *et al.,* 2007). Aluminum sulphate is used as an antimicrobial compound in commercial preservative solutions (Zadeh and Mirzakhani, 2012). Aluminum sulphate acidifies vase solution, diminishes bacterial proliferation and enhances water uptake (Liao *et al.,* 2001). The aims of this study were to determine the effectiveness of some materials (glutamic acid, salicylic acid, calcium chloride and aluminum sulphate) as preservation treatments to reduce Gladiolus cut flowers senescence and increase vase life. Moreover, to find out the most effective concentration among materials used to produce the best quality cut flower with longer vase life.

MATERIALS AND METHODS

Two separated experiments were conducted in the Plant Production Department, Faculty of Agriculture, Saba Basha, Alexandria University in (April and November, 2015) on Gladiolus cut flowers. This study was carried out to study the effect of glutamic acid, salicylic acid, calcium chloride and aluminum sulphate treatments on vase life of Gladiolus cut flowers (Rose Supreme variety). Cut flowers were obtained from a well-known commercial nursery in Cairo. Cut spikes were cut from the field in early morning, wrapped with polyethylene sheet, and then quickly moved to the laboratory, of an average temperature of (18° C ±1) and (50- 60 %) relative humidity and light from a white fluorescent lamp. Each stem was recut to a length of 60cm before postharvest treatments. Leaves of the lower third part of the stem were removed to avoid contamination in the vase solution as recommended by Khimani *et al.* (2005). After that, flower stems were pulsed in freshly solutions which prepared at the start of experiments from (concentrations of glutamic acid, salicylic acid, calcium chloride, aluminum sulphate) in plastic container for 24 hours. Then the flowers were moved to glass containers (vases) which contained 300 ml of tap water to calculate the vase life and the tested parameters.

Treatments and design

The treatments were arranged in a factorial experiment with Randomized Complete Block Design (RCBD) in two factors (Materials and Concentrations) with 3 replications. All data obtained throughout the course of this study were statistically analyzed by the analysis of variance as described by Steel and Torrie (1980), all analysis were done by Average of SAS (2002) statistical software. Cut flowers were pulsed in concentrations of glutamic acid (100, 200 and 300 mg/l), salicylic acid (200, 400 and 600 mg/l), calcium chloride (1000, 2000 and 3000 mg/l) and aluminum sulphate (100, 200 and 300 mg/l) with Litter tap water and 1% sucrose in the same time, control cut flowers pulsed in 2liter tap water and 1% sucrose for 24 hours.

Vase Life (days):

Was determined when the seventh floret in the spike wilted as recommended by Badr *et al.* (2008).

Total Fresh Weight (g):

The average fresh weight of fresh stems carrying leaves and the flowers were calculated at the full opening stage (Barakat, 2013).

Water Uptake (g):

The volume of water uptake was calculated by subtracting the volume of water evaporated from a control vase without cut flowers and the amount of water decreased in vases containing flowers (Zamani *et al.,* 2011).

Chlorophyll Index (SPAD):

Chlorophyll index was measured by chlorophyll meter (SPAD- 502, Minolta Co. Japan). Average of 3 measurements from different spots of a single leaves was considered (Yadava, 1986).

Number of Bacteria (CFU/ml):

Bacterial contamination was determined in the keeping solution at the end of experiment. The samples of the preservative solutions were taken (1 ml of each) and diluted using sterilized distilled water. One ml of each diluted solution was streaked on nutrient agar into Petri dishes. Cultures were incubated 2 days at 28°C and the colonies appearing on the plates were counted. This experiment was repeated two times with 3 replicates in each treatment at the laboratory of Microbiology Department, Faculty of Agriculture, Saba Basha, Alexandria University (Gendy and Mahmoud, 2012).

Determination of Proline Content in Leaves (µg proline/g):

Proline colorimetric determination proceeded according to Bates *et al.* (1973), Marin *et al.* (2009) based on proline's reaction with ninhydrin. For proline colorimetric determinations, a 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. The reaction was arrested in an iced bath and the cromophore was extracted with 4 ml toluene and its absorbance at 520 nm was determined in a Bio Mate spectrophotometer (Thermo Spectronic).

RESULTS AND DISSCUSSION

Total Fresh Weight (g)

Data in Tables 1 and 2 generally, revealed that, all treatments significantly increased fresh weight compared with the control in first and second experiment (April and November, 2015). In addition, the statistically analyzed data indicated that glutamic acid (300 mg/l), salicylic acid (400 mg/l), calcium chloride (3000 mg/l) and aluminum sulphate (300 mg/l) were more effective on increasing fresh weight than other treatments. The change in fresh weight of Gladiolus cut flowers was increased with increasing vase life periods.

Results are similar to those of Zamani *et al.* (2011) on Rose cut flower and Mazher *et al.* (2011) on *Codiaeum variegatum* L. plants, they observed that treatments with glutamic acids had a significant effect on fresh weight. These results seemed to be due to the reduction of MDA (malonyldialdehyde) accumulation, the microbial populations on vase solution of cut flower and ACC oxidase activity (Aminocyclo propane carboxylate oxidase) and improved membrane stability confirmed by Aran *et al.* (2011) similarly Kazemi *et al.* (2012c).

With regard to salicylic acid treatments, the findings proved to be in accordance to results of Mashhadian *et al.* (2012) on Chrysanthemum and Marandi *et al.* (2011) on Gladiolus, they reported that salicylic acid enhanced the fresh weight also, Sabzi *et al.* (2012) and Ashtari *et al.* (2013) on Rose cut flowers. We could return this increase of fresh weight by treatments of salicylic acid to its antimicrobial activity (inhibiting vascular blockage), it increases water uptake and decrease transpiration rate, thereby enhancing water balance of cut flowers which might be because of the possibility of salicylic acid to decrease pH of vase solution and consequently, the growth and proliferation of bacteria is reduce, which increase water uptake as proved by Soleimany-Fard *et al.* (2013).

Similarly, Sardoei (2014) and Ibrahim *et al.* (2011) on *Narcissus tazetta* and *Gerbera jamesonii*; respectively indicated that the effect of calcium chloride on fresh weight of cut flowers was significant. These results may be referred to the role of calcium in maintenance and modulation of various cell functions as the main role of integrated biocide in floral preservatives is to sustain clarity in vase solution and to avoid blockage of xylem elements by microorganisms as confirmed by Sardoei (2014). Moreover, Cortes *et al.* (2011) indicated that calcium increases tissue resistance by slowing senescence because it inhibits the synthesis or action of ethylene.

On other hand, Viradia *et al.* (2015) and Seyf *et al.* (2012) observed that aluminum sulphate significantly enhanced the fresh weight for longer period in Tuberose and Rose cut flowers compared to control treatments. In general, aluminum sulfate had significant effect on fresh weight loss, this might be related to solution uptake enhancement, improved water relations and prevent vascular blockage by microorganisms which finally resulted extension in vase life as indicated by Mohammadi *et al.* (2012) and Hussen and Yassin (2013).

Regarding the effect of vase life periods on the change in fresh weight of Gladiolus cut flowers it was found that fresh weight increased with increasing vase life periods and the differences among all tested vase life periods were statistically significant, except for the last sampling date (20 days) where the difference was significantly decreased in two separated experiment compared with initial time. The increase in fresh weight might be due to the improvements in water balance which is a major factor determines quality and longevity of cut flowers. It is influenced by water uptake and transpiration, being balance between these two processes. Low water uptake is often due to occlusions located mainly in the basal stem end and microbes are common cans of stem end blockage as described by Hajizadeh *et al.* (2012) and Sardoei (2014) who observed that obstruction of the xylem by bacteria, therefore, inability of water absorption by flower steams is one of the current problems that lead to decrease in flowers postharvest longevity and also early welter of them**.**

Table (1). Effect of some preservative solutions on fresh weight (g) of Gladiolus cut flowers "Rose Supreme" in the first experiment (April, 2015).

Total Fresh Weight (g) (April, 2015)						
Treatments	**Vase Life (Days)**					
	Initial Time	**5**	**10**	**15**	**20**	**Average**
Control	31.87e	53.04g	51.81e	37.13 d	14.07f	**37.58f**
Glutamic acid 100 mg/l	33.15d	55.49cde	53.55bcd	39.33bc	16.36cde	**39.58de**
Glutamic acid 200 mg/l	33.43c	55.72cd	54.25b	39.59bc	16.31cde	**39.86cd**
Glutamic acid 300 mg/l	33.81a	56.52a	56.41a	41.58a	18.25a	**41.31a**
Salicylic acid 200 mg/l	33.62b	55.34def	52.97cde	39.42bc	16.24cde	**39.52de**
Salicylic acid 400 mg/l	33.9a	56.25ab	55.77a	41.43a	18.03a	**41.08a**
Salicylic acid 600 mg/l	33.36c	55.67cd	52.50de	39.21bc	15.37e	**39.22e**
Calcium chloride 1000mg/l	33.12d	55.14ef	53.33bcd	39.26bc	15.71de	**39.31e**
Calcium chloride 2000mg/l	33.39c	55.73cd	53.69bcd	39.38bc	16.76bcd	**39.79cd**
Calcium chloride 3000mg/l	33.64b	56.21ab	55.64a	39.85b	17.65ab	**40.60b**
Aluminum sulphate 100mg/l	33.05d	55.00f	52.99cde	38.57c	16.20de	**39.16e**
Aluminum sulphate 200mg/l	33.34c	55.36def	53.37bcd	39.58bc	16.50cd	**39.63de**
Aluminum sulphate 300mg/l	33.58b	55.85bc	54.11bc	40.16b	17.34abc	**40.21bc**
Average	**33.33d**	**55.49 a**	**53.88 b**	**39.58c**	**16.52e**	**39.76**
LSD at 0.05	**T: 0.47**		**D: 0.29**		**T×D: 1.07**	
T: Treatments	**D: Vase Life**		**T×D: Interaction**			

Table (2). Effect of some preservative solutions on fresh weight (g) of Gladiolus cut flowers "Rose Supreme" the in second experiment (November, 2015).

Total Fresh Weight (g) (November, 2015)						
Treatments	Vase Life (Days)					
	Initial Time	5	10	15	20	Average
Control	31.19c	55.77g	51.05e	31.98c	22.42g	**38.48d**
Glutamic acid 100 mg/l	31.24c	58.31edf	52.72cde	36.06ab	25.18cd	**40.70bc**
Glutamic acid 200 mg/l	31.54b	59.40cde	53.25cd	36.09ab	25.04cde	**41.06bc**
Glutamic acid 300 mg/l	31.89a	63.00a	57.67a	37.34a	28.83a	**43.74a**
Salicylic acid 200 mg/l	31.35bc	58.51def	53.18cd	36.18ab	23.84ef	**40.61bc**
Salicylic acid 400 mg/l	31.84a	61.28ab	56.93a	37.26a	27.52b	**42.97a**
Salicylic acid 600 mg/l	31.55b	58.57def	52.28de	33.95bc	23.25fg	**39.92c**
Calcium chloride 1000 mg/l	31.21c	57.01fg	54.48bc	36.15ab	23.95ef	**40.56bc**
Calcium chloride 2000 mg/l	31.39bc	58.02ef	54.48cd	34.84ab	25.28cd	**40.66bc**
Calcium chloride 3000 mg/l	31.57b	59.92bcd	56.18ab	34.11bc	25.96c	**41.55b**
Aluminum sulphate 100 mg/l	31.35bc	57.01fg	53.40cd	35.73ab	24.56de	**40.41bc**
Aluminum sulphate 200 mg/l	31.51b	58.47def	53.49cd	33.93bc	24.17def	**40.31bc**
Aluminum sulphate 300 mg/l	31.39bc	60.61bc	53.8cd	34.80ab	24.1def	**40.94bc**
Average	**31.46d**	**58.91a**	**54.02b**	**35.26c**	**24.93e**	**40.92**
LSD at 0.05	**T: 1.34**		**D: 0.83**		**T×D: 3.05**	
T: Treatments	**D: Vase Life**		**T×D: Interaction**			

Water Uptake (g)

Data in Tables 3 and 4 revealed that all used materials significantly enhanced water uptake compared with the control in first and second experiment (April and November, 2015).Data also showed that glutamic acid (300 mg/l), salicylic acid (400 mg/l), calcium chloride (3000 mg/l) and aluminum sulphate (300 mg/l) caused the greatest water uptake. The increase of water uptake in glutamic acid pulsed stems might be due to decreasing accumulation of bacteria in vase solution which increased water absorption and ACC- oxidase activity (Aminocyclo propane carboxylate oxidase) which relatively has affected on the senescence process as proved by Aran *et al.* (2011). These results are compatible with the findings of Kazemi *et al.* (2012a) on Gerbera, who indicated that glutamine treatments increased cut flowers water absorption.

The enhancing effect of salicylic acid on water uptake may be related to the role of salicylic acid in reducing the microbial population in vase solution of cut flowers and/or positive regulatory role of SA on stomatal closure which regulates the rates of transpiration and increases the water-retaining capacity of leaves and petals as demonstrated by Kazemi *et al.* (2011a, b, and c) and

Khenizy *et al.* (2013). In addition, the role of salicylic acid is evident in ion uptake and transport and also photosynthetic rate, stomatal conductance and transpiration Khan *et al*. (2003). Similar results were obtained by Kazemi *et al.* (2011c) on Gerbera, Zadeh and Mirzakhani (2012) on Carnation and Soleimany-Fard *et al.* (2013) on Alstroemeria cut flower, they revealed that salicylic acid increased water absorption compared to control.

Concerning calcium chloride, results are similar with Sardoei (2014) findings on Narcissus (*Narcissus tazetta*), Ibrahim *et al.* (2011) on Gerbera, Farahat and Gaber (2009) on *Monestera deliciosa* and Cortes *et al.* (2011) on Rosa hybrid, they revealed that calcium chloride treatments increased water uptake compared to control. Thus seemed to be referred to the important role of calcium in increasing tissue resistance and delaying senility through preventing ethylene synthesis and it's processing. It was shown that the use of calcium in vase solutions increases water flow through the stem by association with pectin in the xylem cell walls (Zadeh and Mirzakhani 2012).

Regarding aluminum sulphate, results are consistent with Nader *et al.* (2015), Seyf *et al.* (2012) on Rose cut flowers. These significant effect of aluminum sulphate on water uptake which observed in Tables 3 and 4 might be attributed to the action of aluminum sulphate which inhibited vascular blockage and increased absorption of water, ultimately increased the uptake of water in the spike Viradia *et al.* (2015) and Tsegaw *et al.* (2011).

Results also indicated that water uptake of Gladiolus cut flowers was increased with increasing vase life periods and the differences among all tested vase life periods were statistically significant, except for the last sampling date (20 days) where the difference was significantly decreased in both experiments compared with initial time. The decrease in water uptake of cut flowers during vase period was probably due to growth of microbes and vascular blockage suggesting that adding a suitable germicide in vase solution can prevent the growth of microbes and can increase water uptake as confirmed by Anjum *et al.* (2001). Hashemabadi *et al.* (2015) demonstrated that enhancement of vase life can be described with antimicrobial properties of the mentioned above compounds, so that water absorption improved with prevention of vascular blockage and it delays water deficiency related wilting and reported that anti-ethylene compounds and also antibiotics increase water absorption, significantly.

Table (3). Effect of some preservative solutions on water uptake (g) of Gladiolus cut flowers "Rose Supreme" in the first experiment (April, 2015).

water uptake (g) (April, 2015)						
Treatments	Vase Life (Days)					
	Initial Time	5	10	15	20	Average
Control	30.01e	58.91d	50.66d	40.26e	16.64d	**39.30e**
Glutamic acid 100 mg/l	32.13d	61.88bc	54.37bc	42.21bcd	18.43bc	**41.80cd**
Glutamic acid 200 mg/l	32.26cd	62.07bc	54.96bc	42.51bcd	19.25b	**42.21cd**
Glutamic acid 300 mg/l	33.65a	65.00a	55.98ab	45.53a	20.87a	**44.21a**
Salicylic acid 200 mg/l	32.31cd	62.93b	57.03a	42.17bcd	19.04bc	**42.70cd**
Salicylic acid 400 mg/l	33.15abc	65.02a	56.16ab	45.66a	20. 60a	**44.12ab**
Salicylic acid 600 mg/l	32.51bcd	61.37bc	53.36c	40.82de	18.60bc	**41.33d**
Calcium chloride 1000mg/l	31.96d	62.12bc	53.59c	41.74cde	18.25c	**41.5cd**
Calcium chloride 2000mg/l	32.15d	61.13bc	54.64bc	41.79cde	18.51bc	**41.33cd**
Calcium chloride 3000mg/l	33.14abc	62.79b	55.58ab	43.39bc	18.86bc	**42.75bcd**
Aluminum sulphate 100mg/l	32.06d	60.76cd	54.65bc	42.13bcd	18.29c	**41.58cd**
Aluminum sulphate 200mg/l	32.02d	61.9bc	53.51c	43.41bc	18.76bc	**41.92cd**
Aluminum sulphate 300mg/l	33.28ab	62.62bc	55.22abc	43.65b	19.02bc	**42.76bc**
Average	**32.36d**	**62.19a**	**54.60b**	**42.72c**	**18.86e**	**42.14**
LSD at 0.05		T: 1.42	D: 0.88		T×D: 3.21	
T: Treatments		D: Vase Life	T×D: Interaction			

Table (4). Effect of some preservative solutions on water uptake (g) of Gladiolus cut flowers "Rose Supreme" in the second experiment (November, 2015).

water uptake (g) (November, 2015)						
Treatments	Vase Life (Days)					
	Initial Time	5	10	15	20	Average
Control	27.07f	64.03d	42.24e	28.10d	18.57e	**36.00f**
Glutamic acid 100 mg/l	28.24cde	65.78cd	45.83bc	29.71c	19.20de	**37.75de**
Glutamic acid 200 mg/l	28.48bc	66.15cd	45.47cd	30.00c	19.86cd	**37.99cd**
Glutamic acid 300 mg/l	29.21a	70.77a	48.62a	32.58a	21.31a	**40.49a**
Salicylic acid 200 mg/l	28.25cde	67.14bc	45.43cd	29.58c	19.09de	**37.90cde**
Salicylic acid 400 mg/l	29.21a	71.26a	48.41a	32.6a	21.05ab	**40.51a**
Salicylic acid 600 mg/l	28.44bcd	65.08cd	43.64de	29.70c	18.84e	**37.14e**
Calcium chloride 1000 mg/l	27.99e	66.65bc	44.01cde	29.54c	19.01de	**37.44de**
Calcium chloride 2000 mg/l	28.20cde	66.47c	45.52cd	30.07c	19.45cde	**37.94cde**
Calcium chloride 3000 mg/l	28.78b	69.01ab	47.55ab	31.25b	19.88cd	**39.29b**
Aluminum sulphate 100 mg/l	28.04de	65.33cd	44.63cd	29.60c	19.29de	**37.38de**
Aluminum sulphate 200 mg/l	28.52bc	65.95cd	45.22cd	29.90c	19.82cd	**37.88cde**
Aluminum sulphate 300 mg/l	28.82ab	67.03bc	45.69bc	31.12b	20.22bc	**38.58bc**
Average	**28.40d**	**66.97a**	**45.56b**	**30.29c**	**19.66e**	**38.18**
LSD at 0.05		T: 0.81	D: 0.50		T×D: 1.83	
T: Treatments		D: Vase Life	T×D: Interaction			

Total Chlorophyll (SPAD)

Data of the present investigation, listed in Tables 5 and 6 showed that in both experiments (April and November, 2015), all treatments caused delay in degradation of total chlorophyll and preserved total chlorophyll content

compared with the control. Moreover, statistical analysis of these data proved that the most significant increase in chlorophyll leaf content than other treatments was recorded by treatment of glutamic acid (300 mg/l), salicylic acid (400 mg/l), calcium chloride (3000 mg/l) and aluminum sulphate (300 mg/l). On the other hand, our data in the first experiment (April, 2015) revealed that the change in total chlorophyll content of Gladiolus cut flowers was increased with increasing vase life periods.

We could attribute the delay of chlorophyll degradation in flowers which pulsed in glutamic acid to its action on inhibiting ACC-oxidase activity (Aminocyclo propane carboxylate oxidase) that is the direct precursor of ethylene and decrease ROS (reactive oxygen species) with increase enzyme antioxidant activity decrease number of bacteria and ACC-oxidase activity (Kazemi and Ameri, 2012a). These results were found in agreement with those of Kazemi *et al.* (2012a) and are in accordance with those of Kazemi and Ameri (2012a) on Carnation. Similarly, Zamani *et al.* (2011) on Rose cut flowers.

Likewise, results of salicylic acid were found in accordance to those of Bayat *et al.* (2011) and Kazemi *et al.* (2012c) on Carnation cut flowers and Mohammadi *et al.* (2014) on Gladiolus cut flowers. The effect of salicylic acid might be due to the effect of salicylic acid that affect postharvest life of cut flowers probably via the declining bacterial growth, reducing vascular blockage, reducing transpiration, preventing ethylene formation and inducing antioxidant system in treated cut flowers thereby delaying the senescence process as reported by Danaee *et al*. (2013).

With respect to calcium chloride, results were found in conformity with those of Abdolmaleki *et al.* (2015) on Rose and Zadeh and Mirzakhani (2012) on Carnation, they indicated that calcium chloride have significantly increased chlorophyll content in leaves. These results may due to the important role in increasing tissue resistance and delaying senility through preventing ethylene synthesis and its processing as found by Zadeh and Mirzakhani (2012) and Cortes *et al.* (2011). On the other hand, this salt is already known to lower the respiration as confirmed by Anjum *et al.* (2001). In addition, the calcium ion also seems to affect ethylene action on cell membranes by inhibiting ion leakage and reducing the effect of ethylene on senescence as proved by Asfanani *et al.* (2008).

Effectiveness of aluminum sulphate on delaying the degradation of chlorophyll might be referred to the role of aluminum sulphate as a microbial inhibitor, reducing the transpiration rate and stimulate the minimum ethylene production as reported by Hashemabadi *et al.* (2015), Mohammadi *et al.* (2012) and Tsegaw *et al.* (2011). They are in harmony with findings of Jowker *et al.* (2012) and Hajizadeh *et al.* (2012) on Rose, they stated that aluminum sulphate lead to a considerable delay in degradation of chlorophyll compared to control.

On the other hand, present data in the first experiment (April, 2015) revealed that the change in total chlorophyll content of Gladiolus cut flowers was increased with increasing vase life periods and the differences among all

tested vase life periods were statistically significant, except for the last three dates of sampling (10,15 and 20 days) where the difference was significantly decreased compared with initial time, while the data showed a significant increase in chlorophyll content with increasing periods of the life of flowers of Gladiolus cut flowers in the second experiment (November, 2015). Moreover, there were significant differences between all tested stages of vase life, except for the latest stage (20 days) which decreased compared with initial time. The greatest value in chlorophyll content was obtained by 5th day compared to 0 day and 20th day in both experiments of Gladiolus (10th and 15th day in first experiment). Leaf yellowing is a form of senescence caused by an internal hormone imbalance, such as a lack of cytokinins as confirmed by Ferrante *et al.* (2004). The maintenance of green color in the leaves is an important quality propertys in these economically significant ornamental plants. Previous study had revealed that the leaf yellowing of cut flowers is associated with chlorophyll breakdown and loss, thereby decreasing significant vase life Jahanbazi *et al.* (2014).

Table (5). Effect of some preservative solutions on chlorophyll index (SPAD) of Gladiolus cut flowers "Rose Supreme" in the first experiment (April, 2015).

Chlorophyll index (SPAD) (April, 2015)						
Treatments	**Vase Life (Days)**					
	Initial Time	**5**	**10**	**15**	**20**	**Average**
Control	46.63c	48.43f	46.01d	42.91c	40.01e	**44.80f**
Glutamic acid 100 mg/l	47.96b	50.43cde	48.22c	44.73bc	42.89cd	**46.85de**
Glutamic acid 200 mg/l	48.58b	50.67bcde	48.58c	45.04ab	43.41cd	**47.26de**
Glutamic acid 300 mg/l	51.56a	52.63a	50.17a	48.54a	45.9a	**49.75a**
Salicylic acid 200 mg/l	48.08b	50.96bc	48.49c	44.82bc	43.49cd	**47.17de**
Salicylic acid 400 mg/l	51.19a	52.93a	49.88ab	48.49a	46.12	**49.72a**
Salicylic acid 600 mg/l	50.38a	50.81bcd	48.59c	44.39bc	42.58cd	**47.35cde**
Calcium chloride 1000 mg/l	48.26b	49.97e	48.35c	43.83bc	42.92cd	**46.67de**
Calcium chloride 2000 mg/l	48.58b	50.68bcde	48.36c	43.98bc	43.36cd	**46.99de**
Calcium chloride 3000 mg/l	50.83a	51.27b	48.83c	45.02bc	44.17c	**48.02bc**
Aluminum sulphate 100 mg/l	48.03b	50.07de	48.51c	44.62bc	41.97d	**46.64e**
Aluminum sulphate 200 mg/l	48.62b	50.97bc	48.86bc	45.72b	42.80cd	**47.39cd**
Aluminum sulphate 300 mg/l	50.36a	51.45b	49.02bc	46.04ab	44.22bc	**48.22b**
Average	**49.16b**	**50.87a**	**48.60c**	**45.24d**	**43.37e**	**47.45**
LSD at 0.05	**T: 0.74**		**D: 0.46**		**T×D: 1.67**	
T: Treatments	**D: Vase Life**		**T×D: Interaction**			

Table (6). Effect of some preservative solutions on chlorophyll index (SPAD) of Gladiolus cut flowers "Rose Supreme" in the second experiment (November, 2015).

Chlorophyll index (SPAD) (November, 2015)						
Treatments	**Vase Life (Days)**					
	Initial Time	**5**	**10**	**15**	**20**	**Average**
Control	48.94f	61.04f	57.93c	51.04c	48.18d	**53.43g**
Glutamic acid 100 mg/l	49.85de	62.87def	60.48b	54.11b	48.74d	**55.21f**
Glutamic acid 200 mg/l	50.21bcd	63.44de	60.54b	55.65ab	49.09bcd	**55.79def**
Glutamic acid 300 mg/l	51.00a	67.30a	64.33a	56.81a	51.51a	**58.19a**
Salicylic acid 200 mg/l	50.02cde	63.66cde	60.21bc	55.3ab	49.01bcd	**55.64def**
Salicylic acid 400 mg/l	50.97a	66.99a	64.19a	55.12ab	51.13a	**57.68ab**
Salicylic acid 600 mg/l	50.10cd	63.11de	60.12bc	55.55ab	49.14bcd	**55.60def**
Calcium chloride 1000mg/l	49.51e	63.81cde	60.33b	53.90b	48.34d	**55.18f**
Calcium chloride 2000mg/l	49.68de	65.52abc	60.93b	55.75ab	48.98cd	**56.17cde**
Calcium chloride 3000mg/l	50.50abc	66.12ab	61.81b	56.66a	49.87bc	**56.99bc**
Aluminum sulphate 100mg/l	49.91de	62.58ef	59.92bc	55.58ab	48.82d	**55.36ef**
Aluminum sulphate 200mg/l	49.95de	64.46bcde	60.79b	55.78ab	48.78d	**55.95def**
Aluminum sulphate 300mg/l	50.73ab	64.54bcd	59.67bc	56.52a	50.00b	**56.29cd**
Average	**50.11d**	**64.27a**	**60.87b**	**55.21c**	**49.35e**	**55.96**
LSD at 0.05		**T: 0.88**	**D: 0.55**		**T×D: 1.99**	
T: Treatments		**D: Vase Life**	**T×D: Interaction**			

Determination of Proline (µg proline/g)

The effect of some preservative solutions on accumulation of proline in leaves of Gladiolus cut flowers in both experiments (April and November, 2015) are presented in Table (7). It has been found that all studied materials had remarkable significant effect on decreasing accumulation of proline compared to control in both experiments. Except for glutamic acid at (100 and 200 mg/l), the last concentration of salicylic acid (600 mg/l) and calcium chloride at (1000 and 2000 mg/l) which did not show significant differences in comparison with control in the first experiment. Moreover, there were no significant differences between the other treatments, except the differences between intermediate level of aluminum sulphate (200 mg/l) compared to the first level of aluminum sulphate (100 mg/l) in the first experiment. While, the greatest effect on decreasing accumulation of proline was recorded by the higher concentration of glutamic acid (300 mg/l) in the second experiment.

Similar results were found by Kazemi *et al.* (2012c) on Carnation, they showed that glutamic acid decrease proline accumulation significantly. These effect of glutamic acid might be referred to the decreases in ACO activity (Aminocyclo propane carboxylate oxidase that is the direct precursor of ethylene as confirmed by Kazemi *et al.* (2012a). We could return the effect of

salicylic acid to improving the antioxidant system and reducing oxidative stress damages during flower senescence as observed by Gerallio and Ghasemnezhad (2011). In addition the inhibition of ethylene biosynthesis and prolong vase life (Marandi *et al.,* 2011). These result are in conformity with those of Kazemi and Shokri (2011) on Lisianthus flowers.

Furthermore, the significant effect of calcium chloride is in agreement with Ibrahim *et al.* (2011) on Gerbera and Zadeh and Mirzakhani (2012) on Carnation. They showed that calcium chloride improved vase life and leaf quality. These effectiveness might be related to calcium chloride ability to inhibit the synthesis or action of ethylene. In addition, its role of increase tissue resistance and lower the rate of respiration as proved by Cortes *et al.* (2011) and Anjum *et al.* (2001). Regarding the effect of aluminum sulphate, results are similar to those of El-Quesni *et al.* (2012) on Schefflera, they found that aluminum sulphate increased vase life and quality. May be this results due to its action as an antimicrobial agent in the solution Hussen and Yassin (2013) and Mohammadi *et al.* (2012) Jowker *et al.* (2012) they found that aluminum sulphate improved postharvest visual quality by retaining leave freshness even at the end of vase life.

Number of Bacteria (CFU /ml)

The effect of some preservative solutions on the number of bacteria of Gladiolus cut flowers "Rose Supreme" in first and second experiment (April and November, 2015), are shown in Table (7), proved that number of bacteria in vase solution decreased significantly by using all studied materials compared to control. Moreover, the best effect on decreasing number of bacteria was obtained by glutamic acid at (300 mg/l), salicylic acid at (400 and 600 mg/l), calcium chloride at (3000 mg/l) and all concentrations of aluminum sulphate (100, 200 and 300 mg/l) in the first experiment. Whilst glutamic acid at 300 mg/l resulted in the greatest effect on decrease number of bacteria in vase solution of Gladiolus cut flowers in the second experiment.

The positive effect of glutamic acid may be that glutamic acid is readily metabolized by plants but not by many microorganisms, so it considered to using it as a possible substitute for sucrose according to Aran *et al.* (2011) and Kazemi and Ameri (2012a). Similar results were obtained by Kazemi *et al.* (2012c) on Carnation cut flowers, who found that glutamic acid decreased microbial population on vase solution significantly. As for main effect of salicylic acid, results are in accordance with those of Kazemi and Ameri (2012a) and Kazemi *et al.* (2012c) on Carnation. They observed a significant effect of salicylic acid on bacterial population. Effectiveness of salicylic acid might be referred to its ability to decrease pH of vase solution which reduce the growth and proliferation of bacteria as confirmed by Soleimany-Fard *et al.* (2013).

On the other hand, the best effect on decreasing the number of bacteria by calcium chloride may be attributed to the absence of a carbon source which inhibited bacterial growth as proved by Cortes *et al.* (2011).These results are in harmony with those of Hashemabadi *et al.* (2015) on Carnation cut flowers who reported that anti-ethylene compounds and also antibiotics increase water absorption, significantly. The significant control on number of bacteria that

obtained in aluminum sulphate pulsed flower stems may be related to not only limited to lowering the pH of vase solution but also Its effect is based at least in part, on its action as an antimicrobial agent in the solution (Hussen and Yassin 2013).These results are in agreement with those of Jowker *et al.* (2012) on Rose cut flowers who showed that aluminum sulphate inhibited microbial proliferation in vase solution.

Vase Life (Day)

Data presented in Table (7) cleared the effect of some preservative solutions on vase life of Gladiolus cut flowers "Rose Supreme" in first and second experiment (April and November, 2015). It has been proved from our results that, in general the four compounds which used in the two experiments showed positive effect on increasing vase life of Gladiolus cut flowers in comparison with control in both experiments. On the other hand, the last concentration of glutamic acid at (300 mg/l) and the intermediate concentration of salicylic acid (400 mg/l) resulted in the highest increase in the vase life in first experiment. Whereas, the best effect was found in glutamic acid at (200 and 300 mg/l), salicylic acid at (400 mg/l) and aluminum sulphate at (200 and 300 mg/l) in the second experiment.

With respect to glutamic acid effects, results are similar to the findings of Kazemi *et al.* (2012) on Carnation cut flowers, Kazemi *et al.* (2012a) on Gerbera and Zamani *et al.* (2011) on Rose. They showed that treatments of glutamine significantly increased vase life and delayed flower senescence compared distilled water (control). These enhancement on vase life duration may be due to glutamic acid ability to control microbial contamination, so that improved water absorption, prevented vascular blockage and delayed water deficiency related wilting and control ethylene production that extend vase life of cut flowers (Hashemabadi *et al.,* 2015). Similarly, Zamani *et al.* (2011) on Rose. Further, Kazemi and Ameri (2012a) stated that all glutamine concentrations prolonged Carnation vase life, while decreasing accumulation of bacteria in vase solution and ACC- oxidase activity (Aminocyclo propane carboxylate oxidase). Furthermore it has been proved that the observed decrease in ACC activity could be at least one mechanism through which relatively has affected on the senescence process (Aran *et al.,* 2011).

These significant increase in vase life may be due to that salicylic acid (SA), a natural phenolic secondary metabolite, in various aspects of vital processes like ethylene biosynthesis, stomatal conductance, respiration, senescence and the activation of defense systems against different pathogens is well An and Mou (2011). SA treatments were effective to affect postharvest life of cut flowers probably via the declined bacterial growth, reduced vascular blockage, reduced transpiration, prevented ethylene formation and induced antioxidant system in treated cut flowers thereby delaying the senescence process confirmed by Danaee *et al.* (2013). These results are in accordance with those of Roodbaraky *et al.* (2012), Kazemi *et al.* (2012) on Carnation and Soleimany-Fard *et al.* (2013) on Alstroemeria flowers. They indicated that salicylic acid increased the vase life of cut flowers compared to control.

The improving effect of calcium chloride on vase life might be attributed to possibility of calcium to decrease the respiration rate, osmotic adjustment and stability of cell membrane and increase water flow through the stems by association with pectin in the xylem cell walls, also calcium play important role in increasing tissue resistance and delaying senility through preventing ethylene synthesis and it's processing as confirmed by Sardoei (2014). Similar results were obtained by Amiri *et al.* (2009) and Ibrahim *et al.* (2011) on Gerbera, Zadeh and Mirzakhani (2012) on Carnation, Cortes *et al.* (2011), Abdolmaleki *et al.* (2015) on Rose and Seyedi *et al.* (2013) on Lilium cut flowers, they proved that treatments with calcium chloride increased longevity of cut flowers to highest values and delayed senescence.

We could return the effect of aluminum sulphate on increasing vase life of cut flowers to the antimicrobial effect, increase water uptake, inhibit ethylene production and action and reduce transpiration rate as reported by seyf *et al.* (2012);Mohammadi *et al.* (2012); Jowkar *et al.* (2012) and Hashemabadi *et al.* (2015).These results in agreement with those of Jowkar *et al.* (2012); seyf *et al.* (2012), Basaki *et al.* (2013) and Nader *et al.* (2015) on Rose, Hashemabadi *et al.* (2015) on Carnation, Mohammadi *et al.* (2014) on Tuberose and Amiri *et al.* (2009) on Gerbera cut flowers. They showed that the effect of aluminum sulphate on life of flower was significant.

During senescence, marked changes occur in the biochemical and biophysical properties of the cell membranes. Ethylene plays a central role in the senescence of many cut flowers as proved by Reid (1989). Flower vase life is affected by respiration, carbohydrates deterioration, disease inoculation, water uptake etc. During vase life of cut flowers, ethylene synthesis plays a major role in senescence. Similarly carbohydrates and soluble sugars in the petals also help in quality retention of cut flowers for longer period proved by Hussen and Yassin (2013). There was a direct relationship between vase life and, increasing of relative fresh weight and water uptake. Obstruction of the xylem by bacteria, therefore, inability of water absorption by flower steams is one of the current problems that lead to decrease in flowers postharvest longevity and also early wilting of them as observed by Sardoei (2014).Various studies have found that bacterial contamination is one of the most important factors in reducing postharvest life of cut flowers with the negative impact on respiration, photosynthesis and water uptake, also with increasing the evaporation, caused water imbalance and indirectly stimulates ethylene production and shortens postharvest life of cut flowers found by Mohammadi *et al.* (2012). According to the scientific findings, the postharvest life of different ornamental cut flowers could be affected by the application of various chemicals as preservatives as reported by Danaee *et al.* (2013). Anti-ethylene and antimicrobial compounds due to their stem end bacterial contamination control, can stimulate ethylene production indirectly and can control ethylene production and extending vase life of cut flowers confirmed by Hashemabadi *et al.* (2015).

Table (7). Effect of some preservative solutions on proline (µg proline/g), number of bacteria (CFU, Colony Forming Unit) and vase life (days) of Gladiolus cut flowers "Rose Supreme" in the first and the second experiments (April and November, 2015).

Treatments	First experiment (April, 2015)			Second experiment (November, 2015)		
	Proline µg proline/g	Number of bacteria Log 7 CFU/ ml	Vase life (days)	Proline µg proline/g	Number of bacteria Log 7 CFU/ ml	Vase life (days)
Control	943.3a	20.5a	14.92d	426.66a	11a	17.5d
Glutamic acid 100 mg/l	708.5ab	6.33bcd	19.67bc	186.86e	7.67c	21.58b
Glutamic acid 200 mg/l	688.3ab	7.33bc	20.42b	264.72c	5d	22.25ab
Glutamic acid 300 mg/l	190.4cd	5cde	22.17a	54.50i	3e	23.17a
Salicylic acid 200 mg/l	349.3cd	6.33bcd	20.58b	147.93gh	8.33bc	19.42c
Salicylic acid 400 mg/l	209.0cd	3.67def	22.17a	367.49b	5.33d	22.67ab
Salicylic acid 600 mg/l	928.1a	4def	19.75bc	264.72c	6d	19.35c
Calcium chloride 1000mg/l	817.5a	8b	18.5c	362.82b	9.67ab	19.42c
Calcium chloride 2000mg/l	915.5a	7bc	18.83c	266.27c	8.67bc	20.08c
Calcium chloride 3000mg/l	301.5cd	4.67cde	19.42bc	152.60f	8c	20.25c
Aluminum sulphate 100mg/l	479.6bc	2.67ef	19.17bc	214.89d	9bc	19.75c
Aluminum sulphate 200mg/l	129.9d	2.33ef	19.92bc	130.80gh	7.67c	21.5b
Aluminum sulphate 300mg/l	360.2cd	1.33f	20.58b	115.23h	5.67d	22.17ab
LSD at 0.05	**303.44**	**2.83**	**1.43**	**17.29**	**1.40**	**1.17**

CONCLUSION

In conclusion, the present study demonstrates that all treatments showed significant effect on quality parameters and flowers longevity compared to control in Gladiolus experiments. Glutamic acid with last concentration (300 mg/l) and intermediate concentration of salicylic acid (400 mg/l) were more effective on increasing vase life compared to control than other treatments in both experiments, except for treatments of glutamic acid (200 mg/l) and aluminum sulphate (200 and 300 mg/l) in second experiment. Our findings support for wider testing and use of the natural, cheap, safe and biodegradable compounds.

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الملخص العربى

تأثير بعض محاليل الحفظ على عمر أزهار الجلاديولس المقطوفة

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تعتبر المحافظة على جودة وعمر الأزهار من العوامل الهامة لتقييم جودة زهور القطف فى كل من الأسواق المحلية وأسواق التصدير. تهدف هذه الدراسة إلى تحديد فعالية بعض محاليل الحفظ مثل حمض الجلوتاميك (١٠٠ و٢٠٠ و٣٠٠ مجم/لتر) و حمض الصفصاف (٢٠٠ و٤٠٠ و٦٠٠ مجم/لتر) وكلوريد الكالسيوم (١٠٠٠ و٢٠٠٠ و٣٠٠٠ مجم/لتر) وكبريتات الألومنيوم (١٠٠ و٢٠٠ و٣٠٠ مجم/لتر) على معايير الجودة لأزهار الجلاديولس صنف "Rose Supreme". أظهرت النتائج أن كل المعاملات أدت إلى زيادة معنوية فى عمر الأزهار والوزن الطازج و كمية الماء الممتص ومحتوى الكلوروفيل مع خفض عدد البكتيريا وتراكم البرولين مقارنة بالكنترول. تم الحصول على أعلى زيادة فى الوزن الطازج وكمية الماء الممتص ومحتوى الكلوروفيل من قبل حمض الجلوتاميك (٣٠٠ مجم/لتر) وحمض الصفصاف (٤٠٠ مجم/لتر) وكلوريد الكالسيوم (٣٠٠٠ مجم/لتر) وكبريتات الألومنيوم (٣٠٠ مجم/لتر)، فى حين أسفرت المعاملة بحمض الجلوتاميك (٣٠٠ مجم/لتر) وحمض الساليسلك (٤٠٠ مجم/لتر) عن أطول مدة بقاء للأزهار فى كلا التجربتين، بالإضافة إلى حمض الجلوتاميك (٢٠٠ مجم/لتر) وكبريتات الألومنيوم (٢٠٠ و٣٠٠ مجم/لتر) فى التجربة الثانية. علاوة على ذلك، كان لحمض الجلوتاميك (٣٠٠ مجم/لتر) الأثر الأكبر على تقليل عدد البكتيريا وتراكم البرولين فى كلا التجربتين، بالإضافة إلى حمض الساليسلك (٤٠٠ و٦٠٠ مجم/لتر) وكلوريد الكالسيوم (٣٠٠٠ مجم/لتر) وكبريتات الألومنيوم (١٠٠ و٢٠٠ و٣٠٠ مجم/لتر) على عدد البكتيريا و حمض الساليسلك (٢٠٠و ٤٠٠ مجم/لتر) وكلوريد الكالسيوم (٣٠٠٠ مجم/لتر) وكبريتات الألومنيوم (١٠٠و ٢٠٠و ٣٠٠ مجم/لتر) على تراكم البرولين فى التجربة الأولى مقارنة بالكنترول.

Removal of Some Heavy Metals From Aqueous Solution Using Biosorbent Materials and Nanoparticles

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ABSTRACT: A batch sorption experiments were carried out to study the role of biosorbent and nano-particles on removal of some heavy metals from aqueous solution. The rice straw waste was used as biosorbent material(by-product of agricultural activity). In addition, Nano-Hydroxyapatite (60 nm) and nano-Zero Valent Iron (25 nm) were used as nanoparticles. The batch sorption was done using rice straw waste (RSW) with three sizes as 0.125, 0.25 and 0.5 mm with Pb^{2+} and Ni^{2+} with concentrations up to 28.14 and 100 mg/l, respectively. Also, the sorption experiment was conducted using nano-particles (n-HAP and n-ZVI) and phosphate rock for Pb^{2+} and Ni^{2+} with concentration up to 20 mg/l for Ni and 18.0 mg/l for Pb. The results indicated that RSW (0.250 mm) was best size for heavy metals removal. The rice straw waste has more ability for retention of heavy metals such as Ni and Pb according to linear and Freundlich sorption models. Also, the results indicated that maximum sorption capacity of heavy metals was occurred on Nano-Hydroxyapatite (n-HAP) for both Pb and Ni. These results may be due to the functional groups found in Nano-Hydroxyapatite. When RSW mixed with n-HAP, the results indicated maximum removal percentage of heavy metals. The removal percentage of Pb (99.70%) was more than Ni (38.25%) indicated that the ability of Pb to bind with RSW or n-HAP more than Ni. The current results are very useful in the treatment of wastewaters for the removal of heavy metals, making them suitable for agricultural purposes. The present study recommends a future studies to understand the role of nanomaterials on removal the different heavy metals under different conditions.

Keywords: heavy metals, batch sorption, biosorbents, biosorption, nano-particles, n-HAP, n-ZVI, phosphate rock.

INTRODUCTION

The presence of high levels of heavy metals in the environment may cause long-term health risks to humans and ecosystems. It is therefore mandatory that their levels in drinking water, wastewater and water used for agricultural and recreational purposes must be reduced to within the allowable concentrations recommended by national and international health authorities such as the World Health Organization. Adsorption is an effective physicochemical purification and separation technique used in water and wastewater treatments. It is considered as preferred method for removal, recovery and recycling of toxic heavy metals. Cost is an important parameter for comparing the sorbent materials. Biosorbent or biomaterials able for adsorption of heavy metals on their active surfaces and can be used with safe for removal of heavy metals from wastewaters up to concentration of 200 mg/l.

Treatment of metal bearing industrial wastewater is of height ended interest (Pakshirajan and Swaminathan, 2009; Acheampong *et al*., 2013). This is mainly because heavy metals are toxic even at a very low concentration to human, animal and plant species.

Natural wastes such as agricultural solid wastes have surface active materials can be able to remove or reduce the concentration of heavy metals from polluted ground and surface water. Natural clay and hydroxyapatite will be used also for heavy metals adsorption then; the treated water can be used for irrigation purposes.

Amongst the different known techniques to remove heavy metals from wastewater, biosorption seems to be cost effective as it involves only a passive process for heavy metal sequestration by using mostly dead/inactive and cheap biomass (Nadeem *et al*., 2014). Also, among the different biomass types available for heavy metal removal, the cyanobacterial biomass is particularly attractive owing to its complex biomass structure, minimum nutrient requirement, abundant growth within a short time period, ability to grow well under environmentally stressed conditions etc. The use of dead cyanobacterial biomass is also advantageous because it doesn't require any kind of special media for its growth (Gupta and Rastogi, 2008). Further, it has been reported that chemical pretreatment of such biosorbents show a large ability to form complex with metal ions, thus aiding in their efficient removal from aqueous solution (Afkhami *et al*., 2007).

Industrial heavy metal pollution has become a serious environmental and sanitary problem all over the world in recent years. Heavy metals can not only have toxic and harmful effects on organisms living in water, but also accumulate throughout the food chain and may also affect human beings (Martins *et al*., 2004; Sari and Tuzen, 2008). Heavy metals such as cadmium, lead and nickel among others, are commonly detected in industrial effluents. A variety of syndromes, renal function hypertension, hepatic injury, lung damage and teratogenic effects may result from cadmium toxicity (Sari *et al*., 2008). Lead may cause mental disturbance, retardation, and semi-permanent brain damage (Paulino *et al*., 2008). The occurrence of heavy metals especially cadmium and nickel in industrial effluents beyond permissible limits brings serious environmental pollution, threatening human health and ecosystem. Therefore, these pollutions must be removed to an acceptable level before being released into water ecosystem.

Many techniques, such as ion exchange, precipitation, adsorption, membrane processes, reverse osmosis, sedimentation, electro-dialysis, etc., have been employed for separation of heavy metals from wastewater (Perez-Quintanillo *et al*., 2007; Amini *et al*., 2009). With the increase in environmental pollution, there is a growing demand to develop new adsorbents of higher efficiency for heavy metal ions removal from aqueous media than those commercially available (Matlock *et al*., 2002).

The present study adopts a non-expensive and safe technology for removal of heavy metals from industrial wastewater using biosorbents and nanoparticles.

MATERIALS AND METHODS

Biosorbent

Rice straw waste (RSW) was collected from an agricultural farm in Damnhour, El-Beharia Governorate. The RSW was ground to powder and was sieved with three different sized meshes to obtain three different grade particle sizes namely; 0.125 mm, 0.125-0.25 mm, and 0.25-0.5 mm. The properties of the used rice straw are presented in Table (1).

Nanoparticles

Zero valent Iron nanoparticle **(**n-ZVI), consisting of Fe (0) surface stabilized nanoparticles, 98% Pure, APS: 25 nm and hydroxyapatite nanoparticles (n-HAP), $Ca_{10}(PO_4)_6(OH)_2$, Calcium phosphate tribasic, 96% Pure, APS: 60 nm were used. Morphology: Needle like. The materials were purchased from M K Impex Corp. Division: MKnano 6382 Lisgar Drive, Mississauga, Ontario L5N 6X1, Canada. The properties of the used Zero Valent Iron nanoparticle and hydroxyapatite nanoparticles.

Phosphate Rock

The Phosphate rock crushed and passed through an 0.063 mm sieve. Analysis of phosphate rock was carried out according the methods described in Safi *et al* (2006), Hassan *et al.* (2013) and Aissa *et al.* (2014). The properties of the phosphate rock are illustrated in Table (2).

Table (1). Some chemical properties of the rice straw waste (RSW)

Parameters	Unit	Value
pH	-	7.73
EC	dS/m	7.2
Ni	mg/ kg	0.35
Co	mg/ kg	0
Cd	mg/ kg	0
Pb	mg/ kg	3.05
B	mg/ kg	21.45
Cu	mg/ kg	1.45
Zn	mg/ kg	9.75
Mn	mg/ kg	9.9
Fe	mg/ kg	87.6
Ca	%	0.12
Mg	%	0.05
K	%	0.59
P	%	0.04
N	%	0.6
OM	%	79.5
Cellulose	%	38.3
Hemi cellulose	%	29.7
Lignin	%	10.2
Ash	%	4.8

Table (2). Some chemical properties of the phosphate rock (PR)

Parameters	Unit	Soluble	Total
N	%	0.059	2.10
P_2O_5	%	0.11	8.24
K_2O	%	0.02	0.02
Ca	%	0.62	19.30
Mg	%	0.03	0.60
Fe	%	0.00	1.10
Mn	%	0.07	0.55
Zn	%	0. 013	0.35
Cu	mg/kg	0.0	9.0
Ni	mg/kg	2.5	23.0
Cd	mg/kg	1.0	4.5
Pb	mg/kg	3.7	330
Co	mg/kg	0.0	0.0

Batch experiments

Batch biosorption of RSW

The stock solutions of the Pb^{2+} and Ni^{2+} used in this study was (1000 mg/l). Subsequent dilutions of (2.94, 3.53, 6.81, 11.92, 20.88 and 28.14 mg/l) and (2, 5, 10, 20, 50 and 100 mg/l), respectively were prepared by suitably diluting the stock solution with double distilled water. The experiments were performed in 100 ml flasks containing 25 ml of Pb^{2+} or Ni^{2+} of different concentration plus 0.5 g rice straw waste with three different sizes, and with three replicates for each experiment. The mixture was shaken in a rotary shaker at 50 rpm for one hour followed by filtration using Whatman filter paper (No.1). The filtrate containing the residual concentration of Pb or Ni was stored for analysis.

Batch nanoparticle and phosphate rock sorption

The stock solutions of the Pb^{2+} and Ni^{2+} used in this study was (1000 mg/l). Subsequent dilutions of (1.25, 2, 5, 11.97, 13.53 and 18) and (1, 2, 5, 10, 15 and 20 mg/l), respectively were prepared by suitably diluting stock solution with double distilled water. The experiments were performed in 100 ml flasks containing 25 ml of Pb^{2+} or Ni^{2+} of different concentration plus 0.1 g of Zero Valent Iron nanoparticle (n-ZVI), hydroxyapatite nanoparticles(n-HAP) or 0.2 g of phosphate rock, and with three replicates for each experiment. The mixture was shaken in a rotary shaker at 50 rpm for one hour followed by filtration using Whatman filter paper (No.1). The filtrate containing the residual concentration of Pb or Ni was saved for analysis.

Batch sorption of hydroxyapatite nanoparticles and rice straw waste mixture

Batch equilibrium experiments were performed at room temperature in 100 ml Erlenmeyer flasks containing 25 ml of either Pb^{2+} or Ni^{2+} of known concentration, 20 mg/l. An accurately weighed 1g of rice straw, size of 0.25 mm and 0.1g of hydroxyapatite nanoparticles was added to each flask with three replicates. The mixture was shaken in a rotary shaker at 50 rpm for 30 minutes followed by filtration using Whatman filter paper (No.1). The filtrate containing the residual concentration of Pb or Ni was saved for analysis.

Heavy metal sorption models

According to Sethuraman and Balasubramanian (2010), the percentage of Pb^{2+} and Ni^{2+} removal was calculated using the following equation:

$$\text{Removal of } Pb^{2+} \text{ or } Ni^{2+} (\%) = \frac{C_0 - C_e}{C_0} * 100$$

Where, Co and Ce represent initial and equilibrium concentrations of Pb^{2+} and Ni^{2+}.

According to (Vieira and Volesky, 2003; Vijayaraghavan *et al*., 2006) the equilibrium sorption capacity of Pb^{2+} or Ni^{2+} was calculated using the following equation:

$$S = \frac{(C_0 - C_e) \times V}{m}$$

Where;

S = equilibrium Pb^{2+} or Ni^{2+} ions capacity (mg/g),
V = suspension volume (l),
m = mass of pomelo material (g),
C_e = Pb^{2+} or Ni^{2+} ions concentration at equilibrium (mg/l), and
C_0 = initial ions concentration (mg/l)

To study and compare the sorption of aqueous Ni and Pb on rice straw waste, n-HAP, n-ZVI and phosphate rock, the sorption data were fitted to some sorption models such as linear and Freundlich isotherm models using software IsoFit (Matott, 2007).

Linear isotherm model

$$S = K_d \times C_e$$

Freundlich Isotherm model

$$S = K_f \times Ce^{1/n}$$

Where:

K_d: partition parameter (L/kg),
C_e: equilibrium concentration (mg/l)
K_f : Freundlich isotherm parameter, ((mg/kg)/(mg/l)) $^{(1/n)}$), and
1/n: Freundlich exponent (no units).

RESULTS AND DISCUSSION

1. Characteristics of sorbents

The rice straw waste (RSW), nanomaterials (n-HAP and n-ZVI) and natural material (phosphate rock, PR) were subjected to analysis by Scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR) and thermal analysis (TA) to study its surface texture and surface functional groups. The SEM graphs of the all materials are presented in Figures (1 to 4).

1.1. Scanning Electron Microscopy

SEM Images of n-HAP is presented in Figure (1), Surface morphology and the size distribution of the particles was investigated with the Scanning Electron Microscope (SU 1510) operated at 20kV, magnification x20,000. The solid samples were sprinkled on the adhesive carbon tape which is supported on a metallic disk. The sample surface images were taken at different magnifications. The scale was about 60 nm.

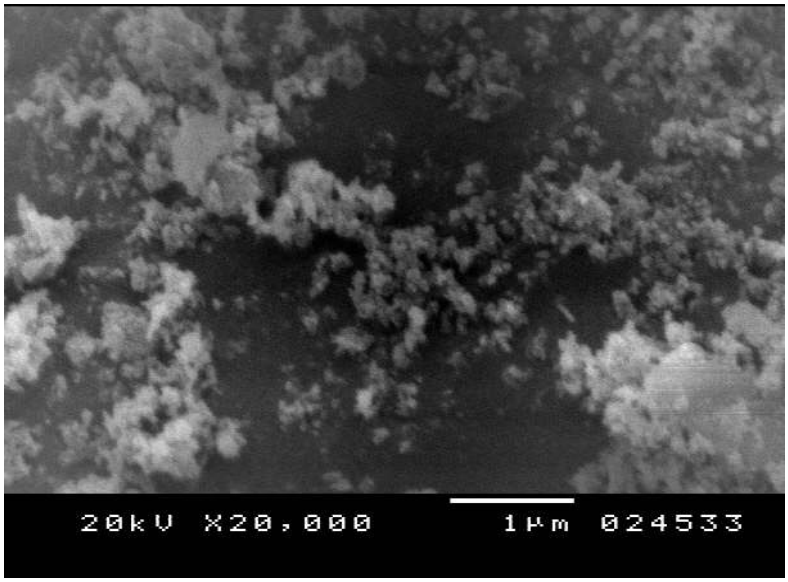

Figure (1). SEM micrograph of n-HAP particles (60 nm)

Figure 2 shows the SEM image of freshly synthesized zero valent iron nanoparticles. It can be observed that the zero valent iron nanoparticles are in the form of nanospheres, which exist in contact with each other and form chains having diameters of 25 nm.

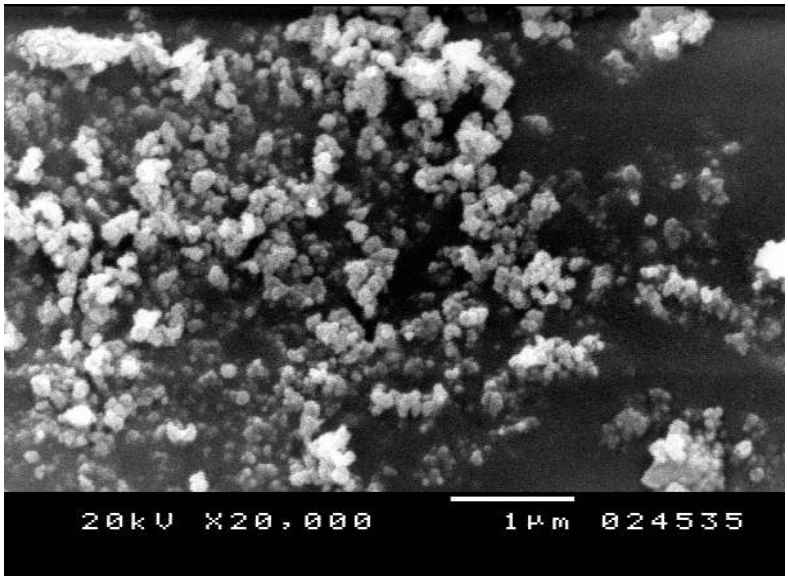

Figure (2). SEM micrograph of n-ZVI particles (25 nm)

SEM Images of phosphate rock particles is presented in Figure (3). The spheres having diameters of around 0.063 mm can be distinguished from each other and is in agreement with SEM results.

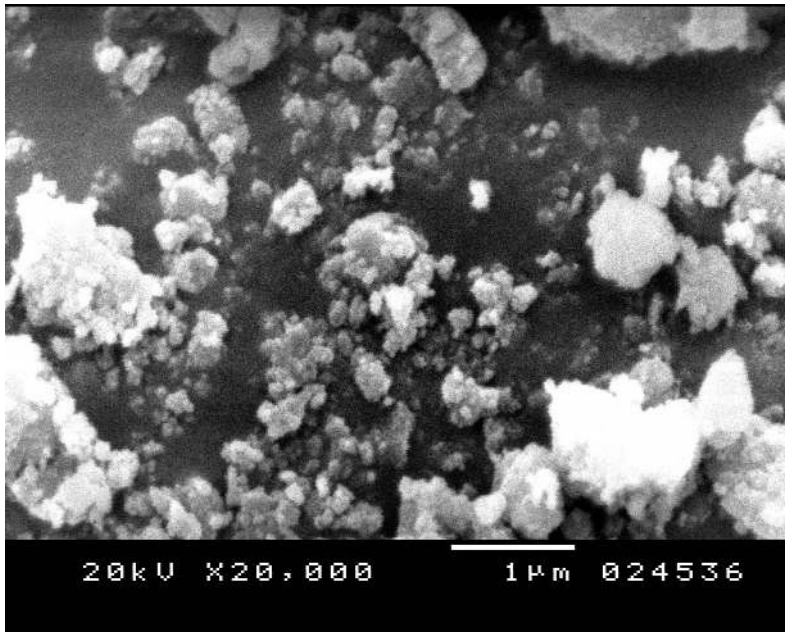

Figure (3). SEM micrograph of phosphate rock particles (0.063 mm)

SEM Images of rice straw waste powder is presented in Figure (4). The spheres having diameters of around 0.25 mm can be distinguished from each other and is in agreement with SEM results.

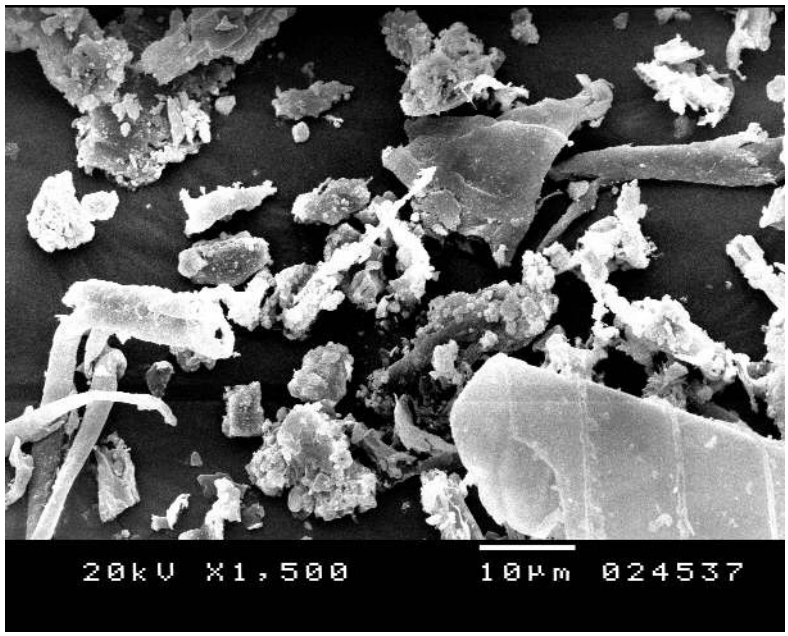

Figure (4). SEM micrograph of rice straw waste powder (0.25 mm)

1. 2. The Fourier Transform Infrared (FTIR)

The Fourier Transform Infrared spectra (FTIR) of rice straw waste (RSW), nanomaterials (n-HAP and n-ZVI) and natural material (phosphate rock, PR) are presented in Figures (5 to 7). Firstly, FTIR spectra of n-HAP, n-HAP particles after the sorption of aqueous Ni and Pb are illustrated in Figures (5, 6 and 7), respectively.

As interpreted from the data of FTIR analysis, the data show the function group of n-HAP type according to wave number. The main peaks are seeing in the n-HAP. The functional groups were identified using the peak assignments. A strong peak at 1638.66 cm^{-1} was assigned to the $R\text{-}C^{=O}\text{-}NR_2$ stretching in N-monosubstituted amides in solid state group.

The strong band at 1038.837 cm^{-1} was assigned to P-O-Alkyl stretching alkane group. The alkyl halide stretching C-Br at 567.759cm^{-1} was also observed. However, adsorption cases about Ni and Pb documented decrease in transmittance and more a symmetric stretching. It is obvious that the intensity of the peaks has increased after adsorption of Ni and Pb. The intensity of the peaks has increased after adsorption of Ni and Pb in stretching vibrations of the $R\text{-}C^{=O}\text{-}NR_2$ at 1640.475 and 1639.336 cm^{-1}, respectively.

The intensity of the peaks has increased after adsorption of Ni and Pb in stretching vibrations of the P-O-Alkyl at 1043.22 and 1039.899 cm^{-1}, respectively, and stretching vibrations of the C-Br at 568,203 and 567.927 cm^{-1}, respectively.

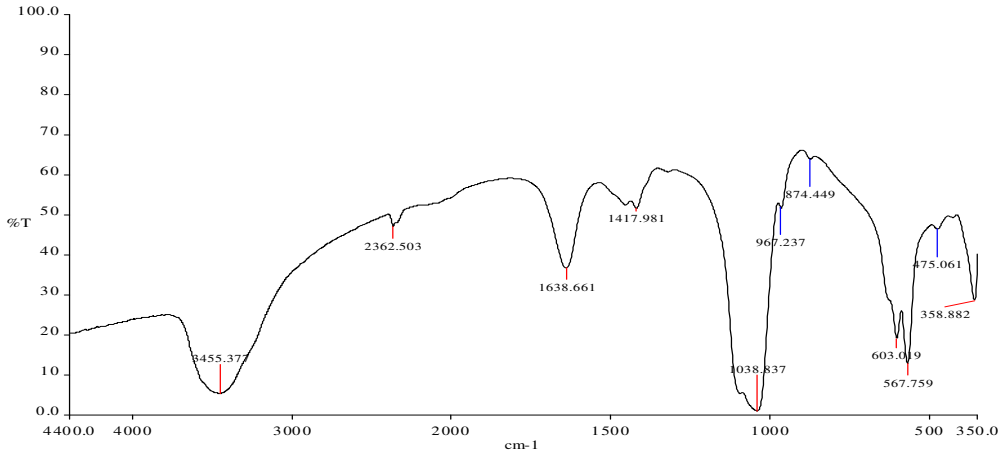

Figure (5). FTIR Spectra of n-HAP particles

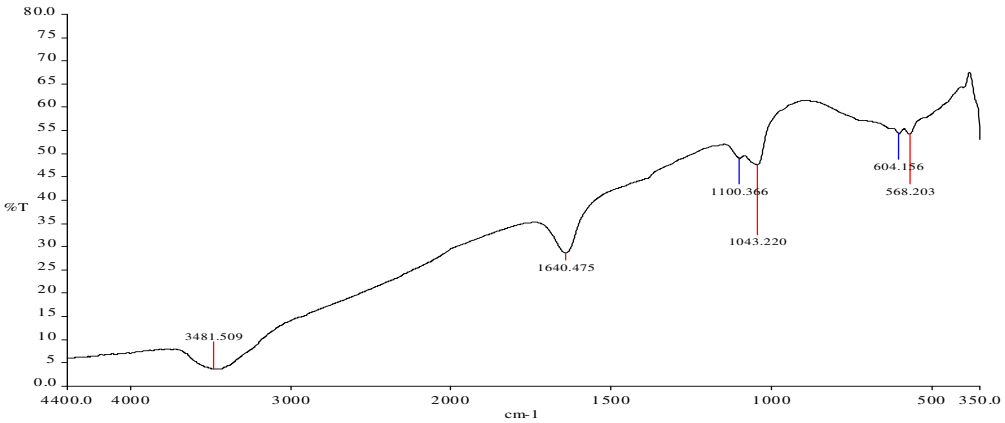

Figure (6). FTIR Spectra of n-HAP particles after the sorption of aqueous Ni

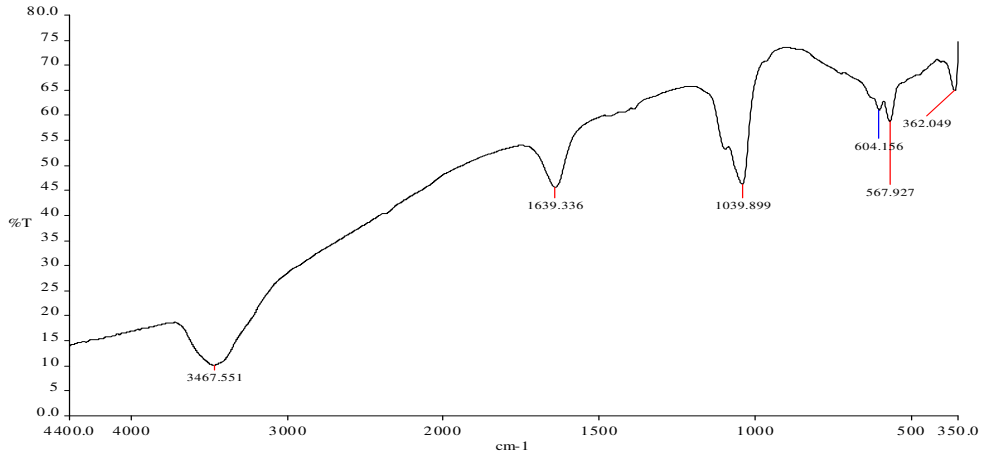

Figure (7). FTIR Spectra of n-HAP particles after the sorption of aqueous Pb

The FTIR spectra of n-ZVI, n-ZVI particles after the sorption of aqueous Ni and n-ZVI particles after the sorption of aqueous Pb, respectively are shown in in Figures (8, 9 and 10). As interpreted from the data of FTIR analysis, the function group of n-ZVI type according to wave number. The sorption peaks at 3458.407 and 1636.48 cm^{-1} are ascribed to stretching vibrations of –CONH– groups and the R-$C^{=O}$-NR_2 stretching in N-monosubstituted amides in solid state, stretching in N-N disubstituted amides, respectively. By comparing the FTIR spectra of n-ZVI before and after adsorption, there were remarkable shifts in some bands. These bands are the function groups of n-ZVI participate in Ni and Pb biosorption. It is obvious that the intensity of the peaks has increased after adsorption. Moreover, the wave number of the –CONH– was shifted from 3458.407 to 3467.397 cm^{-1} after the sorption of Ni. The wave number of the –CONH– was shifted from 3458.407 to 3476.189 cm^{-1} after the sorption of Pb. The intensity of the peaks has increased after sorption of Ni or Pb in stretching vibrations of the R-$C^{=O}$-NR_2 at 1638.022 and 1637.352 cm^{-1}, respectively.

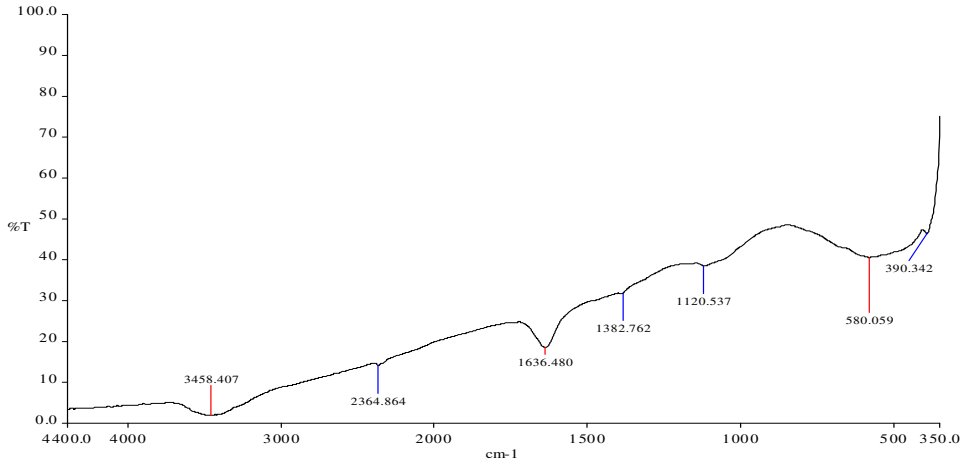

Figure (8). FTIR Spectra of n-ZVI particles

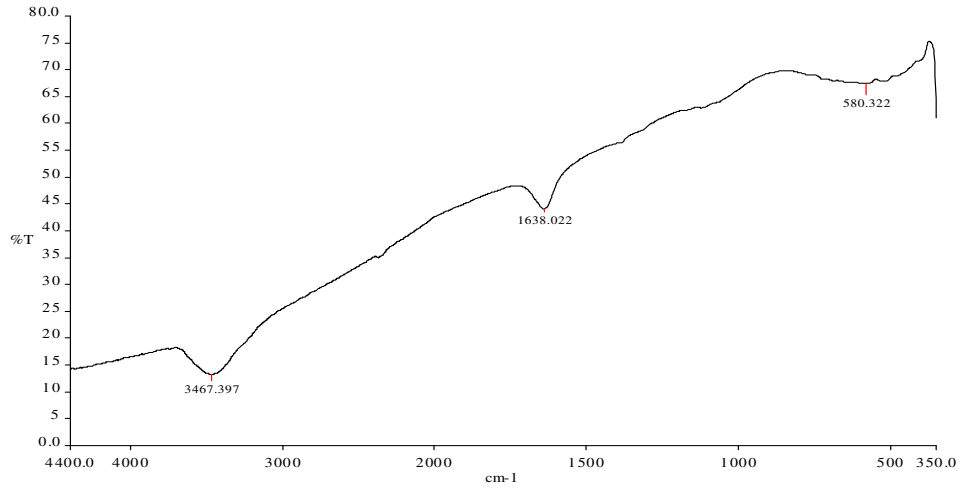

Figure (9).FTIR Spectra of n-ZVI particles after the sorption of aqueous Ni

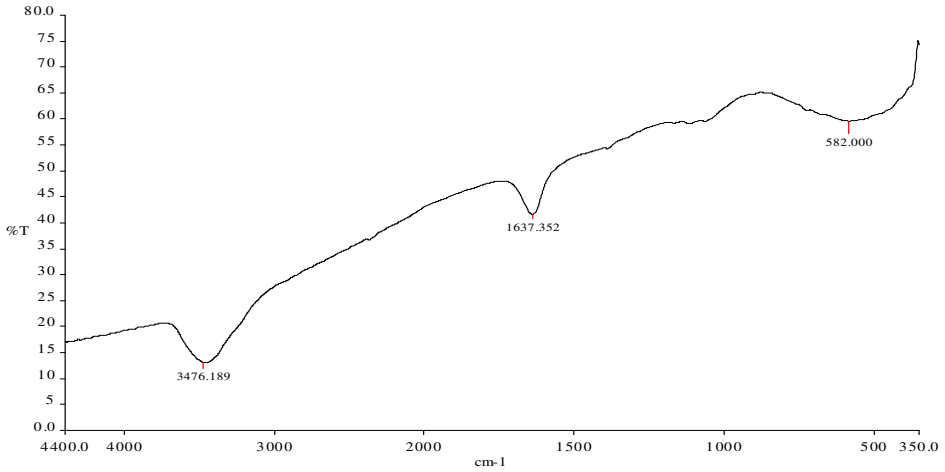

Figure (10). FTIR Spectra of n-ZVI particles after the sorption of aqueous Pb

FTIR spectra of phosphate rock particles, phosphate rock particles after the sorption of aqueous Ni and phosphate rock particles after the sorption of aqueous Pb are illustrated in Figures (11 to 13), respectively. As interpreted from the data of FTIR analysis, the function group of n-HAP type according to wave number, the main peaks are noticed in the phosphate rock particles. The strong bands at 3463.04,1428.878 and 1049.536 cm^{-1} are due to symmetric stretching mode of –CONH–, RO–SO4–OR and –C–O–H, respectively. The wave number of the –CONH–was shifted from 3463.04 to 3451.547 cm^{-1} after the sorption of Ni, and from 3463.04 to 3455 cm^{-1} after the sorption Pb. The wave number of the RO–SO4–OR was shifted from 1428.878 to 1427.139 cm^{-1} after the sorption of Ni, and from 1428.878 to1423.105 cm^{-1} after the sorption Pb. The wave number of the –C–O–H was shifted from 1049.536 to 1054.342 cm^{-1} after the sorption of Ni, and from 1049.536 to1048.541cm^{-1} after the sorption Pb.

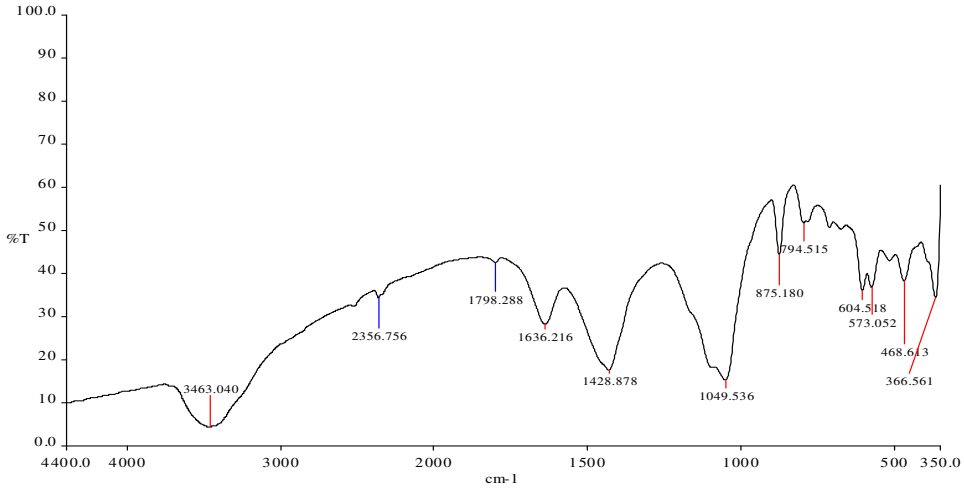

Figure (11). FTIR Spectra of phosphate rock particles

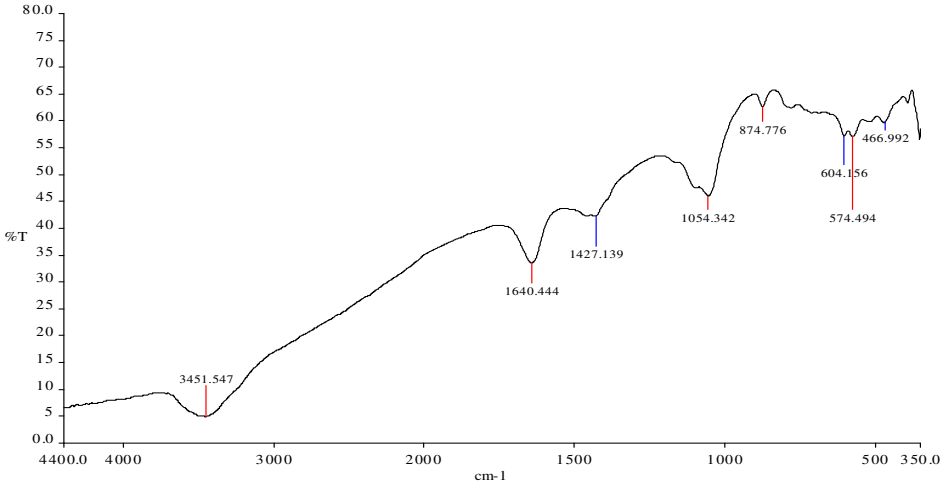

Figure (12). FTIR Spectra of phosphate rock particles after the sorption of aqueous Ni

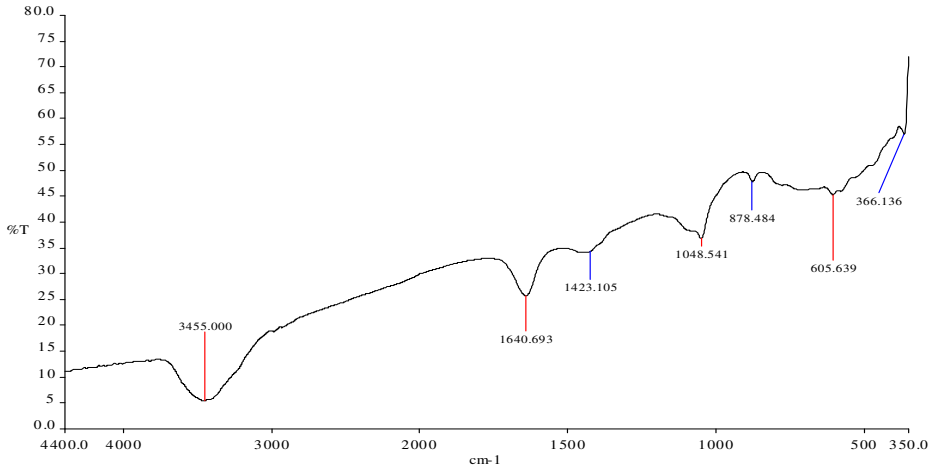

Figure (13). FTIR Spectra of phosphate rock particles after the sorption of aqueous Pb

The Figures (14 to 16) show the FTIR spectra of rice straw waste (RSW) powder, RSW powder after the sorption of aqueous Ni and RSW powder particles after the sorption of aqueous Pb, respectively. As interpreted from the data of FTIR analysis, the functional groups of RSW powder were sorted according to wave number. The main peaks are noticed in the RSW powder. A strong peak at 1639.189 cm^{-1} was assigned to the O-amino – stretching in hydroxyarylketones groups, R-$C^{//O}$-NR_2 stretching in primary amides in solid state, the R –$C^{=O}$-NR_2 stretching in N–mono-substituted amides in solid state, stretching in N-N di-substituted Amides, –C=$C^{/C=O}$ and –O–NO_2. By comparing the FTIR spectra of RSW powder before and after sorption, there were remarkable shifts in some bands. These bands are the function groups of RSW powder in Ni and Pb biosorption. It is obvious that the intensity of the peaks has increased after sorption. Moreover, the wave number was increased from 1639.189 cm^{-1} to 1641.451 cm^{-1} after the sorption Ni. Also. the wave number of the –CONH– was shifted from 1639.189 to 1640.27 cm^{-1} after the sorption of Pb.

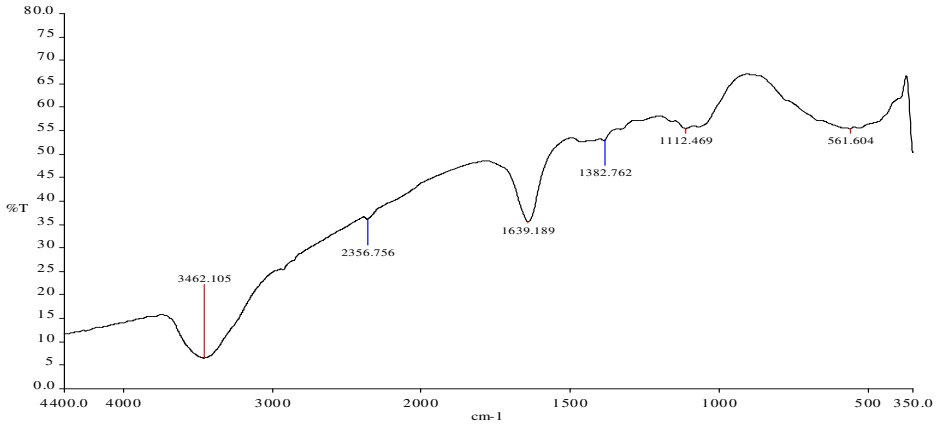

Figure (14). FTIR Spectra of rice straw waste (RSW) powder

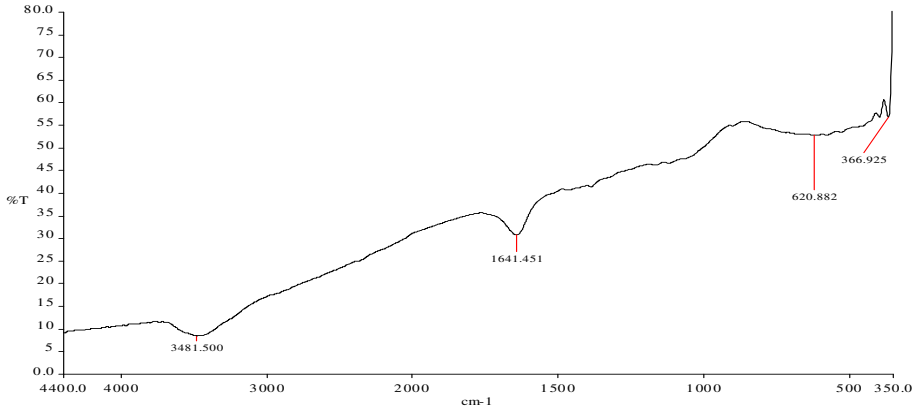

Figure (15). FTIR Spectra of rice straw waste particles after the sorption of aqueous Ni

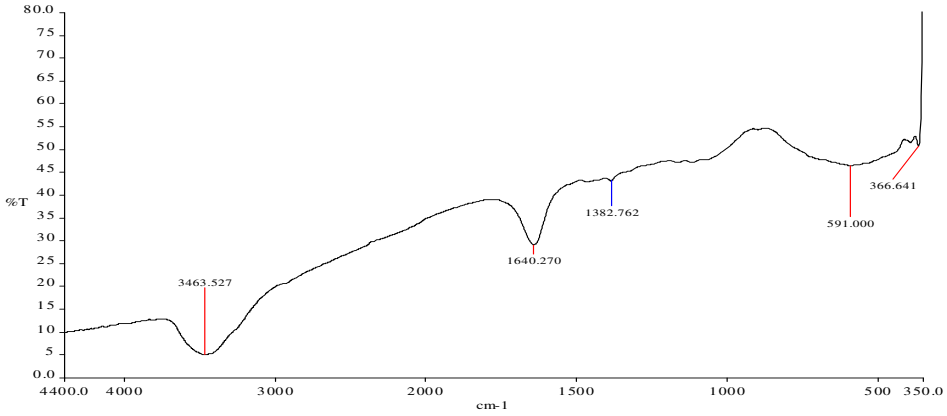

Figure (16). FTIR Spectra of rice straw waste particles after the sorption of aqueous Pb

1.4. X-ray analysis

X-ray analysis graphs of the n-HAP are presented in Figure (17). A broad single peak was observed in the X-Ray diffraction spectrum of raw bone at 2θ of 32.7°, confirming that the n-HAP is amorphous.

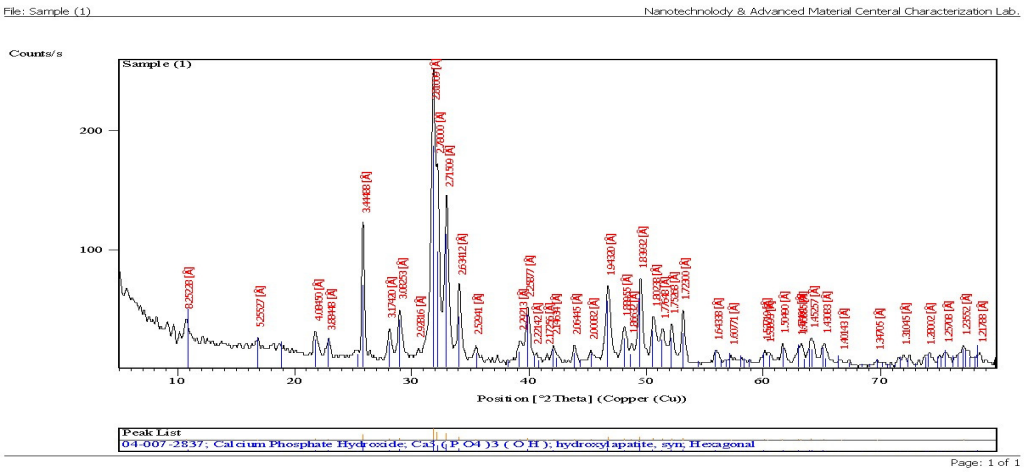

Figure (17). X-ray analysis graph of n-HAP particles

X-ray analysis graphs of the n-ZVI are presented in Figure (18). The peak at 2θ of 44.25^0 indicates the presence of n-ZVI nanoparticles.

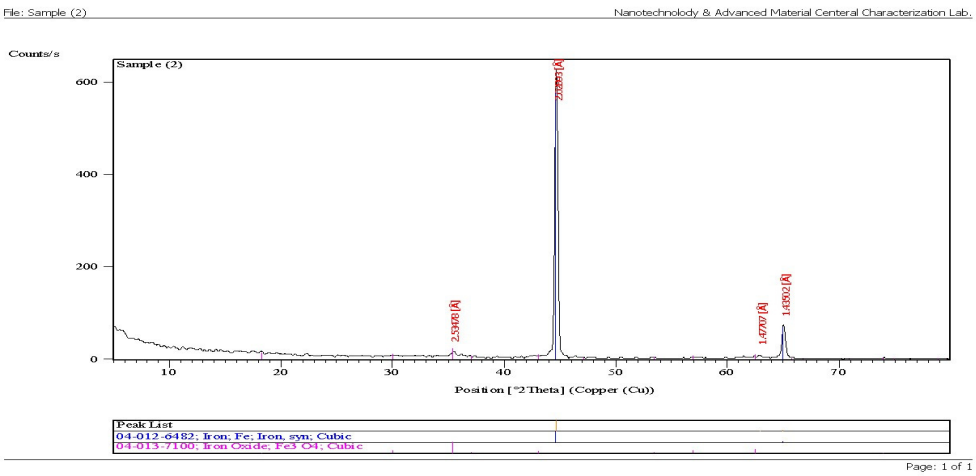

Figure 18. X-ray analysis graph of n-ZVI particles

The diffract gram, presented in Figure 19, shows that phosphate rock was composed of different phases with different intensities. For each sample, the quantitative analysis of its diffract gram using MATCH program reveals the presence of the following phases: calcium carbonate phosphate $Ca_5(PO_4)_3(CO_3)$ (2θ: 32.10°; 33.24°; 33.87°; 46.96°; 49.57), calcite $CaCO_3$ (2θ: 29.42°; 35.99°; 39.43°), dolomite Ca $Mg(CO_3)_2$ (2θ: 30.96°; 41.17°) and quartz SiO_2 (-;2θ: 26.65°; 40.17°). It appears that the recorded dolomite, calcite and quartz phases due to their nature exogangue, while the interference of these phases with fluorapatite are due to their natural endogangue.

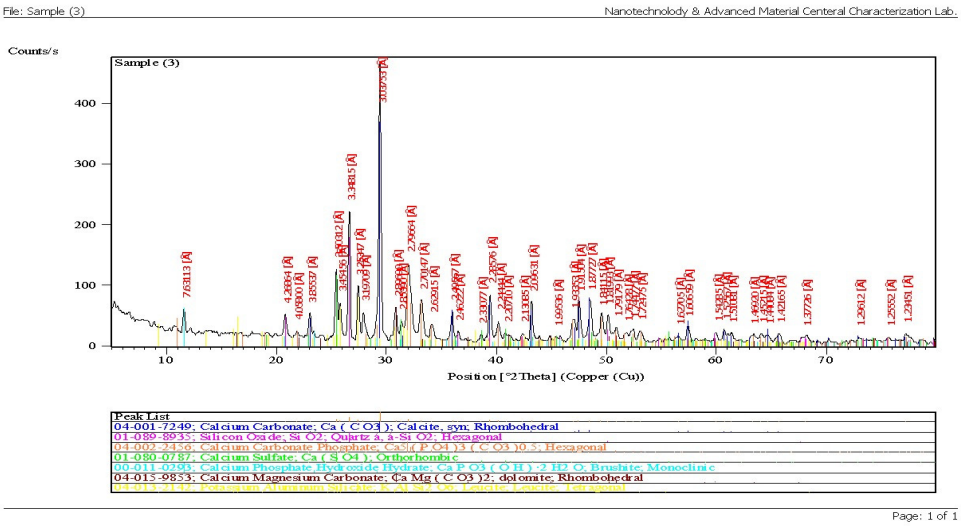

Figure (19). X-ray analysis graph of phosphate rock (PR) particles

2. Batch sorption of rice straw waste (RSW)

Biosorption capacity of rice straw waste (RSW) with three different sizes (0.125, 0.25 and 0.5 mm) increased with an increase in the initial concentration of Pb and Ni as shown in Tables (3 and 4). It suggested that the biosorption capacity of Ni (at 100 mg/l) increased with decreasing the particle size of RSW (0.125, 0.25 and 0.5 mm). It is account as 2840.5, 2826.0 and 2749.5 mg/kg, respectively. The biosorption capacity (S) of Pb (at 28.14 mg/l) in rice straw with three different sizes (0.125, 0.25 and 0.5 mm) were 1160.3, 1179.75 and 1157.0 mg/kg, respectively. The results indicated that optimum size of rice straw waste is 0.25 mm for maximum sorption of both Pb and Ni. These results may be due to the functional groups present on their cell wall offer certain forces of attractions for the metal ions and provide a high efficiency for their removal in RSW.

Similar results have been reported by Suemitsu *et al.* (1986); Wong *et al.* (2003) and El-Sayed *et al.* (2010). Rice straw waste possesses high oxygen; which is largely fixed in hydroxyl group of polysaccharides. These groups help in biosorption process by making a complex between metal ions (present in aqueous solutions) and oxygen of hydroxyl group (Hafiza *et al.*, 2010). The low particle size, have more surface area which plays an important role in the sorption process (Hayam, 2015). The adsorption capacity and the heavy metal efficiency are enhanced with the increase in the surface area because of the functional groups carboxylic groups responsible for the chelation of heavy metals.

Table (3). Equilibrium concentration (mg/l) of Ni at different sizes of RSW

Concentration (C_0) mg/l	0.5 mm		0.25 mm		0.125 mm	
	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg
0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.0	1.5	25.6	1.3	33.6	1.1	44.3
5.0	2.5	125.6	2.4	131.5	3.0	102.5
10.0	5.6	220.8	5.3	237.4	4.2	290.6
20.0	11.0	450.0	10.9	398.5	10.8	461.0
50.0	28.4	1079.3	20.0	1075.5	22.8	1494.0
100.0	45.0	2749.5	43.5	2826.0	40.0	2840.5
Average removal %	**43.86**		**49.29**		**49.69**	

Table (4). Equilibrium concentration (mg/l) of Pb at different sizes of RSW

Concentration (C_0) mg/l	0.5 mm		0.25 mm		0.125 mm	
	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg
0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.94	0.34	130.09	0.31	131.79	0.37	128.85
3.53	0.45	153.95	0.58	147.51	0.67	143.13
6.81	0.76	302.35	0.90	295.35	0.90	295.35
11.92	1.65	513.50	1.60	516.00	1.65	513.50
20.88	3.55	866.50	3.10	888.85	3.06	891.10
28.14	5.00	1157.00	4.55	1179.75	4.93	1160.30
Average removal %	85.98		85.92		84.91	

The experimental data (sorption isotherm) of heavy metals sorption on rice straw waste were modeled using various simple adsorption models such as linear and Freundlich models and the equation parameters are presented in Table (5).

Table (5). Parameters of some sorption isotherm models of Ni and Pb on rice straw waste

Sorption model	Size of rice straw waste	Parameters	Ni^{2+}	Pb^{2+}
Linear	0.125 mm	K_d	59.20	299.84
		R^2	0.9987	0.9306
	0.250 mm	K_d	66.50	322.61
		R^2	0.9927	0.9571
	0.5 mm	K_d	58.13	276.04
		R^2	0.9830	0.9926
Freundlich	0.125 mm	K_f	55.47	302.31
		1/n	1.02	0.937
		R^2	0.9988	0.9955
	0.250 mm	K_f	65.82	309.11
		1/n	1.00	0.896
		R^2	0.9928	0.9966
	0.500 mm	K_f	20.33	287.33
		1/n	1.29	0.967
		R^2	0.9991	0.9929

The results indicated that the optimum size of rice straw waste is 0.25 mm; it has maximum sorption of both Ni and Pb. The rice straw waste has more ability for retention of heavy metals such as Ni and Pb according to linear and Freundlich models (Table 5). The ability of RSW for removing heavy metals such as Ni and Pb ranging from 46.86 to 49.69% for Ni related to 0.125, 0.25 and 0.5 mm RSW.

Effective sorption of heavy metals using agricultural products and by-products may be documented such as modified rice husk (Lee *et al*., 1998 and Kumar and Bandyopadhyay, 2006), rice husk activated carbon (Guo *et al*., 2002) and maize husk (Jogi and Ansari, 2003). In general, an adsorbent can be termed as a low cost adsorbent if it requires little processing, is abundant in nature, or is a by-product or waste material from another industry (Khan *et al*., 2004).

The present results are in agreement with Aqeel *et al*. (2011). The removal efficiencies of lead, copper and zinc after applying low cost agricultural by-product as an adsorbent, increase as their initial concentrations in the polluted solution decrease. Khan *et al.* (2004) mentioned that the sorption capacity is depended on the type of the investigated period of contact adsorbent and the nature of the wastewater.

Also, natural low cost material that have been studied for the removal of heavy metals by adsorption include rice straw (Hui *et al*., 2008), black gram husk (Holan and Volesky, 1994), sugarcane bagasse (Volesky and Holan, 2008) and wheat bran. Rice straws proved to be the best biosorbent for Pb (II) and Ni(II) in aqueous solution. The biosorption characteristics fit well with Langmuir and Freundlich isotherm (Khalid *et al*., 2010). Also, Mathew (2008) stated that rice husk showed a maximum removal efficiency of 99.5% for Ni (II), 80.0 % for Cd (II), 72.8% for Cr (VI), 56.2% for Cr (III) and 40.0% for Cu (II).

Rice straw and sugarcane bagasse are abundant agro-residues. The sugarcane bagasse is currently used as a biofuel and in the manufacture of pulp and building materials. On the other hand, open field burning of rice straw frequently causes serious air pollution (Nelson *et al*., 1980). Thus a new technology for utilization of these agro-residues to a more value added material should be developed. Many researchers proposed the use of lignocellulosic waste as biosorbents for the removal of heavy metal ions in waste water (Lee and Rowell, 2004).

Rice straw possesses high oxygen; which is largely fixed in hydroxyl group of polysaccharides. These groups help in biosorption process by making a complex between metal ions present in aqueous solution and oxygen of hydroxyl group( ).

Biosorption has been demonstrated as an efficient and economical method for the removal of heavy metals in wastewaters (Benguella and Benaissa, 2002). Several naturally available biomasses, such as seaweeds and wheat straw, can be used as biosorbents. Non-living microorganisms, seaweeds, crab shells and other waste biomasses have also been tested and shown as promising sources of biosorbents (Gadd, 1992; Vieira and Volesky, 2003). The availability of a biomass at a low cost is a key factor dictating its selection for a biosorption process. Biosorption was earlier considered to follow a mechanism similar to that of adsorption (Wagner, and Jula, 1981). Several governing mechanisms of metal uptake by a biomaterial have been proposed, including chemisorption, complexation, chelation of metals, ion exchange, adsorption and micro-precipitation (Volesky, 2000). The metal binding depends on the biosorbent type, the metal ion species and concentration, temperature, pH, and ionic interference by other metal ions in the solution

3. Batch sorption of nanoparticles and phosphate rock

The effect of initial metal concentration (Pb^{+2} and Ni^{+2}) on adsorption onto biosorbents (Nano- hydroxyapatite, Nano- Zero Valente Iron and phosphate rock) are illustrated in Tables (6 and 7). The increase in biosorption capacity of Nano- Hydroxyapatite, Nano-Zero Valente Iron and phosphate rock was corresponded with increasing in the concentrations of Pb and Ni (up to 18 and 20 mg/l, respectively). The maximum Biosorption capacities of Pb by these ingredients were 4496.3, 4017.5 and 2152.5 mg/kg, respectively. The maximum Biosorption capacity of Ni^{+2} were 1283.5, 1026.8 and 757.1 mg/kg, respectively. The results indicated that maximum sorption capacity of heavy metals was occurred on Nano-hydroxyapatite for both Pb and Ni. These results may be due to the functional groups found in Nano-Hydroxyapatite. Similar results have been reported by Chen *et al*. (2010) and Yuan *et al*. (2010).

The experimental data (sorption isotherm) of heavy metals sorption on nanoparticles were modeled using various simple adsorption models such as linear and Freundlich models (Table 8). The nanoparticles used in the present study were hydroxyapatite nano-powder and Zero Valente Iron nano-powder, besides phosphate rock.

Table (6). Equilibrium concentration (mg/l) of Pb at nanoparticles and phosphate rock

Concentration (C_0) mg/l	Nano-Hydroxyapatite		Nano- Zero Valente Iron		phosphate rock	
	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg
0.00	0.00	0.0	0.00	0.0	0.00	0.0
1.25	0.02	308.3	0.06	297.0	0.12	141.3
2.09	0.02	517.8	0.13	491.3	0.17	240.4
5.01	0.04	1243.8	0.40	1152.5	0.30	588.8
11.97	0.06	2977.9	1.10	2717.5	0.62	1418.8
13.53	0.06	3367.1	1.32	3052.5	0.65	1610.0
18.07	0.09	4496.3	2.00	4017.5	0.85	2152.5

Table (7). Equilibrium concentration (mg/l) of Ni at nanoparticles and phosphate rock

Concentration (C_0) mg/l	Nano-Hydroxyapatite		Nano- Zero Valente Iron		phosphate rock	
	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg	Ce mg/l	S mg/kg
0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.8	55.8	1.0	0.5	0.6	51.0
2	1.5	132.1	1.9	31.5	1.4	80.9
5	4.0	250.0	4.3	166.0	3.2	223.9
10	7.6	605.3	8.0	500.0	6.9	385.3
15	11.5	876.0	12.0	750.0	10.7	535.0
20	14.9	1283.5	15.9	1026.8	13.9	757.1

Table (8). Parameters of some sorption isotherm models of heavy metals on nanoparticles and phosphate rock

Sorption model	Nanoparticles	Parameters	Pb	Ni
Linear	Nano-Hydroxyapatite (n-HAP)	K_d	51361.15	81.89
		R^2	0.9355	0.9903
	Nano-Zero Valente Iron (n-ZVI)	K_d	2151.16	62.97
		R^2	0.9787	0.9767
	Phosphate Rock (PR)	K_d	2421.58	53.86
		R^2	0.9791	0.9916
Freundlich	Nano-Hydroxyapatite (n-HAP)	K_f	55257.6	94.89
		1/n	1.41	0.95
		R^2	0.9857	0.9849
	Nano-Zero Valente Iron (n-ZVI)	K_f	2439.9	32.22
		1/n	0.75	1.26
		R^2	0.9985	0.9929
	Phosphate Rock (PR)	K_f	2421.58	67.83
		1/n	0.90	0.903
		R^2	0.9791	0.9939

The results show the sorption parameters of heavy metals (Pb^{2+} and Ni^{2+}) on different nanoparticles indicated that maximum sorption of heavy metals was occurred on Nano- hydroxyapatite for both Pb and Ni according to linear and Freundlich sorption models (Table 8). The Nano-hydroxyapatite has more ability for retention of heavy metals such as Pb and Ni. The partition coefficient was 81.89 L/kg for Ni and 51361.15L/kg for Pb, indicated that Pb is more binding on such materials than Ni. The values for n-ZVI were 62.97 and 2151.2 L/kg, respectively. For phosphate rock the values were 53.86 and 2421.6 L/kg, respectively. The more ability of n-HAP to retain the Pb than Ni reflected in high value of partition coefficient.

As reported by Mavropoulos *et al.* (2002); Nzihou and Sharrock (2002) and Mobasherpour *et al.* (2011), calcium hydroxyapatite (CaHAP), $Ca_{10}(PO4)_6(OH)_2$, is used for the removal of heavy metals from contaminated soils, wastewater and fly ashes. The most important properties related to various surface characteristics of HAP, e.g., surface functional groups, acidity and basicity, surface charge, hydrophilicity, and porosity. It has been found that HAP surface possesses 2.6 groups nm^{-2} of P-OH groups acting as sorption sites (Tanaka *et al*., 2005). The sorption properties of HAP are of great importance for both environmental processes and industrial purposes.

In general, HAP selectivity towards divalent metal cations is a result of the ion-exchange process with Ca^{2+} ions (Monteil Rivera and Fedoroff, 2002). Ionic radius of Ni^{2+} (0.72 A˚) slightly differ from that of Ca^{2+} (0.99 A˚), and it can substitute Ca^{2+} in the HAP crystal lattice. No structural changes of nano- HAP were detected by the powder X-ray diffraction analysis and FTIR of the solid residue with maximum amount of uptake capacity of Ni^{2+} and Pb^{2+}. Monteil Rivera and Fedoroff (2002) reported that the reaction mechanism corresponds to equimolar exchange of nickel or lead and calcium yielding $Ca_{10-x}Ni_x(PO_4)_6(OH)_2$, where x can vary from 0 to 10 depending on the reaction time and experimental conditions Ni^{2+} or Pb^{2+} ions are first adsorbed on the nano-HAP surface and substitution with Ca^{2+} ion occurs as described by the following equations:

$Ca_{10}(PO_4)_6(OH)_2 + xNi^{2+}$ ------- $Ca_{10-x}\ Ni_x(PO_4)_6(OH)_2 + xCa^{2+}$

$Ca_{10}(PO_4)_6(OH)_2 + xPb^{2+}$ ------- $Ca_{10-x}\ Pb_x(PO_4)_6(OH)_2 + xCa^{2+}$

The n-HAP is an effective adsorbent for the removal Ni^{2+} or Pb^{2+} from aqueous solutions. The results of XRD analysis and FTIR strongly support the ion exchange as a main mechanism for Ni^{2+} and Pb^{2+} removal by n-HAP. The results show that the Ni^{2+} or Pb^{2+} sorption by nanohydroxyapatite proceeds with a rapid surface complexation of the Ni^{2+} or Pb^{2+} on the -POH site before the formation of a compound of formula $Ca_{10-x}\ Ni_x(PO_4)_6(OH)_2$.

Sorption of heavy metals on HAP has been extensively studied. It has previously shown by researchers that HAP had the greatest sorption capacity for Pb compared to other heavy metals and it could effectively immobilize Pb ions existing in the contaminated soils and waters (Lee *et al*., 2005; Smiciklas *et al*., 2008). High sorption capacity of HAP for Pb is due to the different dominant sorption mechanism for Pb, dissolution– precipitation, whose driving force is rapid dissolution of HAP followed by precipitation of less soluble

hydroxypyromorphite (HPy) (Mavropoulos *et al*., 2002; Zhang *et al*., 2010; Googerdchian *et al*., 2012).

In recent years, iron nanoparticles (INP), amongst other metallic nano-particles; have received much attention for their potential application to the treatment of contaminated soils and waters. Their high surface area to volume ratio and high surface energy (Zhang *et al*.,1998) means that INP offer a greater reactivity than the surfaces of bulk scrap metal or iron filings/granules commonly used for remediation purposes in permeable reactive barriers, injection, etc., Tratynek (1996) and Bigg and Judd (2000) for reviews. The remediation mechanism depends on the nature of the contaminant but in all cases is driven by the oxidation of Fe (0) (Miehr *et al*., 2004).

To date, iron nanoparticles have been shown to be effective remediators of a range of contaminants including chlorinated organics (Nurmi *et al*., 2005; Kanel *et al*., 2005) and inorganic anions (Ponder *et al*. 2000; Zhu, 2010; Wijesinghe *et al*., 2014). In addition, INP have also been shown to successfully remediate solutions contaminated with a range of metals, including Pb, Cr, Cu, As, Ni, Zn, Cd and Ag (Li and Zhang, 2007).

As the present results, n-ZVI has high reactivity with contaminants such as Pb and Ni due to their high surface volume (Cantrell, and Kaplan, 1997 and Saleh *et al*., 2005). However, high reactivity alone is not enough to appoint this promising technology as a good in-situ remediation tool. Simultaneously, n-ZVI needs to be properly dispersed in water (Saleh *et al*., 2005).

4. Batch sorption of Nano- hydroxyapatite and rice straw waste mixture

The effect of rice straw waste and nano-hydroxyapatie as alone, and their mixture on removal percentage of Pb and Ni are presented in Table (9). Data showed that removal percentage of Pb increased as 21.48, 25.67 and 38.25 % for rice straw waste (RSW), nano-hydroxyapatite (n-HAP), and their mixture (RSW+ n-HAP), respectively. The trend of Pb removal was 94.41, 99.67 and 99.69%, respectively.

The results indicated maximum percentage removal of heavy metals was occurred on their mixture of nano- hydroxyapatite with rice straw waste for both Pb and Ni. These results may be due to the many functional groups in RSW and n-HAP.

The removal percentage of Pb was more than of Ni indicated that the ability of Pb to bind with RSW or n-HAP more than Ni. Explanation for this phenomenon may be due to the properties of the metal according to Marilen *et al.* (2007). Which could be due to larger ions might better binding site with two distinct active groups according to Figueiral *et al* (2000).

Table (9). Effect of RSW, n-HAP and mixtures of n-HAP and RSW on percentage of Ni and Pb removal

Treatment	Percentage Removal	
	Ni^{+2}	Pb^{+2}
Rice straw waste	21.48	94.41
Nano-hydroxyapatite	25.67	99.67
Rice straw waste + Nano-hydroxyapatite	38.25	99.69

Hannah and Thompson (2008) stated that the recent revolution of nanoscience and the advanced sophistication in the tools of characterization, shrinking the particle size of the sorbents is expected to influence the sorption capacity and further assist in developing commercial nanosorbents for wastewater treatment. In fact, nanotechnology has a significant impact to deal with legacy environmental pollution and to predict and prevent future environmental problems.

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الملخص العربي

إزالة بعض العناصر الثقيلة باستخدام مواد الادمصاص الحيوى والحبيبات النانوية

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أجريت تجارب الادمصاص لدراسة دور مواد الادمصاص الحيوى والحبيبات النانوية لازالة بعض العناصر الثقيلة (الرصاص والنيكل) من المحاليل المائية . استخدم قش الارز كمادة ادمصاص حيوية (نوائج العمليات الزراعية) . بالاضافة لذلك استخدمت مواد نانونية مثل نانو هيدروكسي اباتيت (٦٠ نانوميتر) ونانو الحديد غير المشحون (٢٥ نانوميتر). وقد استخدم قش الارز باحجام ٠,١٢٥ و ٠.٢٥ و ٠.٥٠ مم لتجارب ادمصاص بعض العناصر الثقيلة بتركيزات تصل الى ١٠٠ مجم/لتر فى حالة عنصر النيكل و ٢٨.١٤ مجم/لتر فى حالة عنصر الرصاص . مع المواد النانونية وكذلك مسحوق صخر الفوسفات (٠.٠٦٣ مم)، أوضحت النتائج ان قش الارز بحجم ٠.٢٥ مم كان الانسب فى ازالة العناصر الثقيلة (الرصاص والنيكل) بتركيزات حتى ٢٠ مجم/لتر فى حالة النيكل و ١٨ مجم/ليتر فى حالة الرصاص وهذا يرجع اللى القابلية العالية للمواد النانوية لادمصاص العناصر الثقيلة كما أوضحته نماذج الادمصاص الخطيي ونموذج فريندليش. كذلك اوضحت النتائج القدرة العالية لحبيبات الهيدروكسي اباتيت النانونية مع قش الآرز لادمصاص الرصاص والنيكل وهذا قد يعود الى المجاميع الفعالة فى قش الارز والحبيبات النانونية . كما ان خليط قش الارز وحبيبات الهيدروكسي اباتيت النانونية كانت ذات قدرة عالية لادمصاص العناصر الثقيلة. اوضحت النتائج ان نسبة ازالة الرصاص وصلت الى ٩٩.٧٠ % وللنيكل ٣٨.٢٥% وهذا يوضح قدرة الرصاص العالية للارتباط بقش الارز وحبيبات الهيدروكسي أباتيت النانونية٠ النتائج الحالية مفيدة جدا فى معالجة المياه العادمة (مثل مياه الصرف الصناعي ومياه الصرف الزراعي) وازالة العناصر الثقيلة وجعلها صالحة للاستخدام الزراعي. كما تلقى الدراسة الحالية الضور على فاعلية حبيبات الهيدروكسى أباتيت النانوية فى ازالة أيونات الرصاص والنيكل من المحاليل المائية ومعالجة المياه العادمة. وتوصى الدراسة بعمل دراسات مستقبلية لمعرفة دور المواد النانوية فى ازالة العناصر الثقيلة المختلفة من المياه العادمة مختلفة المصدر.

Response of Sesame Plants Productivity and Seed Quality to Different Fertilization Methods

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ABSTRACT: Two filed experiments were conducted at the Experimental Station Farm of Faculty of Agriculture (Saba Basha), Alexandria University during 2014 and 2015 seasons. The objective of this study was to investigate the response of sesame plants productivity and seed quality to different fertilization methods. The results could be summarized as follows: Foliar spraying of amino acid gave higher plant height, number of pods/plant, weight of pods (g), number of seeds/pods, 1000-seed weight (g), seed yield (g/plant), seed, straw and biological yields (ton/ha), as well as, oil yield (t/ha) in both seasons. Whereas, phosphorus fertilizer at 30 unit resulted in a significant increment in yield components and chemical composition of sesame seed in both seasons. Significant variation was recorded between the tested biofertilizer treatments for yield, yield components and percentages of oil, N, P and K of sesame seeds in both seasons. The effective treatments for all characters and chemical composition were obtained from foliar spraying of amino acid with phosphorein inoculation in both seasons. The effective treatments for oil percentage, phosphorus percentage and potassium percentage in both seasons were obtained from applying phosphorus at 30 units with phosphorein inoculation.
Keywords: Seasme, nitrogen, phosphorus, Biofertilizers, yield, Chemical composition.

INTRODUCTION

Sesame (*Sesamum indicum* L.), is as important oil seed crops grown in the tropics and subtropics, however, most of its cultivated area are grown in developing countries where usually grown by small holders. Sesame crop has an important advantage because it could be grown under fairly high temperature low supply and low levels of others inputs (Ghosh and Mohiuddin 2000 and El-Habbasha *et al.,* 2007).

In Egypt, sesame is consider as food crop rather than oil seed crop because most of its seeds production is used for snacks, confectionery bakery products, tehena and halawa purposes. The cultivated area of sesame in Egypt increased markedly during the last few years, while, the productivity was not increased by the same relative.

Nitrogen and phosphorus are essential nutrients required by the plants for their growth and vigor. Nitrogen is considered as an essential element of bio-molecules such as amino acids, proteins, nucleic acids, phytohormones and a number of enzymes and coenzymes. N strongly stimulates growth, expansion of the crop canopy and interception of solar radiation (Mengel and Kirkby, (2001). Similarly, phosphorus is an essential nutrient both as a part of several key plant structure compounds and as catalysis in the conversion of numerous key flower formation and seed production, more uniform and earlier crop maturity , improvements in crop quality and increased resistance to plant diseases (Bill, 2010 and Kashani *et al.,* 2015).

Recently, under Egyptian condition, a great attention is being devoted to reduce the high rates of mineral fertilizers, the cost of production and decrease environmental pollution via reducing doses of chemical fertilizer by using bio-forming systems (El-Habbasha *et al.,* 2007 and El-Nagdy *et al.,* 2010).

Using bio-fertilizers for non-legume crops (a symbiotic N-fixing bacteria, phosphate dissolving bacteria and bio-control) had a marked influence and had a positive effect on seed yield and recorded significant increases in all growth and yield tested parameter compared to uninoculation plants (Kumar *et al.,* 2009, Ziedan *et al.,* 2011 and Mahrous *et al.,* 2015). The aim of this study was to examine the effect of the different fertilization methods on the seed yield, its components and oil content of sesame crop.

MATERIALS AND METHODS

Two filed experiments were carried out at the Experimental Farm of the Faculty of Agricultural (Saba Basha), Alexandria University, during the two successive summer seasons of 2014 and 2015 seasons. Filed experiments were conducted to study the response of sesame plant productivity and seed quality to different fertilization methods (cv. shandawel 3).

Soil samples of the experimental sites were taken at the depth of (0-30 cm), physical and chemical analysis are presented in Table (1) were done according to Chapman and Pratt (1978). The split-split plot designs with three replicates were used. The main plot included three nitrogen application methods (soil application N, foliar spraying of amino acid and mixture soil + foliar), while, the phosphorus fertilizer (i.e. control, 15 and 30 kg P_2O_5/fed) was arranged in the sub plots. Bio-fertilizers (uninoculation, phosphorein and ceraline) were allocated to sub-sub plots.

The experimental unit area was 10.5m^2 (i.e. 1/400 feddan) consisting of five rows (3.5 m long and 60 cm between rows). Sesame seeds were sown on May 18th and 15th in the first and second season, respectively. The sesame seeds (Shandawel 3 cv.) was coated just before sowing with biofertilizers (phosphorein and ceraline), using arabic gum as an adhesive agent amounted 5kg/feddan. The preceding winter crop was wheat (*Triticum aestivum,* L.) and barssem (*Trifolium alexandrinum*, L.) in the first and second seasons, respectively. Sesame was manually harvested on September 22th and 24th in the first and second seasons, respectively.

Data recorded: At harvest, a random sample of 10 plants from the two central rows in each plot was taken to determine the studied characters

- Plant height (cm), Number of pods /plant, weight of pods / plant (g), Number of seeds/pods, 1000-seeds Weight (g), Seed yield /plant (g), Seed, straw and biological yield ton/ha and seed oil yield ton/ha. were estimated.
- Two random seed samples were drawn from each subplot to determine oil content using soxhlet apparatus and n-hexane (60°C) as an extraction solvent according to A.O.A.C. (1980) and seed content of N, P and K were determined according to A.O.A.C. (1980).

Data were subjected to statistical analysis of variance as described by Gomez and Gomez (1984). Mean value of the recorded data were compared by using the least significant differences (L.S.D. 5%).

Table (1). Some Physical and chemical properties of the experimental soil in 2014 and 2015 seasons

Soil properties	Season	
	2014	**2015**
A) Mechanical analysis :		
Clay %	38	37
Sand %	32	33
Silt %	30	30
Soil texture	Clay loam soil	
B) Chemical properties		
pH (1 : 1)	8.20	8.31
EC. (dS/m)	3.80	3.70
1) Soluble cations (1:2) (cmol/kg soil)		
K^+	1.52	1.54
Ca^{++}	9.40	8.70
Mg^{++}	18.3	18.5
Na^{++}	13.50	13.8
2) Soluble anions (1 : 2) (cmol/kg soil)		
$CO_3^{--} + HCO_3^-$	2.90	2.80
Cl^-	20.4	19.80
SO_4^{--}	12.50	12.60
Calcium carbonate (%)	6.50	7.00
Total nitrogen %	1.00	0.91
Available phosphate (mg/kg)	3.70	3.55
Organic matter (%)	1.41	1.40

RESULTS AND DISCUSSIONS

A. Yield and its components:

Results recorded in Tables (2 and 3) revealed that plant height (cm), number of pods /plant, weight of pods/ plant (g), number of seeds/pods, 1000-seeds Weight (g), Seed yield/ plant (g), Seed yield (t/ha), straw yield (t/ha), biological yield (t/ha) and oil yield (t/ha) in both seasons were significantly affected by application nitrogen.

The highest values of yield and its components were obtained by foliar spraying of amino acids and without significant between foliar spraying and mixture application soil+ foliar on number of seeds/pods, Seed yield (t/ha), straw, biological and oil yield (t/ha) in both seasons. This may be due to the provision of nutrients at latter stages which might have enhanced accumulation of assimilate of the seeds and thus resulting in hover seeds of sesame. Such findings is in agreements with those of El-Nkhlawy and Shaheen (2009), Shehu *et al.* (2010), Haruna (2011), Ulmar *et al.* (2012) and Kashani *et al.* (2015).

Data in Tables (2 and 3) revealed significant differences between the phosphorus fertilizer units in yield components i.e. plant height (cm), number of

pods /plant, weight of pods / plant (g), number of seeds/pods, 1000-seeds weight (g), seed yield /plant (g), seed yield (t/ha), straw yield (t/ha), biological yield (t/ha), as well as, oil yield (t/ha) in both seasons.

Phosphorus application at 30 units significantly surpassed (zero, without phosphorus) in all characters under study. The positive effect of phosphorus fertilization on yield components of sesame might be attributed to the soil of the experimental site, which was very poor in its phosphorus content. Also, P plays important role in enhancing translocation of metabolites which might be the reason for the increases observed on yield component (Hafiz and El-Bramawy, 2012). These results are in harmony with those reported by Okpara *et al.* (2007), Shehu *et al.* (2010) and Haruna (2011).

With regarded to the effect of bio-fertilization on sesame yield and its components, the results were given in Tables (2 and 3). These results reveal generally showed that all characters under this study were significantly affected by inoculation of sesame seeds with phosphorein when compared with uninocultion (control treatment).

Result presented in Tables (2) show the effect of phosphorein (bacterial) inoculation on plant height, number of pods /plant, weight of pods / plant (g), number of seeds/pods of sesame plants.

The obtained results might be attributed to better development of inoculted plants compared to uninoculated ones creating a more favorable environment in terms of natural and concentration of root exudates for cell growth and metabolic activities of rhizospheric microorganisms (El-Khawas, 1990).Many investigators reported the positive effect of biofertilization on these characters El-Habbasha *et al.* (2007), Babajide *et al.* (2012), Abdullahi *et al.* (2013) and Wayase *et al.* (2014).

The effect of the interaction between N application and phosphorus fertilizer units on plant height (cm), number of pods /plant, weight of pods / plant (g), number of seeds/pods, 1000-seeds weight (g), seed yield /plant (g), seed yield (t/ha), straw yield (t/ha), biological yield (t/ha), as well as, oil yield (t/ha) were significant in both seasons (Tables 2 and 3).

The effect of the interaction between N application and biofertilization on yield and its components were significant (Tables 2 and 3).

The effect of the interaction phosphorus fertilizer and biofertilization were significant for yield and its components in both seasons Tables (2 and 3). Application phosphorus at 30 units gave the highestmean value of yield and its components under using inoculation phosphorein.

Also, the foliar spraying of amino acids and application phosphorus at 30 units with inocultion phosphorein gave the highest mean value of yield and its components.

B. Seed quality

Results recorded in Table (4) revealed that percentage of oil, nitrogen, phosphorus and potassium in seeds were significantly affected by adding nitrogen by spraying methods.

The highest means values of all chemical compositions character were obtained using foliar spraying (amino acids) in both seasons, while, the lowest ones was recorded by soil application nitrogen. On the other hand, without in significant variations foliar spraying and mixture soil + foliar on oil % and nitrogen (%) in the second season. The present results are in line with those obtained by Shehu *et al.* (2010) and Haruna (2011).

Data illustrated that in Table (4) showed that the mean values of oil, nitrogen, phosphorus and potassium percentages of sesame plants were significantly increased using zero to 30 unit phosphorus fertilizers in both seasons. Application phosphorus at 30 units gave the highest value of oil, N, P and K percentages than the control treatment. Thus phosphorus is an important nutrient for seed development and seed filling contributing to better yield formation (Shrawat and Islam, 1990). These results in agreement with Mian *et al*. (2011), Khaled *et al*. (2012) and Kashani *et al*. (2015).

Data in Table (4) indicated that percentages of oil, nitrogen, phosphorus and potassium significantly increased by inoculation of sesame seeds with phosphorein and cerealine when compared with uninoculation (control treatment) during the two seasons. The maximum increment was obtained by using phosphorein followed by cerealine. The increment percentages attained 13.67 and 9.08% for oil % 1.78 and 39.40% for nitrogen %, 17.81 and 12.10% for phosphorus% and 16.67% and 10.22% for potassium % as an average two seasons for treatment (phosphorein + cerealine), respectively, compared with uninoculation treatment. This may be due to the role of phosphorus dissolving and nitrogen fixation bacteria on increasing the endogenous phytohormonas (IAA, GAs and CKs), which play an important role in formation a big active root system, increasing the nutrient uptake and photosynthesis and translocation, as well as, accumulation within different plant parts (El-Khawas, 1990). These results are in agreement with those obtained by El-Habbasha *et al.* (2007), Hasonpour *et al.* (2012), and Abdullahi *et al.* (2013).

The interaction between N-application and phosphorus fertilizers on percentages for oil, N, P and K in both seasons was significant as presented in Table (4). Foliar spraying with phosphorus application at 30 units gave the highest oil, N, P and K percentages in both seasons.

The interaction between N-application and bio-fertilizer (AXC) significant for oil (%), nitrogen (%), phosphorus (%) and potassium (%). It is clear from data in Table (4) that application P at 30 with inoculation phosphorein significantly increase all studied chemical composition characters.

The highest value of oil (%), nitrogen (%), phosphorus (%) and potassium (%) were recorded by foliar spraying of amino acids and phosphorus at 30 units with phosphorein inoculation.

Table (2). Effect of N-application, phosphorus units and bio-fertilization on some yield and its components for sesame plants during 2014/2015 seasons.

Treatments	Plant height (cm)		Number of pods /plant		Weight of pods / plant (g)		Number of seeds/ pods		1000-Seeds weight (g)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
N-applicaction (A)										
Soil application	141.52b	140.63b	103.05c	111.33c	226.70c	249.79c	36.79b	39.08b	3.60c	3.96c
Foliar application	145.10a	147.00a	115.38a	127.13a	254.24a	286.30a	41.18a	44.26a	4.10a	4.55a
Soil + Foliar	138.25c	141.08b	112.07b	123.28b	246.80b	272.16b	41.47a	44.05a	3.96b	4.37b
L.S.D.(0.05)	**1.45**	**1.60**	**1.80**	**2.05**	**7.80**	**6.70**	**0.72**	**0.55**	**0.09**	**0.10**
P- units (B)										
Control	135.50c	134.50c	101.74c	112.00b	224.47c	249.34c	35.12c	37.15c	3.54c	3.92c
15 unit	140.55b	143.34b	108.88b	120.46b	241.88b	266.18b	40.39b	42.63b	3.89b	4.28b
30unit	147.83a	150.87a	118.99a	128.68a	261.78a	288.02a	44.50a	47.60a	4.24a	4.67a
L.S.D.(0.05)	**1.90**	**2.20**	**2.10**	**2.30**	**8.40**	**9.10**	**1.02**	**1.09**	**0.12**	**0.14**
Bio-fertilization (C)										
Uninoculation	119.30b	136.92c	100.43c	110.83c	221.62c	243.76c	32.96c	35.27c	3.46c	3.80c
Phosphorein	145.14a	148.03a	119.22a	128.95a	262.32a	288.57a	45.08a	47.70a	4.23a	4.69a
Cerealine	144.49a	143.69b	110.86b	121.96b	244.20b	269.22b	42.68b	44.63b	3.98b	4.39b
L.S.D.(0.05)	**1.50**	**2.30**	**2.30**	**2.50**	**9.30**	**8.90**	**1.10**	**1.15**	**0.15**	**0.16**
Interactions										
Ax B	*	*	*	*	*	*	*	*	*	*
AxC	*	*	*	*	*	*	*	*	*	*
BxC	*	*	*	*	*	*	*	*	*	*
AxBx C	*	*	*	*	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference (L.S.D.)
*: Significant at 0.05 level of probability.

Table (3). Effect of N-application, phosphorus units and bio-fertilization on some yield and its components for sesame plants during 2014/2015 seasons.

Treatments	Seed yield /plant (g)		Seed yield (t/ha)		Straw yield (t/ha)		Biological yield (t/ha)		Oil yield (t/ha)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
N-applicaction (A)										
Soil application	94.57c	104.04c	1.83b	2.08b	3.97b	4.13b	5.84b	6.17c	1.18b	1.31b
Foliar application	110.83a	122.25a	2.23a	2.45a	4.09a	4.55a	6.30a	7.01a	1.27a	1.40a
Soil + Foliar	105.14b	116.11b	2.21a	2.41a	4.15a	4.51a	6.35a	6.91a	1.26a	1.38a
L.S.D.(0.05)	**8.75**	**4.50**	**0.05**	**0.07**	**0.06**	**0.05**	**0.07**	**0.10**	**0.03**	**0.04**
P- units (B)										
Control	89.02c	98.26c	1.89c	2.09c	4.00c	4.29b	5.88c	6.41c	1.17c	1.31c
15 unit	104.18b	114.67b	2.11b	2.30b	4.10b	4.39a	6.15b	6.53b	1.24b	1.31b
30unit	117.37a	129.42a	2.33a	2.58a	4.17a	4.42a	6.44a	6.97a	1.30a	1.43a
L.S.D.(0.05)	**4.50**	**5.40**	**0.06**	**0.11**	**0.04**	**0.05**	**0.10**	**0.12**	**0.04**	**0.03**
Bio-fertilization (C)										
Uninoculation	89.07c	97.85c	1.86c	2.05c	3.91c	4.34b	5.79c	6.41c	1.11b	1.23b
Phosphorein	115.35a	127.29a	2.28a	2.51a	4.17a	4.53a	6.44a	6.84a	1.31a	1.44a
Cerealine	106.47b	117.21b	2.19b	2.38b	4.13b	4.38b	6.27b	6.73b	1.29a	1.41a
L.S.D.(0.05)	**5.70**	**5.50**	**0.07**	**0.10**	**0.03**	**0.06**	**0.11**	**0.09**	**0.04**	**0.05**
Interactions										
Ax B	*	*	*	*	*	*	*	*	*	*
AxC	*	*	*	*	*	*	*	*	*	*
BxC	*	*	*	*	*	*	*	*	*	*
AxBx C	*	*	*	*	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference (L.S.D.)
*: Significant at 0.05 level of probability.

Table (4). Oil and macronutrients (N, P and K) percentages as affected by N-application, P- units and bio-fertilization on some yield and its components for sesame plants during 2014/2015 seasons.

Treatments	Seed oil (%)		N%		P%		K%	
	2014	2015	2014	2015	2014	2015	2014	2015
N-applicaction (A)								
Soil application	44.86c	45.25c	2.70c	2.84b	0.413c	0.453c	1.77c	1.95c
Foliar application	48.31a	49.29a	2.95a	3.06a	0.450a	0.539a	2.03a	2.24a
Soil + Foliar	47.58b	48.82b	2.89b	3.06a	0.434b	0.522b	1.98b	2.15b
L.S.D.(0.05)	**0.80**	**0.85**	**0.04**	**0.05**	**0.09**	**0.10**	**0.04**	**0.04**
P- units (B)								
Control	44.39c	45.06c	2.46c	2.78c	0.418c	0.460c	1.73c	1.91c
15 unit	46.32b	47.21b	2.92b	3.01b	0.449b	0.492b	1.93b	2.13b
30unit	50.02a	51.13a	2.98a	3.18a	0.504a	0.560a	2.12a	2.33a
L.S.D.(0.05)	**1.05**	**1.10**	**0.04**	**0.06**	**0.011**	**0.014**	**0.06**	**0.05**
Bio-fertilization (C)								
Uninoculation	43.78c	44.43c	2.29c	2.42c	0.417c	0.459c	1.77c	1.95c
Phosphorein	49.63a	50.64a	3.04b	3.17b	0.490a	0.541a	2.06a	2.27a
Cerealine	47.98b	48.25b	3.21a	3.37a	0.469b	0.513b	1.95b	2.15b
L.S.D.(0.05)	**0.95**	**0.05**	**0.06**	**0.07**	**0.015**	**0.016**	**0.05**	**0.06**
Interactions								
Ax B	*	*	*	*	*	*	*	*
AxC	*	*	*	*	*	*	*	*
BxC	*	*	*	*	*	*	*	*
AxBx C	*	*	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference (L.S.D.)
*: Significant at 0.05 level of probability.

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الملخص العربي

إستجابة إنتاجية نباتات السمسم وجودة البذور لطرق التسميد المختلفة

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أجريت تجربتان في مزرعة كلية الزراعة (سابا باشا) جامعة الأسكندرية خلال موسمي ٢٠١٤، ٢٠١٥ لدراسة إستجابة إنتاجية نباتات السمسم (صنف شندويل) وجودة البذور لطرق التسميد المختلفة.

أوضحت النتائج مايلي:

١. أعطت الإضافة بالرش بالأحماض الأمينية أعلي إرتفاع نبات ، عدد القرون/نبات، وزن القرون (جم)، عدد البذور/قرن، وزن ١٠٠٠ بذرة، محصول البذور(جم)/ نبات ، محصول البذور، القش، البيولوجي (طن/هكتار) وأيضاً محصول الزيت (طن/هكتار) في كلا الموسمين، كما ان التسميد الفوسفوري عند ٣٠ وحدة أنتجت زيادة معنوية في المحصول ومكوناته والمكونات الكيمائية لبذور السمسم في كلا الموسمين.

٢. سجلت إختلافات معنوية بين معاملات التسميد الحيوي المختبرة للمحصول ومكوناته والنسبة المئوية للزيت، النيتروجين، الفوسفور، البوتاسيوم لبذور السمسم في كلا الموسمين.

٣. الإختلافات المعنوية لجميع صفات المحصول ومكوناته والمكونات الكيمائية تم الحصول عليها من معاملة الإضافة بالرش بالأحماض الأمينية مع التلقيح بالفوسفورين في كلا الموسمين. الفروق المعنوية في النسبة المئوية للزيت، الفوسفور، البوتاسيوم في كلا الموسمين تم الحصول عليها من إضافة الفوسفور عند ٣٠ وحدة مع التلقيح بالفوسفورين.

Effect of Nitrogenous Fertilization, A- mycorrhizal Inoculation and Effective Microorganisms on Sunflower Productivity

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ABSTRACT: Two filed experiments were carried out at the Experimental Farm of the Faculty of Agriculture (Saba Basha) at Abees region, Alexandria University, Alexandria, Egypt, during the two growing seasons 2014 and 2015 to study the effect of nitrogenous fertilization, A-mycorrhizal inoculation, and effective microorganism (EM) on sunflower cv. Sakha 53 productivity and its oil quality. Experimental design was split- plot with three replicates. The main results could be summarized as follows: 1) The application of nitrogen fertilizer at 96 kg N/ha., significantly increased stem diameter, head diameter, head weight, number of seeds/head, 100-seed weight, seed yield (ton/ha), straw yield (ton/ha.), biological yield (ton/ha.), Harvest index (%) and Oil yield (ton/ha.), 2) A-Mycorrhizal inoculation + foliar application of EM biofertilizer, significantly, increased yield and its components, as well as, oil yield (ton/ha.), chemical composition (N, P and K) and crude protein content in both seasons, 3) interaction between nitrogen fertilizer of 96 kg/ha and A-mycorrhizae + EM gave the highest values of all yield and its components compared and control treatment (without N fertilizer) with control (without biofertilizer) in both seasons. Also, oil yield (ton/ha.), chemical composition (N, P and K) and crude protein was, significantly, increased due to application 96 kg/ha. + A-mycorrhizae + EM. The present investigation suggest the need for more studies concerning the effect of mineral and biofertilizer, as well as, applying NPK on sunflower plants under different environmental conditions using different types of soils especially newly reclaimed soil, to reach the optimum combination of mineral and biofertilizer to achieve the highest yield and quality of seed oil content.
Key words: sunflower; nitrogen; A-mycorrhizae; Effective microorganisms; yield; oil.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important annual crops in the world grown for edible oil. It receives considerable attention in Egypt due to its short growing season and it can be grown well under the low fertility soils in the newly reclaimed areas. So, sunflower could be one of the main suggested oil crops to solve edible vegetable oil shortage in the country. Seeds contain 24-49 % oil and the cake contains 25-35% of protein (Henen, 2011).

Nitrogen plays an important role in plant growth and it is considered as the most important fertilizer element needed for maximum yield in most field crops, as well as, sunflower, and it should be applied at the optimum level to obtain the highest seed yield (Abou-Khadrah, *et al.,* 2002).

Mycorrhizae (VAM) fungi increases significant amount of nutrients to the plants such as copper, zinc, phosphorus and sulphur by producing widely extended hyphal network on the upper or lower side of the soil layer (Tilak and Singh, 1994).

In addition, biofertilization is one of the most important factors used to produce products free from mineral fertilizer that cause environmental pollution problems and high rates of it lead to decrease in the potential activity of micro flora and the mobility of organic matters. Hence, the attention has been focused on the researches of bio-fertilization as a safe attemative for the chemical fertilizers **(**Namvar *et al.,* 2012). Also, bio-fertilizers play vital role for increasing the number of microorganisms and accelerate certain microbial process in the rhizosphere of inoculated soil plants which can change the available form of nutrients into plants (Abou-Khadrah *et al*., 2002; Bassal, 2003 and Radwan *et al.,* 2015). Biofertilizers, significantly, increased yield attributes of sunflower, viz. thalamus diameter, weight of thalamus, filled seed/capitulum, and 100 seed weight (g), as well as seed biological yields and oil content. The combined inoculation of PSB + VAM + *Azotobacter* recorded higher values of these parameters, as compared to PSB + *Azotobacter* and VAM + inoculation (Patra *et al.,* 2013). Also, Pramanik and Bera (2013) concluded that inoculation of biofertilizers, significantly, increased test weight, weight of thalamus, number of filled seeds per capitulum as well as seed yield, biological yield and oil content of sunflower. The combined inoculation of PSB +VAM + Azotobacter recorded higher seed yield over Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation.

Therefore, the objective of this study was to evaluate the effect of nitrogen levels and biofertilizers on yield and quality of sunflower (*Helianthus annuus* L.).

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm, the Faculty of Agriculture (Saba- Basha) at Abees region, Alexandria University, Egypt, during 2014 and 2015 seasons. The experiments were carried out to study the effect of nitrogenous fertilizer levels and biofertilizer on yield and quality of sunflower "*Helianthus annuus*, L." cv. Sakha 53.

The experimental design was split- plot with three replicates. Three nitrogen fertilizer levels (Control = without N applied, 48kg N/ha. and 96 kg N/ha) were allocated to main plots while four biofertilization treatments (without biofertilizer (control), Mycorrhizal inoculation, Effective microorganisms (EM) and "Mycorrhizal inoculation + EM") were allocated to sub- plots in both seasons.

Analysis of physical and chemical properties of the experimental soil site for the depth of (0 - 30 cm) as shown in Table (1) were carried out according to the methods reported by Page *et al*. (1982).

Table (1). Some physical and chemical properties of the experimental soil in 2014 and 2015 seasons.

Soil properties		
	Season	
	2014	2015
A) Mechanical analysis :		
Clay %	38	37
Sand %	32	33
Silt %	30	30
Soil texture	**Clay loam soil**	
B) Chemical properties		
pH (1 : 1)	8.20	8.31
E.C. (dS/m)	2.80	2.70
1)Soluble cations (1:2) (cmol/kg soil)		
K^+	1.52	1.54
Ca^{++}	9.4	8.7
Mg^{++}	18.3	18.5
Na^{++}	13.50	13.8
2)Soluble anions (1 : 2) (cmol/kg soil)		
$CO_3^{--} + HCO_3^-$	2.90	2.80
Cl^-	20.4	19.80
$SO_4^{—}$	12.50	12.60
Calcium carbonate (%)	6.50	7.00
Total nitrogen %	1.00	0.91
Available phosphate (mg/kg)	3.70	3.55
Organic matter (%)	1.41	1.40

Before planting sunflower seed were inoculated with A-mycorrhizal fungi (*Glomus mocrocarpum*) strain from Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, at a rate of 250 spores of infected roots and was mixed with seeds. The effective microorganisms (EM) sprayed as foliar application at two times after three weeks from planting date and the second spray was after two weeks from the first one at the rate of 4.8 litter/ha. The EM was produced by the General Organization for Agric. Equalization Foundation, Ministry of Agriculture, Egypt.

Nitrogen fertilizer treatments were applied in the form of ammonium nitrates (33.5 % N) at the rates of (control= no N fertilizer added, 48 and 96 kg N/ha) after thinning and before the first irrigation after planting, Phosphorus fertilizer was applied in the form of calcium super phosphate (15.5% P_2O_5) during soil preparation.

Each plot consisted of 5 ridges 3 m long and 60 cm apart with 30 cm space between plants. Two ridges were used to determine seed yield and its components. Sunflower “Sakha 53” seeds were sown in 28th and 26th June of the two growing seasons 2014 and 2015, respectively. In the first and second

season, Egyptian clover (*Trifolium alexandrinum* L.) and wheat (*Triticum aestivum*, L.) were the preceding crops in both seasons.

Hoeing was practiced before the first and second irrigation. The plants were thinned to secure one plant per hill after 10 days from planting other cultural practices for growing sunflower were carried out as recommended.

Recorded data:

A. Yield and yield components:

At harvest, two guarded plants were taken from the 2nd and 3th ridges in each plot to determine the following parameters

- Stem diameter (cm).
- Head diameter (cm).
- Head weight (g).
- Number of seeds/head.
- 100-seed weight (g).
- Seed yield (ton/ha).
- Straw yield (ton/ha.).
- Biological yield (ton/ha.).
- Harvest index (HI %).

B. Oil yield (ton/ha) and chemical compositions:

Oil percentage was determined using duplicated seed samples each of about two grams. Seed samples were oven dried at 65 to 70 ℃ for 24 hours. After weighting the seed samples seeds subjected to a constant pressure of 20000 pounds/square inch using a carve laboratory press which as described by A.O.A.C (1980).

Approximately 70% of the oil in the seed was extracted. The crushed seeds were then placed in oven with solvent petroleum ether stopper and allowed to stand a dry at 35℃. Two changes of solvent were applied at 24 hours intervals. Then oven dried for 24 hours at 65 to 70 ℃ and weighted. The loss in weight of seeds removed by pressing and solvent extraction were combined and oil % was then calculated as follows.

$$\text{Oil \%} = \frac{weight\ \ of\ \ oil}{weight\ of\ \ seed} \times 100$$

Oil yield (ton/ha) was determined by multiplying seed yield (ton/ha.) by seed oil percentage.

C. Chemical composition

Total nitrogen was determined in digested plant material colorimetrically by Nessler`s method **(**Chapman and Pratt, 1978). Protein content was calculated as N % x 6.25. Phosphorus was determined by the Vanadomolyate yellow method as given by Jackson (1973) and the intensity of colour developed was read in spectrophotometer at 405 nm. Potassium was determined

according to the method described by method Jackson (1973) using Beckman Flame photometer.

All the data collected were subjected to standard statistical analysis of variance ANOVA and LSD values to test the differences among the studied treatments means according to Gomez and Gomez (1984). The treatment mean was compared using the least significant differences (L.S.D.) test at 5% level of probability by using the split- model as obtained by CoStat 6.311, (2005) as statistical program.

RESULTS AND DISCUSSION

A) Yield and yield component

The obtained results given in Tables (2 and 3) showed, clearly, that nitrogen fertilizer levels exhibited significant effects on all estimated traits during the two cropping seasons of the study. Notably, increasing nitrogen fertilizer levels resulted in a significant increase in stem diameter, head diameter, head weight, number of seeds/head, 100- seed weight, seed, straw and biological yields (ton/ha), as well as, harvest index and oil yield (ton/ha).

These findings might be attributed to more adsorption of nutrition with reflect more growth substance more cell division and enlargement more of tissues and organs and plant elongation. Also, the nitrogen fertilizer may increase the synthesis of endogenous phytohormones which cause the formation of a big active root system which allow more nutrients uptake. The previous results agreed, more or less with the findings obtained by Abou Khadrah *et al.* (2002), Bassal (2003), El-Sadek (2005) and Radwan *et al.* (2015).

With regard to the effect of biofertilization on sunflower yield and its components. The results are shown in Tables (2 and 3). It could be concluded that inoculation of sunflower seeds with A-mycorrhizal + foliar application of EM encourages the increase of stem diameter, head diameter, head weight, number of seeds/head, 100- seed weight, seed, straw and biological yields (tons/ha), as well as, harvest index and oil yield (tons/ha) when compared with the control treatment in both seasons. This may be due to the effect of A-mycorrhizal inoculation + EM (foliar biofertilizer) which plays an important role in the increasing the nutrients that reflected on enhancing the growth of sunflower characteristic. Also, it could be attributed to the role of plant phytohormones such as IAA, GAs and CKs which promote plant growth, cell division, breaking the opical dominances, hence encouraging the phytosynthesis and assimilate accumulation **(**El-Khawas, 1990). These results are in harmony with the results obtained by Awad (2004), El-Temssah (2008), Henen (2011) and Patra *et al.* (2013).

The interaction between nitrogen fertilizer levels and bio-fertilization was significant for yield and its components in both seasons.

The highest mean values of seed yield (ton/ha.) character were recorded by application of 96 kg N/ha., with inoculation A- Mycorrhizae + Em in both seasons **(**Table 5).

B) Oil %, protein content (%) and chemical compositions

Increasing nitrogen fertilizer levels, significantly, brought an increase for oil %, protein content (%) and (N, P and K %) during both seasons.

The means of oil yield, protein % and N, P and K percentages for seed of sunflower plants increased due to increase nitrogen fertilizer levels up to 96 kg N/ha. Similar results were reported by Mohamed (2003), Ali (2004) and Nasim *et al.* (2012).

Data in Table (4) indicated that percentages of oil, nitrogen, phosphorus, potassium and crude protein, significantly, increased by inoculation of sunflower seed with A-mycorrhizal + EM (biofertilizer) when compared with the control of biofertilizer in both seasons. This may be due to the effect of mixture of A-mycorrhizal + EM (biofertilizer) of the ability of host plant to uptake insoluble nutrients. Particularly nitrogen, phosphorus and potassium and some microelements. These results are in agreement with those reported by Al-Karaki (2006) and Mai and Shamsuddin (2010).

The interaction between nitrogen fertilizer levels and biofertilizer was significant on protein content (%), oil content (%) and N, P and K% in both seasons.

Generally, the application of 96 kg/ha., with inoculation A- mycorrhizal and EM gave the best vegetative growth, yield and chemical composition as well as oil %, oil yield for sunflower variety in Alexandria conditions.

Table (2).Stem diameter (cm), head diameter (cm), head weight (g), number of seeds/head, 100-seed weight of sunflower cv. "Sakha 53" as affected by N-levels and bio-fertilization in 2014 and 2015 seasons.

Treatments	Stem diameter (mm)		Head diameter (cm)		Head weight (g)		Number of seeds/head		100- seed weight (g)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
A) Nitrogen fertilization levels (kg/ha.)										
0 (Control)	27.11	26.85	20.30	20.20	782.41	777.41	820.41	816.41	5.66	5.59
48	28.71	28.30	20.99	20.86	888.16	841.91	856.33	846.25	6.17	6.08
96	31.50	30.99	23.14	23.55	928.33	921.58	891.66	884.00	6.60	6.52
L.S.D. at 0.05	0.43	0.28	0.11	0.24	4.66	10.12	5.64	5.43	0.02	0.04
B)Bio-fertilization										
Without biofertilizer	24.65	24.37	19.06	18.91	640.77	631.77	788.88	784.55	5.37	5.30
Mycorrhizae	28.84	28.22	20.97	20.97	781.55	774.44	845.00	833.88	5.86	5.79
EM	30.56	30.25	22.67	22.43	867.33	865.66	878.88	871.77	6.47	6.37
Mycorrhizae + EM	32.38	32.01	24.00	23.83	1108.88	1116.00	911.77	905.33	6.88	6.79
L.S.D. at 0.05	0.28	0.23	0.30	0.34	4.56	11.83	3.12	5.41	0.03	0.03
Interaction										
Ax B	*	*	*	*	*	*	*	*	*	*

- * : Significant difference at 0.05 level of probability.

Table (3). Seed yield (ton/ha), straw yield (ton/ha), biological yield (ton/ha), harvest index (%) and oil yield (ton/ha) of sunflower cv. "Skaha 53" as affected by N-levels and bio-fertilization in 2014 and 2015 seasons.

Treatments	Seed yield (ton/ha)		Straw yield (ton/ha)		Biological yield (ton/ha)		Harvest index (%)		Oil yield (ton/ha)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
A) Nitrogen fertilization levels (kg/ha.)										
0 (Control)	3.59	3.50	6.48	6.53	10.07	10.10	35.55	35.23	1.44	1.44
48	3.88	3.87	6.84	6.92	10.75	10.80	36.06	35.78	1.97	1.73
96	4.17	4.23	7.17	7.18	11.34	11.42	36.72	36.98	1.98	1.98
L.S.D. at 0.05	0.01	0.01	0.02	0.03	0.05	0.03	0.10	0.13	0.01	0.04
B) Bio-fertilization										
Without biofertilizer	3.19	3.16	5.99	6.03	9.20	9.20	34.69	34.35	1.21	1.18
Mycorrhizae	3.69	3.69	6.88	6.41	10.07	10.11	36.57	36.48	1.60	1.58
EM	4.17	4.24	7.33	7.28	11.50	11.72	36.21	36.13	1.97	1.96
Mycorrhizae + EM	4.48	4.47	7.63	7.59	12.11	12.06	36.97	37.02	2.14	2.14
L.S.D. at 0.05	0.006	0.006	0.01	0.02	0.05	0.02	0.04	0.14	0.01	0.03
Interaction										
Ax B	*	*	*	*	*	*	*	*	*	*

- * : Significant difference at 0.05 level of probability.

Table (4).Protein content (%), oil content (%), macronutrients (N, P and K percentage) of sunflower cv. "Skaha 53" as affected by N-levels and bio-fertilization in 2014 and 2015 seasons.

Treatments	Protein (%)		Oil (%)		N (%)		P (%)		K (%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
A) Nitrogen fertilization levels (kg/ha.)										
0 (Control)	19.04	18.23	40.66	39.88	3.04	2.99	0.615	0.607	2.61	2.61
48	20.26	19.84	44.50	44.60	3.24	3.17	0.639	0.633	2.81	2.79
96	20.98	20.72	47.31	46.49	3.36	3.31	0.713	0.705	3.12	3.08
L.S.D. at 0.05	0.22	0.23	2.68	1.58	0.05	0.03	0.002	0.006	0.01	0.04
B) Bio-fertilization										
Without biofertilizer	17.50	17.28	37.64	37.88	2.80	2.76	0.574	0.568	2.47	2.39
Mycorrhizae	19.51	19.03	43.01	42.78	3.12	3.04	0.648	0.640	2.70	2.68
EM	20.90	20.74	46.76	46.11	3.35	3.31	0.676	0.671	2.94	2.91
Mycorrhizae + EM	22.44	21.99	49.30	47.85	3.59	3.52	0.724	0.714	3.27	3.32
L.S.D. at 0.05	0.27	0.20	1.75	1.20	0.04	0.03	0.003	0.006	0.02	0.03
Interaction										
Ax B	*	*	*	*	*	*	*	*	*	*

- *: Significant difference at 0.05 level of probability.

Table (5). Interactions between nitrogen fertilizer levels and biofertilizer inoculation on seed yield/ha during 2013/2014 and 2014/2015 seasons.

Treatments		Seasons	
N- fertilizer levels (kg/ha.)	**Biofertilizer**	**2013/2014**	**2014/2015**
0	Uninoculated	2.99	2.94
	Mycorrhizae	3.45	3.44
	EM	3.82	3.80
	Myco + EM	4.09	4.07
48	Uninoculated	3.18	3.16
	Mycorrhizae	3.51	3.53
	EM	4.31	4.29
	Myco + EM	4.52	4.51
96	Uninoculated	3.39	3.37
	Mycorrhizae	4.10	4.09
	EM	4.39	4.63
	Myco + EM	4.82	4.82
LSD at 0.05		0.009	0.01

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الملخص العربي

تأثير التسميد النتروجينى والتلقيح بالميكوريزا والكائنات الدقيقة النافعة على إنتاجية عباد الشمس

فتحى ابراهيم رضوان – محمود عبد العزيز جمعة – عصام إسماعيل قنديل – محمد خليفة العكرمى

قسم الانتاج النباتي – كلية الزراعة (سابا باشا) – جامعة الاسكندرية

أجريت تجربتان حقليتان بالمزرعة البحثية لكلية الزراعة سابا باشا – جامعة الاسكندرية – مصر – خلال موسمى الزراعة ٢٠١٤ و ٢٠١٥ ، لدراسة تأثير التسميد النتروجينى والتلقيح بالميكوريزا والكائنات الحية المفيدة على إنتاجية وجودة محصول عباد الشمس "صنف سخا ٥٣" وصممت التجارب باستخدام تصميم القطع المنشقة مرة واحدة في ثلاثة مكررات وكان ملخص النتائج كما يلى:

١-أدت إضافة التسميد النتروجينى حتى ٩٦ كجم نتروجين/هكتار الى زيادة معنوية لقطر الساق ، قطر الرأس ، وزن الرأس ، عدد البذور/الرأس ، وزن ١٠٠ بذرة (الجم) ، محصول البذور (طن/هكتار) ، محصول القش (طن/هكتار) ، المحصول البيولوجى (طن/هكتار) ، دليل الحصاد (%) ن ومحصول الزيت (طن/هكتار) والمكونات الكيميائية لمحصول عباد الشمس (النسبة المئوية للبروتين ، الزيت ، النتروجين ، الفوسفور ، البوتاسيوم فى البذور) خلال موسمى الدراسة.

٢-حقق التليقح بالميكوريزا مع الرش بالكائنات الحية الدقيقة النافعة زيادة معنوية لجميع صفات المحصول ومكوناته وأيضاً محصول الزيت والمكونات الكيميائية خلال موسمى الزراعة.

٣-التداخل بين التسميد النتروجينى حتى (٩٦ كجم/هكتار) مع التلقيح بالميكوريزا والرش بالكائنات النافعة أعطى أعلى قيم لجميع صفات المحصول ومكوناته مقارنة مع المعاملة بالكنترول (بدون تسميد سواء النتروجينى او الحيوي) في كل من الموسمين. أيضاً النسبة المئوية للزيت ومحصول الزيت للزيت والمكونات الكيماوية.

التوصية:

يوصى البحث باستخدام التسميد النتروجينى بمعدل ٩٦ كجم ن/هكتار مع التسميد الحيوي للحصول على أعلى إنتاج لوحدة المساحة من محصول البذور لعباد الشمس صنف سخا ٥٣ وذلك تحت ظروف التجربة.

Effect of Rice Bran on Productivity, Hatchability and Yolk and Blood Lipid Profile of Laying Japanese Quail

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ABSTRACT: A total number of 216 female and 108 male Japanese quail at 14 wks of age were used in a 7-week-trial to investigate the effect of rice bran on the quality and quantity of egg production as well as blood lipid profile. There were three dietary treatment groups: a control group fed on a corn-soybean based diet, 12.5 and 25.0 % rice bran diets. Results showed that egg laying rate, egg weight, egg mass and feed conversion ratio were insignificantly affected by treatments. Egg quality insignificantly affected by different treatments. The results showed significant decrease in egg yolk total lipids and cholesterol due to inclusion of rice bran in the diets in comparison with the control group. Hatchability and fertility was not influenced by different treatments. Blood serum total lipids, triglycerides, low density lipoprotein were significantly decreased, however, high density lipoprotein increased with increasing level of rice bran in the diet. Rice bran treatments showed significant ($P \leq 0.01$) increase in concentration of TAC than control group reached to 32.6 and 66.3 % for birds fed 12.5 and 25 % rice bran containing diets, respectively. Numerical increase in glutathione peroxidase in the group given 12.5 and 25 % rice bran containing diet as compared to the control group. In conclusion, egg laying rate and egg quality insignificantly affected by rice bran, however, the results showed significant improvements in blood and egg lipid profile, beside the antioxidative status as a result of inclusion fresh rice bran in the diet.

Key words: Quail, rice bran, laying performance, egg quality, lipid profile

INTRODUCTION

Chicken eggs are recognized as a perfect source of protein, lipids, vitamins and other valuable nutrients, but eggs also contain a high level of cholesterol, which is strongly associated with cardiovascular diseases. The current recommended level for daily intake of cholesterol is less than 300 mg and people often limit their egg consumption to avoid increases in the blood cholesterol levels (AHA, 1996; Carrillo-Domínguez *et al.,* 2005). Therefore, approach to reduce the cholesterol content in eggs not only helps to improve public health efforts, but it can also be beneficial for the egg industry. Recently, dietary supplementations of probiotic strains (Abdelqader *et al.,* 2013; Lei *et al.,* 2013; Zeweil *et al.,* 2016), vegetable oils (Zeweil *et al.,* 2013) and fiber feed ingredients (Olgun and Yıldız (2015) have already been used to regulate the egg yolk cholesterol concentration.

Rice bran, a major by-product from the rice milling process, has been used as an ingredient in livestock feed. It is probably the most widely used cereal by-product available. The feeding value of rice bran used as an ingredient in poultry feed has been reviewed by Farrell (1994). Because of its high oil content (20%), rice bran is poorly metabolized by young birds (Warren and Farrell, 1990). Rice bran oil contains a high concentration of unsaturated fatty acids (80-85%) which easily become rancid, especially under warm and humid climatic conditions. Hussein and Kratzer (1982) found that the free fatty acid content of rice bran fat was increased from 13.7% before storage to 42.8%

during 3-month storage period. Srinivasan *et al.* (2007) demonstrated that dietary rice bran is an excellent source of phytochemicals with antioxidative properties, such as β-sitosterol and a wide variety of phenolics and carotenoids. Ferulic acid, a well-studied phenolic compound, has been shown to be an effective scavenger of superoxide anion radicals and inhibitor of lipid peroxidation. Calabrese *et al.* (2008) demonstrated that ferulic acid protected against hydrogen peroxide–induced cellular damage through increased cellular levels of heme oxygenase-1 and heat shock protein-70. A limited number of studies have been conducted to evaluate the feeding value of rice bran for Japanese quail. Studies with other poultry species have shown that layer chickens and ducks can tolerate as high as 40% dietary rice bran while broilers can tolerate a maximum of 20% (Farrell, 1994). These differences may be attributed to the extent to which respective species/type of poultry can tolerate the anti-nutrients such as phytate, fibre, anti-proteolytic substances and lipase present in rice bran. Therefore, this study aimed to investigate the effect of rice bran,(not stored more than 14 day) on the quality and quantity of egg production as well as blood lipid profile in Japanese quail laying hens.

MATERIALS AND METHODS

This study was carried out at the Poultry Research Laboratory belonging to Animal and Fish Production Department, Faculty of Agriculture (Saba Basha), Alexandria University.

Three hundred and twenty-four (216 females and 108 males) Japanese quail of 14 weeks-old which had been in production for ٧weeks were weighed and randomly allocated in a completely randomized design considering three treatments groups, 108 birds each (72 females and 36 males), and each in three replicate per treatment. The birds were selected on the basis of more than 70 % egg production rate after a two-week observation period. All birds were reared under similar hygienic and managerial conditions. Rice bran was included at levels of 0, 12.5 and 25.0 % and fed to 21weeks of age. The samples of rice bran were obtained from the milling of rice (*Oryza sativa L.*) namely Sakha 104 a popular short grain Japonica cultivar for the consumption in Egypt. Rice bran obtained from the milling of rice 4 times and each not stored more than 14 days. The composition and calculated analysis of the experimental diets are shown in Table (1). Fresh feed was mixed weekly and not stored for more than one week. The hens were reared in wire batteries under similar environmental conditions. All birds had full access to feed and fresh water. The photoperiod was 16 hours of light per day throughout the experimental period, which lasted for 7 weeks from January to February. Records were kept for egg number, egg laying rate, feed consumption, egg weight and average body weight change. Measurements of egg quality were taken on average of 21 eggs from each treatment and were performed through two consecutive days per month. Yolk cholesterol was extracted and measured by the method of Folch *et al.* (1956) as modified by Washburn and Nix (1974) from three eggs of each replicate.

Table (1). Composition and calculated analysis of the experimental diets.

Ingredients	Rice bran %		
	0	12.5	25
Yellow corn	59.40	50.00	40.05
Soybean meal (44 %)	22.8	20.30	17.50
Concentrate (50 %) **	10.00	10.00	10.00
Di-calcium phosphate	0.30	0.20	0.20
Limestone	5.50	5.25	5.28
Sunflower oil	1.00	0.75	1.00
Vit. and min. mix.*	0.50	0.50	0.50
Salt (NaCl)	0.50	0.50	0.50
Rice bran [1]	0.00	12.50	25.00
Total	100	100	100
Calculated analyses[2]:			
Crude protein, %	20.08	19.93	19.60
ME (Kcal/ Kg diet)	2905	2879	2874
Ether extract, %	2.59	4.30	5.52
Crude fiber, %	3.05	4.07	5.07
Methionine, %	0.41	0.73	0.66
Methionine + cystine, %	0.56	0.57	0.58
Lysine, %	1.03	1.01	1.00
Calcium, %	2.96	2.85	2.85
Av. Phosphorus, %	0.38	0.38	0.39

*Each kg of vitamin and minerals mixture contained: Vit. A, 4,000,000 IU; Vit. D3, 500,000 IU; Vit, E, 16.7 g., Vit. K, 0.67 g., Vit. B1, 0.67 g., Vit. B2, 2 g., Vit. B6, 0.67 g., Vit. Bi2, 0.004 g., Nicotinic acid, 16.7 g., Pantothenic acid, 6.67 g., Biotin, 0.07 g., Folic acid, 1.67 g., Choline chloride, 400 g., Zn, 23.3 g., Mn, 10 g., Fe, 25 g., Cu,1.67 g., I, 0.25 g.,Se, 0.033 g.,Mg, 133.4 g.

** Concentrate: ME (K cal/kg) 2870, Crude protein 50%, Crude fiber 1.19%, Crude fat 6.16%, Calcium 7.3%, Phosphorus 3.2%, NaCl 1.44%, Methionine 1.65%, Methionine & Cystine 1.98%, Lysine 2.58%.

[1] Rice bran contain 14% crude protein; 3980 ME/kg; 0.59% lysine; 0.26% methionine; 0.35% methionine + cystine; 0.07% calcium; 0.22% available phosphorus; 11.4% crude fiber; 13.0% crude fat.

[2] According to NRC (1994).

Eggs were collected during the 21week of age for 7-day period and were stored in an egg room at 15.5° C dry bulb and 70 % relative humidity. They were incubated at 37.6 ℃ and relative humidity was 55-60% and hatched at 37.3 ℃ and relative humidity was 65-70% in automatic incubators. The removed eggs and eggs not hatched on day 18 were broken to differentiate infertile eggs from those containing dead embryos. Fertility was calculated as number of fertile eggs as relative to total number of eggs set; meanwhile hatchability was calculated as number of healthy hatched chicks as relative to total fertile number of eggs set; embryonic mortality percentage expressed as percentage of fertile eggs set was recorded on day 18 to differentiate the first and second embryonic death. Blood samples from the brachial vein of 4 hens in each treatment were drawn and serum were obtained by centrifugation of blood at 3500 r.p.m. for 15 min. and kept at – 18º C until analyzing. Serum total protein, albumin, total lipids, cholesterol, low density lipoprotein , high density lipoprotein, total antioxidant capacity and glutathione peroxidase were, calorimetrically, determined using commercial kits (from Biomerieux, Poains,

France). The proximate analysis of feed was carried out according to A O A C (2000). Data was statistically analyzed according to SAS program (SAS Institute, Inc., 1994) using general linear model (GLM) and the significant differences among treatments were determined using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSIONS

Results presented in Table 2 showed that different levels of rice bran inclusion in the diet had no significant effects on egg laying rate, egg weight, egg mass and feed conversion ratio as compared with the control. However, body weight change was significantly decreased in the groups given 12.5 and 25 % rice bran containing diets in comparison with the control group, even they consumed more feed comparing to the control. These results were in agreement with those of Amoah and Martin (2010) who reported that performance of laying type quail fed 20% full fat rice bran was comparable with those fed control diet. On the other hand, Abeyrathna *et al.* (2014) reported that the total egg production of quail fed diet containing 40% rice bran was significantly lower than that of birds fed 20 and 30% of rice bran.

The results in Table 3 showed that egg weight, specific gravity, percentage of albumin weight, egg shape index, yolk weight, yolk weight percentage and yolk color were not significantly affected by rice bran inclusion in the diet. On the other hand, absolute albumen weight was significantly ($P \leq 0.05$) increased with 12.5 % rice bran containing diet than the group fed control diet, also, the group given 25 % rice bran increased absolute albumin weight, but the increase was not significant. Significant ($P \leq 0.01$) decrease in albumin height in the group received 25 % rice bran was recorded as compared to the group had 12.5 % rice bran, , however, the groups had 12.5 and 25 % rice bran in their diets was not significantly differ in comparison with the control group. Significant ($P \leq 0.05$) increase in yolk index was observed in the group received 25 % rice bran in their diet in comparison with control group, while the group given 12.5 % rice bran diet did not different significantly than the control group. Egg shell weight was significantly ($P \leq 0.05$) deteriorated by increasing the level of rice bran in the diet and this deterioration reached significant with 25 % rice bran diet in comparison with the control group. The results showed also numerical deterioration in egg shell percentage and egg shell thickness due to increasing level of rice bran in the diet. The results obtained by Nobakht (2007) stated that egg quality did not significantly affected by inclusion different levels of rice bran in laying hen diets. The results obtained by Hagnazar and Rezaei (2004) indicated also that there were insignificant difference in egg quality with inclusion rice bran in laying hen diets up to 25 %. The same results were obtained by Filardi *et al.* (2007) reported that the inclusion of rice bran up to 15% in the diet had no negative effect on egg quality of commercial laying hens. On the other hand, Ademola *et al.* (2012) reported that laying hens fed diet containing 20 % rice bran had the best Haugh units, shell thickness, yolk and shell weights as compared to laying hens fed 40 % rice bran containing diet. Also, Samli *et al.* (2006) showed that Haugh units significantly improved by using up to 15 % rice bran in laying hen diets.

Table (2). Performance of laying Japanese quail hens fed on the experimental diets.

Parameters	Rice bran %			P value
	0	12.5	25	
Body weight change, g	8.04±2.68	-6.36±3.82[b]	-6.51±2.84[b]	0.001
Egg production, % hen-da y	76.91±2.11	76.22±2.50	74.18±2.44	0.692
Egg number, hen/day	0.77±0.02	0.76±0.02	0.74±0.02	0.629
Mean egg weight, g	13.23±0.16	13.45±0.14	13.33±0.12	0.429
Egg mass/hen/day, g	10.16±0.25	10.27±0.41	9.87±0.26	0.653
Feed consumed /hen /day, g	32.31±0.50[b]	34.08±0.31	33.43±0.45[ab]	0.026
Feed conversion ratio	3.18±0.08	3.34±0.14	3.39±0.08	0.392

[ab..] Means having the different small letters in each row are differ significantly (P≤0.05)

Table (3). Egg quality and egg yolk lipid profile of laying Japanese quail hens fed on the experimental diets.

Parameters	Rice bran %			P value
	0	12.5	25	
Egg weight, g	13.38±0.19	13.67±0.21	13.14±0.19	0.1737
Egg specific gravity	1.074±0.001	1.074±0.001	1.074±0.001	0.8449
Albumen height, mm	2.62±0.08[ab]	2.91±0.13	2.43±0.12[b]	0.0063
Albumen weight, g	5.85±0.18[b]	6.36±0.15	5.89±0.15[ab]	0.0543
Albumen ,%	43.68±1.13	46.74±1.13	44.85±1.04	0.1550
Yolk weight, g	4.28±0.07	4.30±0.08	4.25±0.08	0.931
Yolk, %	32.12±0.63	31.55±0.55	32.49±0.61	0.541
Yolk color	3.69±0.09	3.51±0.13	3.51±0.11	0.4396
Yolk index	420.92±1.05[b]	449.59±1.02[ab]	472.90±0.66	0.006
Egg shell weight, g	1.151±0.022	1.117±0.019[ab]	1.084±0.017[b]	0.0550
Egg Shell ,%	8.625±0.153	8.219±0.162	8.277±0.133	0.1194
Egg shell thickness, mm	0.246±0.004	0.243±0.004	0.244±0.004	0.8157
Egg yolk lipid profile:				
Total lipids, g/100g yolk	307.05±7.37	270.03±4.40[b]	227.37±1.721[c]	0.0001
Total cholesterol, g/100g yolk	12.57±0.17	11.81±0.21[b]	11.76±0.18[b]	0.005

[ab..] Means having the different small letters in each row are differ significantly (P≤0.05)

The obtained results showed significant decrease (P ≤ 0.01) in egg yolk total lipids and cholesterol due to rice bran inclusion in the diets in comparison with the control group. Our results were in agreement with the results presented by Dung *et al.* (2012) reported that levels of 2.5 and 3% of rice bran oil had beneficial effect on lowering egg yolk cholesterol. But the results of Safamehr and Attarhoseini (2011) and Abeyrathna *et al.* (2014) showed that inclusion of the rice bran in laying hens diets did not significantly influence cholesterol in egg yolk as compared with the control group.

In the present study, hatchability and fertility was not influenced by different treatments (Table 4). Also, the results of Awad *et al.* (2011) showed no significant differences were found among groups fed diets contained rice bran at levels of 16 and 24 % as compared to those of the control for egg fertility, hatchability, early and late embryonic mortality percentages and chick weights.

Table (4). Fertility and hatchability traits of Japanese quail hens fed on the experimental diets.

Parameters	Rice bran %			P value
	0	12	25	
Fertility, %	90.19±0.99	90.83±0.62	90.78±1.12	0.896
Hatchability, %	82.43±1.17	85.43±1.70	83.72±1.15	0.350
Early embryonic death, %	1.87±0.54	2.03±0.58	2.27±0.61	0.901
Late embryonic death, %	3.48±0.79	2.86±0.74	2.83±0.77	0.793
Hatch chick weight,g %	8.97±0.10	9.09±0.19	9.09±0.14	0.880

Blood serum total lipids, triglycerides, low density lipoprotein were significantly decreased, however, high density lipoprotein increased with increasing level of rice bran in the diet (Table 5). The increase in high density lipoprotein concentration reached to 30.5 and 34.4 % for birds fed 12.5 and 25 % rice bran containing diets, respectively. On the other hand, the obtained results showed that different levels of rice bran had insignificant effect on total cholesterol concentrations as compared with the control group. Dung *et al.* (2012) indicated that crude rice bran oil is a rich source of phytoceuticals such as, oryzanol, tocopherols, tocotrinols and linoleic acid. Gamma oryzanol is one of a component having antioxidant property. The role of gamma oryzanol in decreasing plasma or serum cholesterol was showed by Kahlon (1992a, b), lowering cholesterol absorption, decreasing platelet aggregation and lowering LDL-cholesterol were well documented (Patel and Naik, 2004).

Table (5). blood Serum lipid profile, total antioxidant capacity and glutathione peroxidase of laying Japanese quail hens fed on the experimental diets.

Parameters	Rice bran %			P value
	0	12.5	25	
Total lipids (mg/dl)	716.00±29.98	599.88±30.00^{b}	639.44±28.37^{b}	0.007
Triglycerides (mg/dl)	335.22±24.17	208.33±20.40^{c}	258.00±25.28^{b}	0.0001
Cholesterol (mg/dl)	179.44±3.02	173.50±2.78	178.20±4.26	0.232
Low density lipoprotein (mg/dl)	49.66±1.13	49.22±1.52	42.22±1.09^{b}	0.0001
High density lipoprotein(mg/dl)	62.66±3.51^{b}	81.77±5.02	84.22±6.43	0.0051
Totalantioxidantcapacity(mm/L)	0.89±0.04^{c}	1.18±0.14^{b}	1.48±0.09	0.001
Glutathioneperoxidase(nmol/ml)	45.44±5.71	49.56±7.41	54.33±8.06	0.593

$^{ab.}$ Means having the different small letters in each row are differ significantly ($P \leq 0.05$)

The results presented by Abd El-Hady (2013) reported that, full fat rice bran is more effective in cholesterol lowering than either rice bran oil or defatted rice bran, certainly due to the presence of comparatively high levels of tocopherol, tocotrienol and oryzanol as well as unsaponifiables. These results are supported by those of Minhajuddin *et al.* (2005), Wilson *et al.* (2007) and Zigoneanu *et al.* (2008). Also, Abd El-Hady (2013) observed that, full fat rice bran showed slight improvement in serum HDL-cholesterol of rats followed by rice bran oil and defatted rice bran in comparison with the control group.

The results illustrated in Table 5 showed significant (P ≤ 0.01) decrease in serum total antioxidant capacity (TAC) in the control group, however, inclusion of rice bran showed significant (P ≤ 0.01) increase in concentration of total antioxidant capacity surpassed the control one by 32.6 and 66.3 % for birds fed 12.5 and 25 % rice bran containing diets, respectively. The results of Liang *et al.* (2014) reported that lipid oxidation can be prevented by rice bran oil in poultry muscle, due to having different compounds such as: gamma-oryzanol, tocotrienols, tocopherols and squalene. Seifi *et al.* (2015) showed that the decrease of TBARS values in chicken muscle which has been injected by rice bran oil may be due to the increase of the ratio of other components like tocopherol, tocotrienol, and oryzanol in the products that come from rice bran oil. Kim *et al.* (2000) and Chae *et al.* (2002) demonstrated that rancid rice bran reduced TBARS value in chicken meat.

Glutathione peroxidase was insignificantly affected by rice bran levels, however, it was observed numerical increase in glutathione peroxidase in the group given 12.5 and 25 % rice bran containing diet. This increase surpassed the control one by 9.1 and 19.6 %, respectively. Seifi *et al.* (2015) found that the level of glutathione was higher either in muscle or heart in that group which has been injected by rice bran oil into the yolk as compared to control group. These results also confirmed the antioxidant capacity power of rice bran oil (Öztürk-Ürek *et al.,* 2001).

In conclusion, egg laying rate and egg quality insignificantly affected by rice bran, however, the results showed significant improvements in blood and egg yolk lipid profile and diminished oxidative status as a result of inclusion fresh rice bran in the diet. A number of human (Lai *et al.,* 2012; Mäkynen *et al.,* 2012) and animal (Mobarak *et al.,* 2010) studies have shown that rice bran and rice bran oil have hypocholesterolemic effects, results of this experiment suggested that such an effect does exist in laying Japanese quail.

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الملخص العربي

تأثير رجيع الكون على الانتاج ، الفقس، مستوى الدهون فى الصفار والدم فى السمان اليابانى البياض

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أستخدم عدد ٢١٦ انثى ، ١٠٨ ذكر سمان يابانى عمر ١٤ أسبوع فى تجربة أستمرت لمدة ٧ أسابيع لدراسة تأثير أضافة رجيع الكون فى العليقة على جودة ومعدل انتاج البيض بالاضافة الى تأثيره على دهون الدم وصفار البيض. أستخدمت ثلاث معاملات غذائية ، مجموعة كونترول ضابطة تناولت عليقة من الذرة الصفراء وكسب الصويا، مجموعة تناولت عليقة تحتوى ١٢.٥ % رجيع الكون الطازج (لم يخزن أكثر من ١٤ يوم) ، مجموعة تناولت عليقة تحتوى ٢٥ % رجيع الكون الطازج. أوضحت النتائج أن معدل انتاج البيض ، وزن البيض، كتلة البيض، والكفاءة

التحويلية لم تتأثر بالمعاملات المختلفة. لوحظ انخفاض في الدهون الكلية وكولسترول صفار البيضة نتيجة أضافة رجيع الكون فى العليقة مقارنة مع المجموعة الضابطة. لم يتأثر كل من نسبة الخصوبة والفقس بالمعاملات المختلفة. أنخفض كل من الدهون الكلية ، الجلسريدات الثلاثية ، الكولسترول منخفض الكثافة بينما أرتفع الكولسترول مرتفع الكثافة نتيجة لاحتواء العليقة على رجيع الكون الطازج. معاملات رجيع الكون أدت الى زيادة تركيز الصفات الضد تاكسدية مقارنة مع المجموعة الضابطة وصلت الى ٣٢.٦ ، ٦٦.٣ % للطيور التى تناولت ١٢.٥ ، ٢٥ % رجيع الكون على التوالى. تلاحظ زيادة رقمية فى تركيز الجلوتاثيون بيروكسيديز نتيجة لاضافة رجيع الكون فى العليقة مقارنة مع المجموعة الضابطة. وخلاصة الدراسة أن معدل انتاج البيض وصفات جودة البيض لم تتأثر برجيع الكون ولكن تلاحظ تحسن معنوى فى محتوى الدم وصفار البيض من الدهون بجانب الصفات الضد تأكسدية نتيجة لاضافة رجيع الكون الطازج .

Bioremediation Effect of Contaminated Soils with Copper and Lead on Sweet Basil Plants

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ABSTRACT: The effects of phosphorus amendments and arbuscular mycorrhizal (AM) fungi *Glomus intraradices* and *pseudomonas putida* on the sweet basil (*Ocimum basilicum* L.) yield, chemical composition and percent of volatile oil, and metal accumulation in plants and its availability in soil were investigated in field experiment at two seasons 2012 and 2013 under contaminated soil with Pb and Cu. The plant height, herb fresh weight, content of essential oil and shoot and root dry weights of sweet basil were increased by the application of mineral phosphorus as compared to control. Inoculation with AM fungi and bacteria reduced the metal concentration in shoot, recording a lowest value of (33.24, 18.60 mg/kg) compared to the control (46.49, 23.46 mg/kg) for Pb and Cu, respectively. Availability of Pb and Cu in soil were decreased after cultivation in all treatments compared to control. However, metal root concentration increased with the inoculation, with highest values of (30.15, 39.25 mg/kg) compared to control (22.01, 33.57mg/kg) for Pb and Cu, respectively. The content of linalool and methyl chavicol in basil oil was significantly increased in all treatments compared to control, but the interaction was not. We can thus conclude that the AM-sweet basil symbiosis and bacterial inoculation could be employed as an approach to bioremediate polluted soils and enhance the yield and maintain the quality of volatile oil of sweet basil plants.
Keywords: Arbuscular mycorrhizal fungus, *Pseudomonas*, Sweet basil, Heavy metals, Bioremediation.

INTRODUCTION

Aromatic and medicinal plants are an important source of national income and foreign currency in Egypt. Basil is one of the most important species for export among the medicinal and aromatic plants and it has a good reputation in the European countries. The area cultivated with basil in Egypt is about 4-5 thousand feddans, and the exports are more than 4000 tons per year (El-Sayed *et al*., 2003). The genus *O. cimum* comprises more than 150 species and is considered as one of the largest genera of the Lamiaceae family (Evans, 1996). *Ocimum basilicum* L. (sweet basil) is an annual herb, plants is widely used in food and oral care products; the essential oil of the plant is also used as perfumery (Bauer *et al*., 1997) and antimicrobial (Chiang, 2005). Environmental conditions and agricultural practices can significantly alter yield and chemical composition of sweet basil (Sifola and Barbieri, 2006).

Monitoring for toxic heavy metals in medicinal plants has become part of the quality control in the pharmaceutical industry as consumers demand products that are free from potentially harmful constituents (Chizzola *et al*., 2003). Phytoextraction potential of medicinal and aromatic plants grown in heavy metal polluted agricultural soils is a subject of ongoing research (Kovačik *et al*., 2009; Stancheva *et al*., 2014). In the rhizosphere, a synergism between various bacterial

genera such as *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Rhizobium* has been shown to promote plant growth of various plants such as sweet basil (*Ocimum basilicum* L.) (Hemavathi *et al*., 2006). *Pseudomonas putida* has the capacity to improve growth in plants (Rodriguez *et al*., 2014) particularly in sweet basil plants (Ordookhani *et al*., 2011). .Arbuscular mycorrhizal fungi (AMF) symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomass (Smith *et al*., 2010) which improves plant productivity (Fedderman *et al*., 2010). AM fungi can contribute plant growth, particularly in disturbed or metal polluted sites, by increasing plant access to relatively immobile minerals such as P, Zn, and Cu (Ryan and Angus 2003; Christie *et al*, 2004). In addition, they can improve soil structure (Gaur and Adholeya, 2004), stabilize metals in the soil, reduce their uptake, and thus decrease the risk of toxicity to plants growing in polluted substrates (González-Chávez *et al*., 2009).Thus, plants in symbiosis with AM fungi have the potential to take up heavy metals (HM) from an enlarged soil volume (Upadhyaya *et al*., 2010).

The aim of the present study was to examine the potency of two isolates of AMF (*Glomus intraradices*), and *Pseudomonas putida* to reduce metal accumulation in plant organs, and to evaluate how inoculation effect of mycorrhization and essential oil composition of (*Ocimum basilicum* L). cultivated in Pb and Cu contaminated soil.

MATERIALS AND METHODS

Cultivated plant: Sweet Basil (*Ocimum basilicum*) seeds were obtained from Medicinal and Aromatic Plants Department, Agricultural Research Center, Ministry of Agriculture, Egypt. Seeds were germinated in seedling trail with 209 holes containing (1:1, v: v) peat moss and Sand. In case of mycorrhizal treatment (G3, G4), the inoculant was applied to the nursery at the rate of 10% added to the mixture. Seeds were sown in the nursery on April 16th and the seedlings of 2-3 pairs of leaves were transplanted to the field at May 28th with one plant per hill.

Soil initial state analysis: Some physical and chemical properties of surface layer (0-30 cm) of the experimental field and heavy metals content (Pb and Cu) were determined according to (Page *et al*., 1982) and (Klute, 1986). The soil had the following physicochemical properties: clay loam in texture, pH (1:1 soil water suspension) 7.75 and 7.85, electrical conductivity (EC) 1.95 and 1.98 dSm^{-1}, available P (15, 14.7 mg kg^{-1}) soil, and the available Pb and Cu were (109, 100) and (90.3, 88.6) mg/ kg for the two seasons, respectively.

Experimental design: The field experiment was conducted in Abees Experimental Farm, Faculty of Agriculture, Saba Basha, 10th Village, Alexandria University, Egypt during two seasons 2012 and 2013. The experiment was carried out in a randomized split – split plot (10.5m^2, 3.5 x 3m) design in 3 replicates. Phosphorus fertilizer (0%, 75%, and 100% of the recommended dose) was the main plot, and

the two treatments un-inoculated and inoculated with *Pseudomonas putida* were randomly distributed in the sub- plot, while the three treatments with *Glomus intraradices* (control un-inoculated, G3 and G4) were arranged in the sub – sub plots.

Inoculation

Arbuscular Mycorrhizal Fungi: Two mycorrhizal isolates of *Glomus intraradices* were used in this experiment; isolate (G3) was isolated from the Experimental Station of Alexandria University at Abees (Aboul-Nasr, 1993), and isolate (G4) was obtained by Amykor Company, Germany. The inoculum consisted of expanded clay aggregates (2-4 mm in diameter, Leca) containing chlamydospores and fungus mycelium, which had been cullivated on *Tagetes erecta* L. (Aboul-Nasr, 2004). Inoculant was added at the rate of 7.0 g/per hill below basil seedling after transplanted. Control plants received the same amount of heat sterilized expanded clay.

Bacterial treatment: *Pseudomonas putida* obtained from Bio fertilization unit, Faculty of Agriculture, Ain Shams University was grown in liquid broth (LB) medium comprising of (g/L): tryptone, 10; yeast extract, 5; NaCl, 5. The pH of the medium was adjusted to 7.2–7.4 with 1 N HCl or 1 N NaoH and sterilized by autoclaving at 121 ℃ for 15 minutes. Incubation was carried at 35 ℃ for 3 days. When plants were transplanted to the field, 15 ml (1.3×10^6 viable cells/ml) of bacterial suspension were added to each hill.

Mineral fertilizers: Three different rates (zero, 75% and 100%) of the recommended dose (400 kg/fed) of mono- calcium phosphate (15.5 % P_2O_5) was used in one does at seedling transplanted for each growing seasons. Ammonium nitrate (33.5% N) was added at the recommended dose150 kg/fed (50 kg N/ fed) after 30, 60, and 90 days of cultivation. Potassium sulfate (48% K_2O) was applied to all plots after 41 days of cultivation in the rate of 100 kg/fed.

The percentage of mycorrhizal colonization: The percentage of mycorrhization was estimated three times; (first, 4 weeks from germination to confirm the colonization before transfer to the field, second, 9 weeks old 1st cut and third at 16 weeks old 2nd cut). Root samples 1 cm were cleared with 10% KOH and stained with Trypan blue (0.05%) in lactophenol to observe under microscope (Koska and Gemma, 1989). Mycorrhizal colonization was estimated by determining the percentage of length of root segments containing AM fungal structure (arbuscules, vesicles, spores) according to Biermann and Linderman, (1981).

Vegetative parameters: Ten plant samples per plot for each cut (1st and 2nd) were taken to determine the plant height (cm.) and herb fresh weight (g/plant). Five plants were air dried till constant weight and the average dry weight of root and shoot (g/plant) were calculated.

Chemical analysis

Phosphorus content: Plant powder (0.5g) was wet-digested with H_2SO_4 – H_2O_2 mixture (Lowther, 1980). Phosphorus was determined by the Vanadomolybdate yellow method (Jackson, 1967) using Millton Ray Spectronic 21 D.

Essential oil percentage: The essential oil percentage was determined in the air-dried herb according to British Pharmacopoeia (1963) by water distillation of 40 g of air dry herb for 1.5 – 2.0 hours, in order to extract the essential oil.

Essential oil analysis and its major components: The essential oils were diluted in diethyl ether (20 :1, v:v) and analyzed with GC (HP 8644) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5, 25 m in length, 0.32 mm i.d., and 0.5 mm film thickness.133 Helium was used as the carrier gas with a flow rate of 1.6 ml/min; the detector 134 temperature was 260 °C, the oven temperature was programmed to increase from 130 to 260 °C at a rate of 4 °C/min. The split injector was heated at 250 °C; the split 136 ratio was 15:1. Data were processed on a DP 800 integrator. The percentage of major constituents of stander (β-caryophellene, Linalool and Methyl chavicol) was estimated by measuring the peak area of the different compounds of the chromatogram according to (Heftman, 1967) and (Gunther and Joseph, 1978). Standards of the principal components which used as reference compounds for sweet basil were obtained from Ciba Gigi, NY, USA.

Total lead and copper analysis: Heavy metals content in shoots and roots were determined by digesting the dried plant of each plot according to (Lowther, 1980). The disgusted solutions were analyzed for lead and copper using the atomic absorption spectrophotometer (A Analyst 400) (Jackson, 1967). Available lead and copper remain in soil after cultivation were measured by atomic absorption spectrophotometer model (A.A. Spectrometry Thermo Elemental Type Solar 54/2001, ser No. GE 710728) using diethylene triamina penta acetic acid (DTPA) method as described by (Page *et al*., 1982).

Statistical analysis: Data were statistically analyzed by the procedure of variance to test the treatments effect on different measured parameters according to (Snedecor and Cochran, 1981). Data for the percentage of root length colonization % were analyzed using angular transformation (Steel and Torrie, 1982)

RESULTS

Mycorrhizal colonization percentage %

Mycorrhization was determined three times during every growing season before plant transplanting (4 weeks old) and two times after transplanting (9 and 16 weeks) the colonization at the 4 weeks was more than 33.3% that to confirm the colonization before transplanting. Root colonization by indigenous *G. intraradices*

(G3 and G4) were low (Fig 1), but was enhanced by the inoculation with *P. putida*. Addition of P had no effect on mycorrhization in age 9 weeks but increased significantly after 16 weeks (Table1). However, the percent of root colonization was significantly higher in the basil inoculated with *G. intraradices* G3 and G4 than the non-inoculated plants. The AM root colonization in inoculated plants ranged from 32.99 to 55.10% and the native soil AM colonized roots were 7.72 % and 11.53 % at 9 and 16 weeks, respectively. The highest percent (55.10%) of colonization was noticed in plants with 16 weeks old with G3 and *P. putida* in the presence 75% of mineral phosphorus fertilizer. Generally the mycorrhizal root colonization hadn't high value in this contaminated soil this could be attributed to the effect of heavy metals on the mycorrhizal spore germination, hyphal growth and branching.

(a) Plants with 9 weeks old **(b) Plants with 16 weeks old**

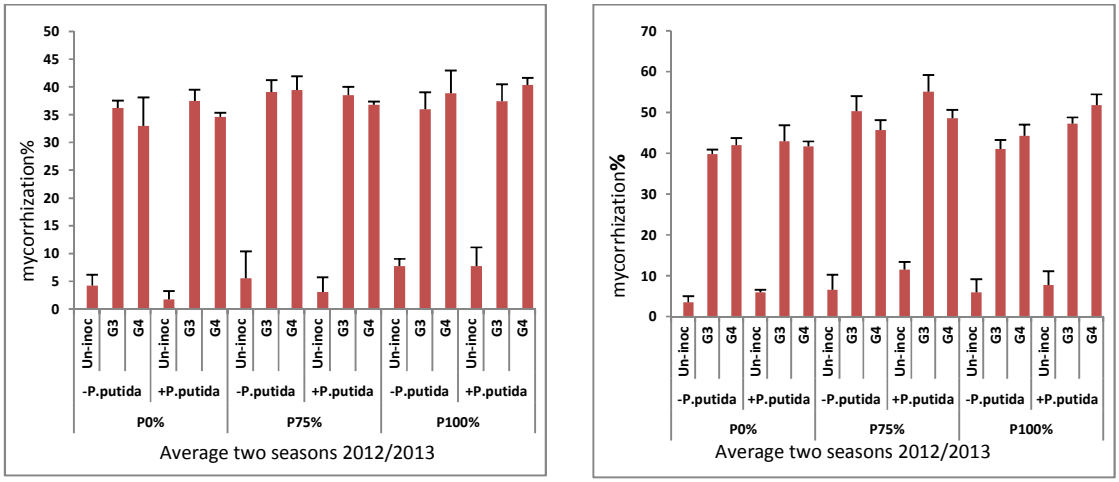

Fig (1). Effect of inoculating Ocimum *basilicum* L with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on mycorrhization after 9 and 16 weeks.

Table (1). A three-way ANOVA (LSD $_{0.05}$) showing the effect of P rates (P), *P. putida* (P.p) and *Glomus intraradices* (G.i) and their interactions.

Factors	Mycorrhization 9 weeks	Mycorrhization 16 weeks
Phosphorus (P)	3.18^{ns}	2.07^{**}
***P. putida* (P.p)**	3.87^{*}	3.22^{**}
***G. intraradices* (G.i)**	1.96^{***}	1.45^{***}
P× P.p	4.89^{ns}	4.57^{ns}
P× G.i	2.13^{**}	2.06^{***}
P.p× G.i	5.12^{ns}	5.07^{ns}
P× P.p× G.i	3.42^{ns}	6.18^{ns}

*: significant; ns: not significant

Vegetative parameters

Plant height (cm) and herb fresh weight (g/plant)

The results presented in Table (2) average values of cut one and cut two for the two seasons showed the growth responses of sweet basil grown in heavy metals polluted soil inoculated with *Pseudomonas putida* and AM fungi (G3 , G4) in the presence of different mineral phosphorus fertilizer rates. Data revealed that the plant height and herb fresh weight significantly increased in case of inoculated plants with both bacteria and mycorrhizal individually or in dual treatments, and the interaction between application of phosphorus and AM fungi had the same trend, additionally the interaction between bacteria and phosphorus or between bacteria and AMF had not affected, also the interaction between bacteria, AMF and phosphorus had the same trend except at the 2nd cut (16 weeks old).

Shoot and root dry weight (g/plant)

Sweet basil shoot and root dry weights (Table 3) responded significantly to phosphors application and AM inoculation, although the bacterial treatment had no significant impact in shoot dry weight in case of the second cut. Best results of shoots dry weight obtained with the addition of $P_{75\%}$ inoculated with *P. putida* and AM isolate G4 in the second cut. However the best results of roots dry weight (17.19 g/plant) obtained with addition $P_{100\%}$ and inoculation with bacteria and G4 in the 1st cut. Additionally no interaction could be observed in shoots or roots dry weight between the factors P nutrition and inoculation with *P. putida* while, root dry weight significantly increased in case of the interaction between the factor of inoculation with *P. putida* and AMF or the interaction between P, *P. putida* and AMF except at 1st cut for the latest interaction.

Phosphorus uptake (mg/g shoot) and oil content %

Data presented in Table (4) clearly show that the P uptake significantly increased, although the interaction between all factors had no impact. Application of phosphorus with different rates increased the phosphorus uptake of sweet basil plants, in the presence of inoculants (AM and bacteria). The highest P uptake observed in plants inoculated with G4 with bacteria in 75% of phosphorus fertilizer was (0.194 mg/g shoot). Although the highest percentage increase 268.8% was clear in G4 with bacteria. On the other hand the applied treatments improved the essential oil percentage compared with untreated plants. AMF isolate G4 was the most effective treatment in increasing the oil content under contaminated soil its value was (0.47%), moreover the interaction had a significant differences between all factors. Table (4) showed that the percentage increase of oil content in plants inoculated with AM isolate G3 and G4 were (80 and 88%) , respectively more than the un-inoculated basil plants. No significant differences were observed between the duel inoculations with bacteria and phosphorus application and between mycorrhizae too.

Table (2). Effect of inoculated *Ocimum basilicum* L. with *Glomus intraradices* and *Pseudomonas putida* on plant height (cm) and herb fresh weight (g/plant) in lead and copper contaminated soil as an average of the two growing seasons 2012 and 2013

Parameter		Plant height (cm/plant)					
Phosphorus (P)	*Pseudomonas putida (P.p)*	Cutst			Cutnd		
		G. intraradices (G.i)			*G. intraradices (G.i)*		
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	22.22	37.46	39.43	23.19	37.92	43.82
	+	30.38	45.62	38.50	21.86	48.92	50.73
P_{75}	-	35.99	40.23	51.88	23.44	40.93	49.88
	+	37.52	49.39	53.81	24.33	48.08	60.13
P_{100}	-	31.91	38.26	43.47	25.98	47.67	51.17
	+	33.37	43.85	46.34	34.33	61.34	54.90
$LSD_{0.05}$							
P			2.18***			2.06***	
P.p			1.18***			3.11***	
G.i			2.14***			1.86**	
P× P.p			6.21^{ns}			7.82^{ns}	
P× G.i			1.58*			2.14**	
P.p× G.i			9.21^{ns}			9.18^{ns}	
P× P.p× G.i			5.42^{ns}			1.92*	
		Herb fresh weight(g/plant)					
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	142.21	302.58	291.75	124.03	275.04	297.09
	+	155.59	335.63	342.78	132.76	325.16	390.97
P_{75}	-	226.70	280.62	321.32	136.71	259.80	350.50
	+	177.70	326.41	360.33	165.01	398.84	381.41
P_{100}	-	152.45	374.49	446.18	198.84	405.56	504.76
	+	195.02	433.49	498.23	215.41	462.52	528.91
$LSD_{0.05}$							
P			9.45***			7.11***	
P.p			11.36*			9.28**	
G.i			5.27***			7.79***	
P× P.p			15.96^{ns}			16.02^{ns}	
P× G.i			9.32**			5.14***	
P.p× G.i			22.11^{ns}			20.41^{ns}	
P× P.p× G.i			20.62^{ns}			5.29**	

Values are means± SE, n= 10 plants,*: significant; ns: not significant

Table (3). Effect of inoculated *Ocimum basilicum* L. with *Glomus intraradices* and *Pseudomonas putida* on shoot and root dry weight (g/plant) in lead and copper contaminated soil as an average of the two growing seasons 2012 and 2013

Parameter		Shoot dry weight (g/plant)					
Phosphorus (P)	*Pseudomonas putida* (P.p)	Cutst			Cutnd		
		G. intraradices (G.i)			*G. intraradices* (G.i)		
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	13.74	18.56	25.12	19.68	24.75	30.86
	+	17.14	25.91	28.49	20.00	29.18	34.00
P_{75}	-	17.95	37.86	35.85	22.77	39.19	41.07
	+	19.41	36.21	37.70	22.36	39.31	41.43
P_{100}	-	19.30	24.46	34.03	22.42	32.31	34.57
	+	15.36	30.99	33.66	24.50	34.20	37.57
$LSD_{0.05}$							
P		2.43***			1.06***		
P.p		1.75*			4.32^{ns}		
G.i		4.27**			1.45***		
P× P.p		3.71^{ns}			4.62^{ns}		
P× G.i		2.89**			1.11***		
P.p× G.i		7.19^{ns}			9.22^{ns}		
P× P.p× G.i		5.89^{ns}			7.56^{ns}		
		Root dry weight (g/plant)					
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	9.07	10.98	10.57	9.68	11.23	11.32
	+	9.43	11.92	12.03	10.41	11.74	11.98
P_{75}	-	10.94	13.17	13.47	11.05	12.76	12.82
	+	11.23	13.98	14.60	10.97	14.13	14.37
P_{100}	-	11.50	14.47	15.35	10.55	13.74	15.12
	+	11.83	16.04	17.19	11.34	15.71	15.93
$LSD_{0.05}$							
P		0.43***			0.165***		
P.p		0.29***			0.128**		
G.i		0.25***			0.341***		
P× P.p		2.18^{ns}			2.58^{ns}		
P× G.i		0.23***			0.267***		
P.p× G.i		0.41**			0.125**		
P× P.p× G.i		2.74^{ns}			0.131**		

Values are means± SE, n= 5 plant**,***: significant; ns: not significant

Table (4). Effect of inoculated *Ocimum basilicum* L. with *Glomus intraradices* and *pseudomonas putida* on phosphorus uptake (mg/g shoot) and oil content% in lead and copper contaminated soil as an average of the two growing seasons 2011/2012 and 2012/2013

Parameter		Second cut					
Phosphorus (P)	*Pseudomonas putida* (P.p)	Phosphorus uptake (mg/g DW of plant)			Oil content %		
		G. intraradices (G.i)			*G. intraradices* (G.i)		
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	0.032	0.082	0.118	0.23	0.32	0.34
	+	0.043	0.129	0.145	0.25	0.36	0.37
	±%	-	156.3	268.8	-	39.1	47.8
	±%	-	200	237.2	-	44	48
P_{75}	-	0.051	0.101	0.175	0.25	0.45	0.47
	+	0.055	0.180	0.194	0.25	0.45	0.47
	±%	-	98.03	243.1	-	80	88
	±%	-	227.3	252.7	-	80	88
P_{100}	-	0.066	0.139	0.166	0.24	0.39	0.39
	+	0.053	0.172	0.192	0.29	0.41	0.43
	±%	-	110.6	151.5	-	62.5	62.5
	±%	-	224.5	262.3	-	41.4	48.3
$LSD_{0.05}$							
P		0.010***			0.018***		
P.p		0.009**			0.014**		
G.i		0.031**			0.018***		
P× P.p		0.21^{ns}			0.17^{ns}		
P× G.i		0.032***			0.016***		
P.p× G.i		0.31^{ns}			0.28^{ns}		
P× P.p× G.i		0.17^{ns}			0.015***		

Values are means± SE, n= 5 plants,*: significant; ns: not significant

Major oil components (Linalool and Methyl chavicol %) of sweet basil plants

Chemical characterization of essential sweet basil oil (Table 5) demonstrated that quality of oil improved on mycorrhization and bacterial treatments in the presence of phosphorus fertilizer. Linalool the major component of sweet basil affected significantly with both isolates of AM G3, G4. However the bacterial inoculation had no significant differences. Methyl Chavicol had the similar trend with inoculation; moreover the interaction showed no significant impact under contaminated soils. Data recorded in Fig (2) showed that the best results were obtained from G4 and bacterial inoculation in the presence of 100% P, it were (52.99% and 7.83%) for linalool and methyl chavicol, respectively.

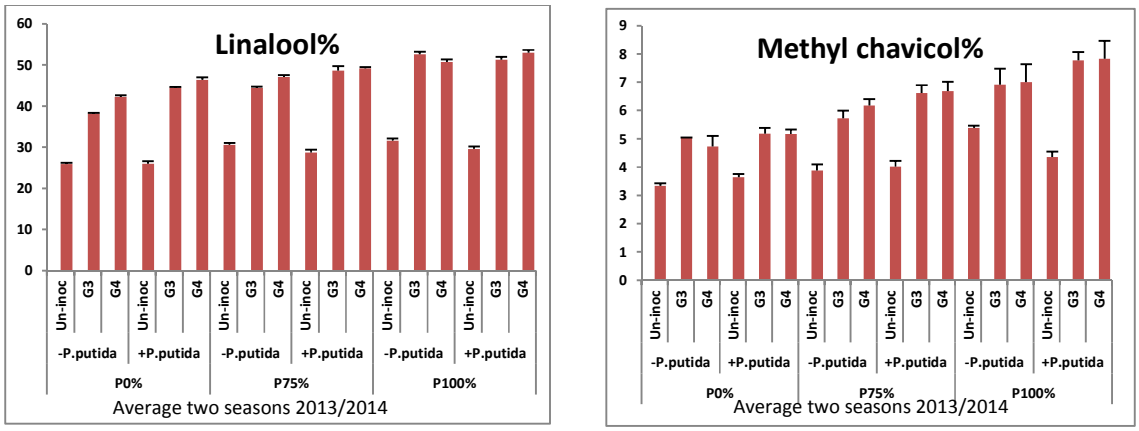

Fig (2). Effect of inoculated *Ocimum basilicum* L with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on linalool% and methyl chavicol% in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (5). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P), *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i), as well as the effect of their interaction.

Factors	Linalool %	Methyl chavicol%
Phosphorus (P)	3.74**	1.85***
P. putida (P.p)	2.46^{ns}	4.17^{ns}
G. intraradices (G.i)	5.22**	3.64**
P× P.p	3.15*	4.18^{ns}
P× G.i	1.27**	5.85^{ns}
P.p× G.i	5.12*	5.62^{ns}
P× P.p× G.i	4.48*	4.96^{ns}

*: significant; ns: not significant

Analysis of heavy metals
Lead concentration mg/kg in shoots and roots

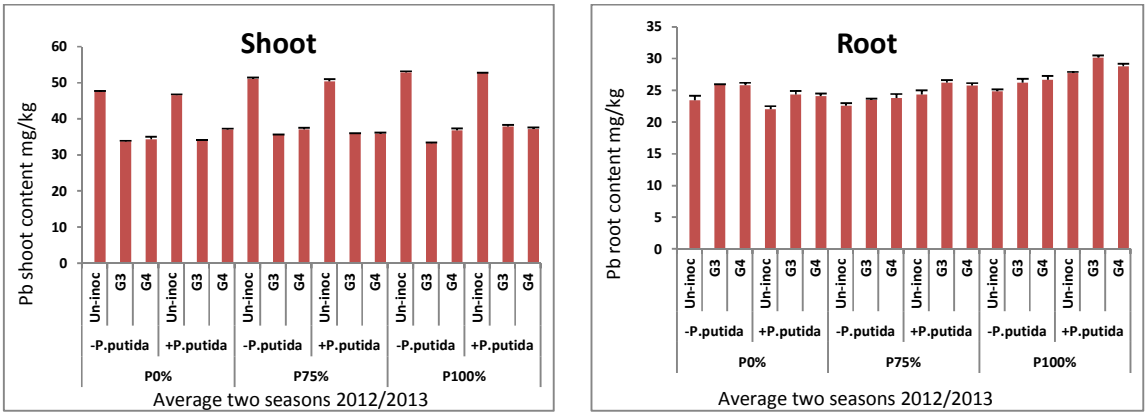

Fig (3). Effect of inoculated *Ocimum basilicum* L with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on Pb content (mg/kg) in shoot and root in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (6). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P), *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i) as well as the effect of their interaction.

Factors	Pb shoot content (mg/kg)	Pb root content (mg/kg)
Phosphorus (P)	2.54***	1.23*
P. putida (P.p)	1.31*	3.15^{ns}
G. intraradices (G.i)	2.14**	1.06**
P× P.p	1.15*	2.84^{ns}
P× G.i	0.85**	0.87*
P.p× G.i	0.94*	0.93**
P× P.p× G.i	1.02*	1.74*

*: significant; ns: not significant

Copper concentration mg/kg in shoots and roots

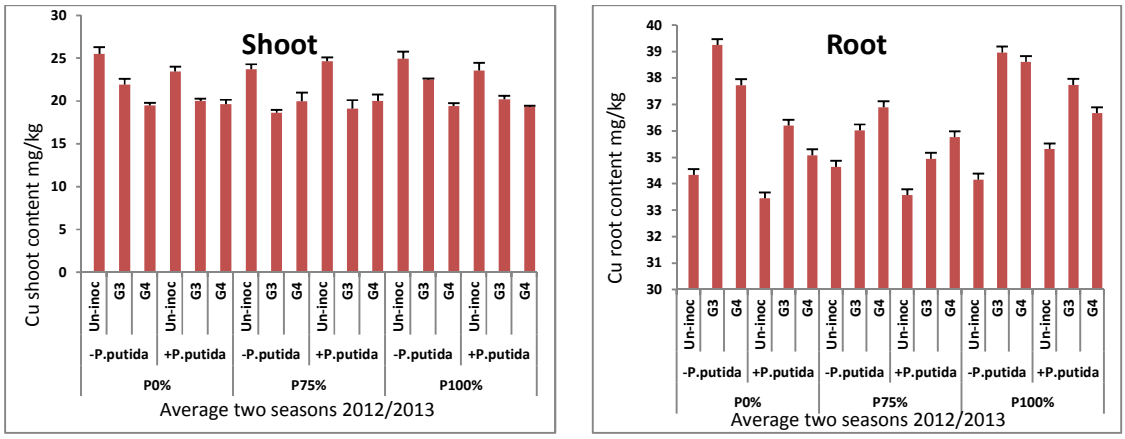

Fig (4). Effect of inoculated (*Ocimum basilicum* L) with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on Cu shoot and root concentration in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (7). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P), *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i) as well as the effect of their interaction.

Factors	Cu shoot content (mg/kg)	Cu root content (mg/kg)
Phosphorus (P)	2.18***	1.38***
***P. putida* (P.p)**	1.67*	0.99*
***G. intraradices* (G.i)**	2.41**	2.83**
P× P.p	3.81^{ns}	3.14^{ns}
P× G.i	2.04*	1.77**
P.p× G.i	2.89^{ns}	2.08*
P× P.p× G.i	3.79^{ns}	3.87^{ns}

*: significant; ns: not significant

Lead and copper available in soil after cultivation

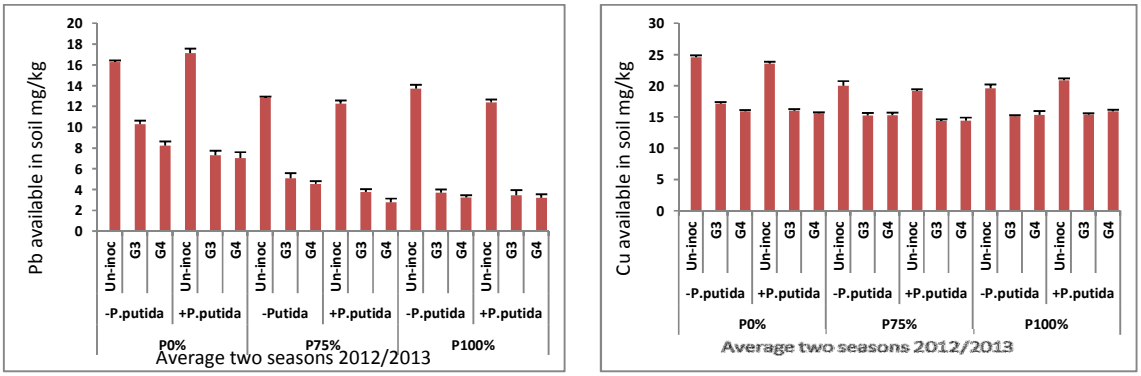

Fig (5). Effect of inoculated (*Ocimum basilicum* L) with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on Pb and Cu available in soil after cultivation (mg/kg) in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (8). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P) *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i) as well as the effect of their interaction.

Factors	Pb available in soil (mg/kg)	Cu available in soil (mg/kg)
Phosphorus (P)	2.13***	2.18**
***P. putida* (P.p)**	1.21*	1.64*
***G. intraradices* (G.i)**	1.67**	3.14***
P× P.p	4.31^{ns}	5.48^{ns}
P× G.i	0.92**	2.87**
P.p× G.i	1.35*	3.66^{ns}
P× P.p× G.i	5.71^{ns}	4.18^{ns}

*: significant; ns: not significant

Data presented in Figure (3, 5) showed the effect of mycorrhizal and bacterial inoculum in the presence of different phosphorus rates on Pb shoot and root content (mg/kg). From the analysis of variance (ANOVA) presented in Tables (6, 8) it was clear that Pb concentrations in sweet basil shoots, were highly significant affected by bacterial and mycorrhizal inoculation as well as the interaction between them. On the other hand Pb concentrations in shoots were increased by increasing phosphorus application and were decreased by the inoculation with AM isolate G3 or G4 and *P. putida*. Shoots of inoculated plants with G3 had the lowest value of Pb (33.24 mg/kg) showed in treatments with $P_{100\%}$ without bacteria. Opposite results were obtained in roots, lead concentration was higher in the inoculated plants than un-inoculated one.

Low levels of Pb were measured in the control basil roots compered to treated plants. Available lead residues in the soil were lower as a result of inoculation with both bacteria and AM mycorrhizal isolate G4 and G3 then the inoculation with bacteria alone. Significant reduction of Pb compared with the controls was observed in both soil and shoot of basil inoculated G3 and G4 isolate. However the highest value of soil available Pb (17.18 mg/kg) was observed in the absences of fungi and with bacteria inoculation and not amended with phosphorus fertilizer. It is clear also, that the AM isolate G4 was more effective mostly than G3 in reducing Pb value in soil. In the same time, there was a significant difference between the Pb values over all treatment for the soil after cropping except the interaction between P*Pp, P*Gi and Pp*Gi Table (6, 8). The same trend was observed in copper concentration copper metal accumulation in shoot and a root tissue of sweet basil was significantly affected by application of AM inoculation Fig. (4, 5). It was accumulated in very low concentration in shoot tissue (< 25 mg/kg) and soil (<12 mg/kg) as compared to accumulation in control plants. Inoculation significantly increased the concentration of Cu in roots than shoots under all phosphorus fertilizer rates. It is clear that the treatments reduce the soil available Cu compared to non-treated plants Table (4, 5).

DISCUSSION

The present study demonstrates that colonization of basil plant root by the AM fungus *G. intraradices* was significantly affected by the application of different phosphorus fertilizer rates and inoculation with *P. putida*. Non-inoculated sweet basil plants showed >10.0% root colonization due to native mycorrhizae in soil and the percent root colonization was significantly enhanced by plant inoculation with AM fungi and bacteria under heavy metals (Pb and Cu) contaminated soil. Similar results were reported by (Banni and Faituri, 2013) who observed that *Glomus* spp. treated plants had higher mycorrhizal colonization rates than other inoculation-treated plants. The data of the present work also showed that shoot and root of basil plant biomass increased in inoculated plant compared to control. Positive effects of mycorrhization on the growth of essential-oil-containing plants were reported with *Ocimum basilicum* L. (Khaosaad *et al*., 2006) and *Salvia officinalis* L. (Geneva *et al*., 2010). The outcome of the plant-AM fungal association is metal-specific and depends on bioavailability of metals in soil and on both plant and AM species (Sudova and Vosatka, 2007).

AM fungal inoculation increased shoot yield, content of essential oil, and root yield of sweet basil probably due to the increase of nutrient uptake (Marschner and (Dell, 1994). (Banni and Faituri, 2013) reported that the two AM fungi species significantly increased the root and shoot dry weights and this species was more effective than non-mycorrhizal treatment in protection the maize plants against Cu toxicity. (Vinutha, 2005) observed increased shoot and root growth weight, biomass and essential oil content of *Ocimum* spp. when inoculated with *Glomus fasciculatum*, *Azotobacter chroococcum* and *A. awamori*. (Hemavathi *et al*., 2006) found similar observations in *Ocimum basilicum*, where plant growth increased after inoculation with *G. fasciculatum*, *Pseudomonas fluorescens* and *Bacillus*

megaterium. In another study, (Ordookhani *et al*., 2011) found an increase in shoot, root dry weight, N, P and potassium (K) content and essential oils in *Ocimum basilicum* inoculated with PGPR *Pseudomonas putida* and *Azotobacter chroococcum* additionally. (Ordookhani, 2011) reported that *P. putida* as PGPR had the capacity to increase *Ocimum basilicum* microelement contents and significant differences between PGPR treatments on essential oil, Fe, Zn, Mn and Cu contents compered to control.

Relatively little is known about the effects of AM colonization on the accumulation of active phytochemicals in shoots of medicinal plants, which are often the harvest products. However, it was reported that *Glomus mosseae* directly increased the essential oil content in shoots in two of three tested oregano genotypes *Origanum* sp grown on industrially Cd and Pb polluted soil (Khaosaad *et al.,* 2006; and Hristozkova *et al.,* 2015). Similar results were obtained by (Copetta *et al*., 2006) in studies with *Ocimum basilicum* L. Our results are in an agreement with the reports of (Prasad *et al*., 2011) that the AM fungal inoculation maintained the level of linalool, methyl chavicol, in sweet basil oil, which were either increased or decreased by the application of heavy metals. (Ordookhani *et al*., 2011) showed that sweet basil inoculated with *P*. *putida* increase oil yield compared to control.

The arbuscular mycorrhiza (AM) fungi can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003), and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that AM fungi can colonize plant roots in metal contaminated soil (Vogel-Mikus and Marjana., 2006), while their effects on metal uptake by plants are conflicting. (Zhang *et al*., 2010) reported that AMF increased lead accumulation in the roots. The same trend was observed in case of inoculation with isolate G3+*P. putida* under $P_{100\%}$ rate in our results. The most important finding of the present research is that AMF can keep metal concentrations in aromatic plants at low levels and alleviate harmful effects caused by heavy metal pollution. The inoculation with the AMF strains of EEZ 54 and EEZ 55, isolated from a place with naturally high levels of metals, reduced the Pb concentration in marjoram shoots and roots (Hristozkova *et a.l,* 2015). The effects of mycorrhizal colonization on remediation of contaminated soils depend on the plant–fungus–heavy metal combination and are influenced by soil chemical and physical conditions (Siddiqui and Pichtel., 2008). According to (Azcon *et al*., 2010), AMF symbiosis may contribute to phytoremediation via strategies such as HM sequestration or accumulation, keeping metal concentrations in the plants below critical values and improving plant growth and nutrition (Pawlowska and Charvat ., 2004). Prasad et al, (2011), demonstrated that AM species could be effective in protecting sweet basil exposed to high levels of metals. Mycorrhizal fungi are reported to protect plants from the toxic effects of high external concentration of several metals, possibly by binding the metals in their hyphae or by reducing the translocation of metals to the plants tops (Mozafar *et al*., 2002). The higher AM-plant metal content of roots could be attributed to fungal metal binding and sequestration in intraradical hyphae, and these metal forms are not bioavailable to

plants (Christie *et al*., 2004). Plant under study accumulated more copper in roots but large reductions in shoots; this is in agreement with Banni and Faituri, (2013) that the comparisons of the two AM fungal species indicate that the AM fungal represented by *Glomus* spp (mixed) can benefit against potentially toxic Cu and therefore play a role in bioremediation of Cu-contaminated soils. Such results underline the importance of indigenous AM fungi, which are presumably more adapted to heavy metals.

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الملخص العربي

تأثير المعالجة الحيوية للأراضي الملوثة بالنحاس والرصاص علي نباتات الريحان

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أجريت تجربة هذا البحث في مزرعة كلية الزراعة (سابا باشا) بأبيس خلال موسمي ٢٠١٢ و٢٠١٣ وذلك بإضافة سماد الفوسفور بثلاث معدلات (صفر %، ٧٥ % ، ١٠٠%) من الكميه الموصي بها والتلقيح بكل من فطر الميكوريزا (*Glomus intraradices*) وبكتيريا (*Pseudomonas putida*) على نبات الريحان وذلك لدراسة المعالجة الحيوية للأراضي الملوثة بكل من عنصري الرصاص والنحاس وأوضحت النتائج أن هناك زيادة معنوية في كل من (طول النبات ، كمية المحصول ، النسبة المئوية للزيت والمواد الفعالة في زيت الريحان ، الوزن الجاف للسيقان والجذور) للنباتات المعاملة بالمقارنة بالنباتات غير المعاملة وأدى التلقيح بكل من فطر الميكوريزا والبكتيريا إلى إنخفاض نسبة عنصري الرصاص والنحاس في المجموع الخضري والتربة حيث سجلت أقل نسبة للمجموع الخضري وهي (٣٣,٢٤ ، ١٨,٦٠ مجم / كجم) لكل من عنصري الرصاص والنحاس علي التوالي بالمقارنه بالنباتات غير المعاملة فكانت (٤٦,٤٩ ، ٢٣,٤٦ مجم/كجم) علي التوالي ، علي الجانب الآخر إتضح زيادة لتركيز عنصري الرصاص والنحاس في المجموع الجذري مع إضافة المعاملات وبلغت (٣٠,١٥ ، ٣٩,٢٥ مجم / كجم) بالمقارنة بالكنترول (٢٢,٠١ ، ٣٣,٥٧ مجم/كجم) علي التوالي.

Response of Two Maize Hybrids to Spatial Distribution and Nitrogenous Fertilization Rates

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ABSTRACT: To investigate the response of two maize hybrids to spatial distribution and nitrogen fertilization rates. In this respect, two filed experiments were conducted at the Experimental Farm, Faculty of Agriculture (Saba Basha), Alexandria University during 2014 and 2015 seasons in a split- split plot design. Whereas, three factors can be illustrated as follows: the main plot included two maize hybrids (30N11 and 31G98), while, plant spacing (20, 30 and 40 cm) was arranged in the sub plots, while nitrogen fertilization (192, 288 and 384 kg N/ha.) allocated in sub- sub plot. The obtained results cleared that maize hybrid 30N11 recorded higher plant height (cm), ear weight (g), grain weight/ear (g), number of grains /ear, 1000-grains weight (g), number of rows/ear, grain yield (t/ha), biological yield (t/ha), harvest index (%), grains NPK and protein contents than the other hybrid 31G98 in the first and second seasons, respectively. The highest means values of yield and chemical composition characters were obtained using nitrogen fertilizer at rate of 384 kg/ha., in both seasons, while the lowest ones were recorded by application of nitrogen at 192 kg/ha., in both seasons. Wider spacing between plants (40 cm) produced the higher yield and its components and protein content and NPK in the two successive seasons than narrower spacing (20 cm) which produced the lowest mean values of these characters.
Key words: maize; hybrids; spatial distribution; nitrogen rates; yield; chemical composition

INTRODUCTION

Maize (*Zea mays* L.) is the third most important staple food crop in terms of area and production after wheat and rice in Egypt. Also, in the world, it is one of the important cereal crops in the world after wheat and rice (Gerpacio and Pingali, 2007).

Improved cultural practices can play an important role in augmenting yield of corn crop. For an optimal yield, the nitrogen supply must be available according to the needs of the plant. On the other hand, suitable plants spacing for optimum leaf growth by controlling water, fertilizer and chemical inputs is essential for improving the growth variables responsible for high yield. Optimum plant densities ensure the plants to grow in their aerial and underground parts through different utilization of solar radiation and nutrients. When the plant density exceeds an optimum level, competition among plants for light above ground or for nutrients below the ground become severe, consequently the plant growth slows down and the grain yield decreases (Hasanuzzaman *et al*., 2009). Plant population is an improtant factor which affects the crop yield. Yield was increased by 4% with increasing plant density (Shapiro and Wortmann, 2006). Higher plant population produce 25% more grain yield and 38% more biomass as compared with low plant population and early sown crop produce 19% more grain yield and 11% more biomass than late planted crop (Abdul *et al.,* 2007).

Maximum crop production can be achieved by development of improved crop hybrids and suitable growing environment and soil with optimum plant

population/ha. Optimum plant population is the prerequisite for obtaining maximum yield (Trenton *et al.,* 2006 and Gustavo *et al.,* 2006).

Hybrids exhibited such variations in their yield attributes as cob length (cm), number of row/cob, number of kernels/row, number of kernels/cob, 100-kernel weight (g), stover yield Mg/ha., grain yield Mg/ha, biological yield ton/ha., and harvest index (%), and protein %. However, plant population 64000 plant/ha., gave the highest mean values for most studied characters and protein %. , and reduced weeds spread. Also, hybrid "TWC 352" recorded the highest values of most studied parameters under Alexandria conditions (Kandil, 2014).

Nitrogen is a key factor for plant photosynthesis, ecosystem productivity and leaf respiration (Johnson, 2001 and Martin *et al.,* 2008). Nitrogen stress may affect the light use efficiency and consequently influence long-term changes in vegetation biomass and carbon sequestration (Peng *et al.,* 2012). Increase nitrogen fertilization levels upto 200 kg ha^{-1} enhanced the plant height, grain yield and straw yield of hybrid maize, whereas increasing nitrogen levels decreased the harvest, grain, and straw ratio (Dawadi and Sah, 2012). The lowest ear weight was related to the lowest nitrogen level, while the highest ear weight was observed by the highest nitrogen level (240 kg N ha^{-1}), while there was no significant difference among nitrogen levels was observed on harvest index (Hoshang, 2012). Nitrogen fertilization levels, maize hybrids and their interactions showed such significant effects on maize growth, crop yield and its components. The maximum plant height, leaf area index (LAI), chlorophyll SPAD unit, number of rows/cob , number of kernels/row, number of kernels cob, 1000 grain weight, stover, grain, biological yields, harvest index and protein content were produced by the application either 429 or 357 kg N/ha (Kandil, 2013). There were gradual and significant increases in all growth parameters and grain yield resulted from foliar spray by raising N- fertilizer upto 288 kg N/ha., in both seasons. The S.C Pioneer 30K09 maize hybrid treated with 288 N/ha., produced the maximum values of plant height and grain yield in both seasons (Faheed *et al*., 2016).

Keeping in view the importance of plant density and nitrogen fertilization, the study was conducted to find out optimum plant spacing and suitable nitrogen fertilization level for getting higher yield of maize hybrid.

MATERIALS AND METHODS

The present study was carried out at the Experimental Farm, Faculty of Agriculture (Saba- Basha), Alexandria University, Egypt, during the two successive growth summer seasons of 2014 and 2015, to study the response of two maize hybrids to spatial distribution and nitrogen fertilization rates in a split-split plot design. Whereas, three factors can be illustrated as follows: the main plot included two maize hybrids (30N11 and 31G98), while plant spacing (20, 30 and 40 cm) was arranged in the sub plots, while nitrogen fertilization (192, 288 and 384 kg N/ha.) allocated in sub- sub plot.

The grains of the tested two hybrids (31G98 and 30N11) were obtained from Maize Research Section Agriculture Research Center, Ministry of Agriculture. The grains were sown on May 8th and 10th 2014 and 2015 seasons, respectively.

Soil texture was clay loam. A surface sample (0-30 cm) was collected before planting to identify some physical and chemical properties of this soil, as shown in Table (1) according to Page *et al.* (1982) and Klute (1986). The preceding crop was Egyptian clover (berseem) in the first season and barley (*Hordium vulgare*, L.) in the second season, respectively.

Each sub sub plot size was 12.60 m^2 included 6 ridges each 3 m in length and 0.70 m in width with the distance between hills as the above treatments mentioned.

Phosphorus fertilizer was added at rate of 100 kg calcium super phosphate (15.5% P_2O_5) just before sowing. Mineral nitrogen fertilizer was fully given the dose in a form of urea (46% N) after thinning before the first irrigation and before the second irrigation.

Table (1).Some Physical and chemical properties of the experimental soil in 2014 and 2015 seasons.

Soil properties	Season	
	2014	**2015**
A) Mechanical analysis :		
Clay %	38	37
Sand %	32	33
Silt %	30	30
Soil texture	Clay loam soil	
B) Chemical properties		
pH (1 : 1)	8.20	8.31
E.C. (dS/m) (1:2)	3.80	3.70
1) Soluble cations (1:2) (cmol/kg soil)		
K^+	1.52	1.54
Ca^{++}	9.4	8.7
Mg^{++}	18.3	18.5
Na^{++}	13.50	13.8
2) Soluble anions (1 : 2) (cmol/kg soil)		
$CO_3^{--} + HCO_3^-$	2.90	2.80
Cl^-	20.4	19.80
SO_4^{--}	12.50	12.60
Calcium carbonate (%)	6.50	7.00
Total nitrogen %	1.00	0.91
Available phosphate (mg/kg)	3.70	3.55
Organic matter (%)	1.41	1.40

Grain yield and yield components as cob length (cm), number of rows cob^{-1}, number of kernels row^{-1}, number of kernels cob^{-1},100- kernel weight (g), stover yield ton ha^{-1}, grain yield ton ha^{-1}, biological yield (ton ha^{-1}) harvest index (H.I.%) are measurements were obtained as an average of 2 ridges from mid of each plot.

Protein percentage was determined by estimating the total nitrogen in the grains and multiplied by 6.25 to obtain the percentage according of grains protein percentage to A.O. A.C. (1990). NPK percentages were determined in the dry grains. Their dry weights were determined following drying in a drying chamber to a constant weight at 75^{o}C for 72 hour according to Tandon (1995). After dryness, the plant samples were milled and stored for analysis as reported. However, 0.5 g of the grains powder was wet-digested with H_2SO_4–H_2O_2 mixture according to (Lowther, 1980) and the following determinations were carried out in the digested solution to determine NPK. Total nitrogen was determined in digested plant material colorimetrically by Nessler`s method (Chapman and Pratt, 1978). Phosphorus was determined by the Vanadomolyate yellow method as given by Jackson (1973) and the intensity of colour developed was read in spectrophotometer at 405 nm. Potassium was determined according to the method described by method Jackson (1973) using Beckman Flame photometer.

Data obtained was exposed to the proper method of statistical analysis of variance as described by Gomez and Gomez (1984). The treatments means were compared using the least significant differences (L.S.D.) test at 5% level probability by using the split- split model as obtained by CoStat 6.311(2005) as statistical program.

RESULTS AND DISCUSSIONS

Results recorded in Tables (2 and 3) revealed that plant height (cm), ear weight (g), grain weight/ear (g), number of grains/ear, 100-grains weight (g), number of rows/ear, grain yield (t/ha), biological yield (ton/ha) and harvest index (%) of two maize hybrids were, significantly, affected by plant spacing and nitrogen fertilizer rates in both seasons.

Results presented in the same tables demonstrated that maize hybrid “30N11” had higher value for the yield and its components i.e. plant height (cm), ear weight (g), grain weight/ear (g), number of grains /ear, 100- grains weight (g), number of rows/ear, grain yield (t/ha), biological yield (ton/ha) and harvest index (%) than the other hybrid “31G98” in the first and second seasons, respectively. The difference may be attributed to genetically differences between two maize hybrids which play an important role for make up the available nutrients and yield for the maize hybrids. These findings are in harmony with those obtained by Kandil (2014).

Results, also demonstrated that spacing between hills (40 cm), significantly, increased the yield and its components than narrower spacing (20 cm). These results are in agreement with those reported by Ahmad *et al.* (2010), Saadat *et al.* (2010), Peykarestan and Seif (2012), Moosavi *et al.* (2012), Lyocks *et al.* (2013) and Kandil (2014) who showed that there was a significant difference among plants spacing on maize characters.

On the other side, results presented in Tables (2 and 3) revealed that increasing nitrogen fertilizer level up to 384 kg/ha., significantly, increased plant height (cm), and yield components of maize i.e. ear weight (g), grain weight/ear (g), number of grains /ear, 100- grains weight (g), number of rows/ear, grain yield (t/ha), biological yield (t/ha) and harvest index (%) than application of 192 kg N/ha. It can be noticed generally that grain yield and its components affected by nitrogen fertilizer which play an important role in plant growth and finally appeard in gigher grain yield for two hybrids of maize. These finding were consistent with those obtained by Kumar (2008), Khan *et al.* (2012), Moraditochaec *et al.* (2012), Nemati and Sharifi (2012) and Kandil (2013).

The interaction between maize hybrids and plant and plant spacing reveal that the highest mean values of straw, and biological yield and harvest index were obtained with 30N11 hybrid at 40 cm. In the contrast, growing 31G98 at 20 cm produced the lowest ones during two cropping seasons (Table 4).

With regard to maize hybrids x nitrogen level interaction, results in Table (5) showed that the maize hybrid “30N11hybrid” with 288 kg N/ha., recoded the highest mean value of grain yield in the second season.

Considering interaction among maize hybrids x spacing x nitrogen fertilization level were significant for yield and its components characters in both seasons as cleared in Table (6). However, results revealed that wider spacing of “30N11” hybrid plants at (40 cm) and fertilized with 384 kg N/ha., produced the highest mean value of grain and straw and biological yield in the two respective seasons.

Table (2). Plant height, yield and its components as affected by two maize hybrids, plant spacing and nitrogen fertilizer rates in 2014 and 2015 seasons.

Treatments	Plant height (cm)		Ear weight (g)		Grain weight/ear (g)		Number of grains /ear		100-grain weight (g)	
	Season									
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Maize hybrids (H)										
31G98	210.59b	211.30b	225.12b	224.43b	170.86b	172.91b	511.81b	518.11b	39.79b	40.37b
30N11	217.33a	219.18a	286.07a	293.90a	223.88a	227.32a	556.07a	564.44a	47.09a	47.56a
LSD at 0.05	0.84	1.50	26.13	0.61	0.65	1.62	2.29	5.38	0.20	0.25
Plant spacing (cm): (S)										
20	209.11c	210.40c	225.91c	222.57c	166.54c	168.35c	507.33c	515.94c	40.55c	40.94c
30	214.66b	216.40b	249.36b	254.63b	189.63b	192.44b	526.55b	532.72b	43.97b	44.63b
40	218.11a	218.94a	291.51a	300.29a	235.94a	239.55a	567.94a	575.16a	45.81a	46.33a
LSD at 0.05	1.85	1.28	13.53	0.75	2.10	0.72	4.21	3.99	0.21	0.20
N- fertilizer levels (kg/ha.)										
92	209.88c	207.78c	222.49c	214.39c	163.83c	165.69c	446.27c	451.16c	40.46c	41.02c
288	211.94b	215.92b	258.66b	267.46b	196.37b	198.67b	524.33b	533.83b	43.43b	43.96b
384	220.05a	222.03a	285.64a	295.64a	231.92a	235.99a	631.22a	638.83a	46.45a	46.91a
LSD at 0.05	1.49	1.20	18.87	0.64	2.00	0.74	3.71	3.71	0.64	0.24
Interaction										
H x S	*	*	*	*	*	*	*	*	*	*
H x N	*	*	*	*	*	*	*	*	*	*
S x N	*	ns	*	*	*	*	*	*	*	*
H x S x N	*	*	ns	*	*	*	*	*	*	*

Means at the same column followed by the same letter are significantly different according to L.S.D. at 0.05 value, ns: not significant and *: significant difference at 0.05 level of probability.

Table (3). Yield and its components as affected by two maize hybrids, plant spacing and nitrogen fertilizer rates in 2014 and 2015 seasons.

Treatment	Number of rows/ear		Straw yield (ton/ha)		Grain yield (ton/ha)		Biological yield (ton/ha)		Harvest index (%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Maize hybrids (H)										
31G98	13.47b	13.70b	9.46b	9.49b	6.39 b	7.08b	15.86b	16.57b	40.20a	42.62a
30N11	14.41a	14.58a	11.43a	11.47a	7.69 a	8.32a	19.12a	19.80a	40.12a	42.00b
LSD at 0.05	0.45	0.11	0.745	0.633	0.395	0.609	1.14	1.24	0.306	0.586
Plant spacing (cm): (S)										
20	13.24c	13.53c	9.11c	9.04c	6.37 c	6.91c	15.48c	15.95c	41.16a	43.26a
30	14.04b	14.19b	10.65b	10.67b	7.08 b	7.71b	17.74b	18.39b	39.67a	41.90b
40	14.54a	14.70a	11.58a	11.73a	7.67 a	8.48a	19.25a	20.21a	39.65a	41.77b
LSD at 0.05	0.07	0.07	0.503	0.426	0.384	0.405	0.697	0.785	1.57	0.806
N- fertilizer levels (kg/ha.)										
92	13.21c	13.45c	9.66b	9.71b	6.17b	6.86c	15.83b	16.57c	38.81b	41.27b
288	13.96b	14.24b	10.59a	10.67a	7.41a	7.78b	17.99a	18.46b	41.15a	42.15b
384	14.46a	14.73a	11.10a	11.06a	7.55a	8.47a	18.65a	19.52a	40.51a	43.52a
LSD at 0.05	0.09	0.10	0.551	0.541	0.541	0.448	0.962	0.909	1.60	1.11
Interaction										
H x S	*	*	*	*	n.s.	*	*	*	*	*
H x N	*	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*
S x N	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
H x S x N	*	n.s.	n.s.	n.s.	*	*	*	*	*	*

Means at the same column followed by the same letter are statistically significantly different to L.S.D. at 0.05 value, ns: not significant and *: significant difference at 0.05 level of probability.

Table (4). Interactions between maize hybrids and plant spacing for grain yield (ton/ha.), straw yield and biological yield and H.I % in 2014 and 2015 seasons.

Hybrid	Plant spacing	Grain yield (ton/ha)	Straw yield (ton/ha)		Biological yield (ton/ha)		Harvest index (H.I %)	
		Season						
		2015	2014	2015	2014	2015	2014	2015
31G98	20	6.16	7.45	7.47	13.28	13.63	43.32	44.70
	30	6.89	9.66	9.51	15.98	16.40	39.12	41.64
	40	8.20	11.28	11.50	18.31	19.70	38.15	41.52
30N11	20	7.66	10.77	10.61	17.69	18.28	38.99	41.82
	30	8.54	11.64	11.84	19.49	20.38	40.21	41.91
	40	8.77	11.88	11.97	20.18	20.73	41.16	42.28
LSD at 0.05		0.573	0.711	0.602	0.986	1.11	2.22	1.14

Table (5). Interactions between maize hybrids and nitrogen fertilizer levels for grain yield (ton/ha) and H.I % in 2014 and 2015 seasons.

Hybrid	N levels(Kg/ha.)	Grain yield (t/ha)	Harvest index (%)	
		Season		
		2015	2014	2015
31G98	192	5.95	38.11	40.71
	288	6.95	40.82	41.91
	384	8.35	41.66	45.24
30N11	192	7.77	39.52	41.82
	288	8.62	41.49	42.39
	384	8.59	39.36	41.80
LSD at 0.05		0.634	2.26	1.56

Table (6). Interactions among maize hybrids, plant spacing, and nitrogen fertilizer levels for grain yield (t/ha), biological yield and harvest index (HI %) in 2014 and 2015 seasons.

Hybrids	Plant spacing	N levels (kg/ha.)	Grain yield (ton/ha)		Biological yield (ton/ha)		Harvest index (H.I. %)	
			Season					
			2014	2015	2014	2015	2014	2015
31G98	20	192	4.12	4.56	10.38	11.12	39.69	41.03
		288	5.92	5.88	13.46	13.24	43.76	44.40
		384	7.44	8.04	16.00	16.52	46.50	48.67
	30	192	4.66	5.50	13.11	13.83	35.51	39.75
		288	6.16	6.16	16.28	15.76	37.74	39.11
		384	8.16	9.00	18.56	19.60	44.10	46.05
	40	192	6.96	7.80	17.76	18.84	39.12	41.35
		288	8.13	8.80	19.85	20.84	40.95	42.22
		384	6.00	8.00	17.33	19.41	34.39	40.99
30N11	20	192	6.14	6.70	16.09	16.46	38.04	40.99
		288	7.52	8.36	18.28	19.36	40.98	43.05
		384	7.10	7.94	18.70	19.02	37.96	41.77
	30	192	7.64	8.25	18.88	19.57	40.46	42.16
		288	8.48	9.12	20.60	21.48	41.17	42.46
		384	7.42	8.26	19.00	20.08	39.01	41.10
	40	192	7.52	8.36	18.77	19.61	40.05	42.64
		288	8.24	8.36	19.48	20.07	42.32	41.65
		384	9.16	9.58	22.29	22.52	41.11	42.54
LSD at 0.05			1.32	1.09	2.36	2.23	3.92	2.71

Results recorded in Table (7) revealed that percentage of nitrogen, phosphorus, potassium and protein in maize grains were, significantly, influenced by adding high level of nitrogen.

Maize hybrid 30N11 recorded higher grains NPK and protein content than the other hybrid 31G98 in the first and second seasons, respectively.these results can be concluded that the ability to transport enough absorbed nitrogen, phosphorus, and potassium percentages in grains plant. These results agreed with those obtained by Amin *et al.* (2003) and Atia and Abdel- Azeem (2005).

The highest values of all chemical compositions character were obtained using nitrogen fertilizer at rate 384 kg/ha., in both seasons, while, the lowest ones was recorded by application nitrogen at 192 kg/ha., as shown in (Table 7) in both seasons. These results indicate that N- fertilization rate increased the capacity of plant in absorbing nutrients. These results are in agreement with others results were reported by Martin *et al.* (2008), El- Gizawy and Salem (2010) and Dawadi and Sah (2012).

Results in Table (7) revealed that wider spacing between plants (40 cm) produced higher protein content and NPK in the two successive seasons than narrower spacing (20 cm) that produced the lowest mean values of these characters.

On the other side, increasing nitrogen fertilizer from 192 to 384 kg N/ha., significantly, increased grain NPK and protein contents in 2014 and 2015 seasons as shown in Table (7). These results are in agreement with those obtained by Sahoo and Mahapatra (2004), Oktem and Oktem (2005), Kar *et al*. (2006), Melkonian *et al*. (2008), El-Gizawy and Salem (2010) and Tang *et al*. (2015).

Table (7). Macronutrients (N, P and K) and protein percentages as affected by maize hybrids, plant spacing and nitrogen fertilizer rates in 2014 and 2015 seasons.

Treatment	N (%)		P (%)		K (%)		Protein (%)	
	Season							
	2014	2015	2014	2015	2014	2015	2014	2015
Maize hybrids (H)								
31G98	1.27b	1.34b	0.634b	0.638b	1. 67b	1.68b	7.79b	8.37b
30N11	1.32a	1.36a	0.713a	0.720a	1. 86a	1.90a	8.28a	8.51a
LSD at 0.05	0.01	0.01	0.003	0.003	0.01	0.05	0.07	0.11
Plant spacing (cm): (S)								
20	1.22c	1.28c	0.648c	0.652c	1.60c	1.61c	7.65c	8.03c
30	1.31b	1.36b	0.673b	0.681b	1.74b	1.76b	8.22b	8.52b
40	1.36a	1.40a	0.699a	0.704a	1.95a	1.99a	8.51a	8.78a
LSD at 0.05	0. 02	0. 01	0. 002	0.003	0. 02	0. 02	0. 13	0. 07
N- fertilizer levels (kg/ha.)								
92	1.21c	1.25c	0.545c	0.551c	1.57c	1.58c	7.57c	7.82c
288	1.30b	1.35b	0.682b	0.685b	1.76b	1.80b	8.14b	8.46b
384	1.38a	1.44a	0.793a	0.800a	1.96a	1.99a	8.67a	9.04a
LSD at 0.05	0.01	0.01	0.001	0.001	0. 01	0. 02	0.09	0.09
Interaction								
H x S	ns	ns	*	*	*	*	ns	ns
H x N	*	ns	*	*	*	*	*	ns
S x N	*	ns	*	*	*	*	*	ns
H x S x N	*	*	*	*	ns	*	*	*

Means at the same column followed by the same letter are statistically equaled according to L.S.D. at 0.05 value, ns: not significant and *: significant difference at 0.05 level of probability.

CONCLUSIONS

Considering the obtained results, it can be concluded that application of 384 kg N ha^{-1} and with wider spacing (40 cm) between plants to the maize hybrid '30N11' is an optimal for obtaining higher grain yield of maize under the agro-metrological conditions of Alexandria, Egypt.

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الملخص العربي

استجابة بعض هجن الذرة الشامية للتوزيع الفراغي ومعدلات التسميد النتيروجيني

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أجريت تجربتان حقليتان بالمزرعة البحثية بكلية الزارعة سابا باشا بمنطقة أبيس جامعة الإسكندرية خلال الموسمين ٢٠١٤ و ٢٠١٥ ،وذلك لدراسة تأثير المسافات بين النباتات ومستويات السماد النيتروجيني علي نمو ومحصول بعض هجن الذرة الشامية. وإستخدام في تصميم القطع المنشقة مرتين في ثلاث مكررات في تنفيذ التجربتان ، حيث احتلت الهجن (30N11) ، (31G98) القطع الرئيسية ، ووزعت المسافات بين النباتات (٢٠ سم، ٣٠ سم،٤٠ سم) فى القطع تحت الرئيسية ، مستويات السماد النيتروجيني (١٩٢ ، ٢٨٨ ، ٣٨٤ كجم/هكتار) كانت فى القطع تحت تحت الرئيسية.

ولخصت أهم النتائج فيما يلي:

- تفوق هجين الذرة الشامية30N11 علي الهجين 31G98 معنوياً في معظم الصفات المدروسة في كلا الموسمين.
- زراعة النباتات علي مسافة ٤٠سم حققت أعلى قيم للصفات تحت الدراسة ، بينما المسافة ٢٠ سم بين نباتات الذرة الشامية أدت لأقل قيم بالنسبة لارتفاع النبات ولصفات المحصول ومكوناته في موسمي الزراعة.
- وأدى معدل التسميد الأعلى من السماد النتروجينى (٣٨٤ كجم/هكتار) للحصول على أعلى قيم لمحصول الذرة الشامية ، ومكوناته مقارنة بباقى معدلات التسميد ، بينما المعدل الأقل (١٩٢ كجم/هكتار) أعطى أقل القيم لهذه الصفات خلال موسمى الدراسة.
- أدى زراعة هجين 30N11علي مسافة ٤٠ سم بين النباتات الذرة الشامية للحصول على أعلى القيم للصفات المحصولية المدروسة خلال موسمى الذرة ، مقارنة بين بالصنف الأخر (31G98) الذى سجل أقل القيم خاصة مع مسافة زراعة ٢٠ سم بين النباتات.
- سجل الهجين 30N11 أعلى استجابة لمعدل التسميد النتروجينى ٣٨٤ كجم/هكتار حيث حقق أعلى قيم لمحصول الحبوب والقش والمحصول البيولوجى خلال موسمى الزراعة.

- زراعة الذرة الشامية على مسافة ٤٠ سم بين النباتات مع معدل التسميد ٣٨٤ كجم نتروجين للهكتار سجل أعلى قيم لمحصول الحبوب ، والقش والمحصول البيولوجى ودليل الحصاد فى الموسمين ٢٠١٤ ، و٢٠١٥.
- أدت إضافة السماد النيتروجيني بمعدل ٣٨٤ كجم/هكتار مع الزراعة علي مسافة ٤٠سم بين النباتات للهجين30N11 إلي الحصول علي أعلي القيم للمحصول (محصول الحبوب، والقش البيولوجي (طن/هكتار) وأيضاً دليل الحصاد (%) في كلا الموسمين.

Response of Barley to Nitrogen Fertilizer Sources, Sulphur Application, Inoculation with Mycorrhizae and Phosphate Slubilizing Bacteria in Salt Affected Soil

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ABSTRACT: This investigation aimed to increase barley grain yield. In this respect, two field experiments were carried out at the Experimental Farm, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt, in a split- split plot design with three replications during 2013/2014 and 2014/2015 growing seasons. Main plot treatments were sulphur rates (0, 200 and 400 kg/ha), however nitrogen fertilizer sources (urea, nitrate ammonium and ammonium sulphate) were allocated in sub-plots and biofertilizers inoculation (control, mycorrhizae and phosphorein inoculation) were distributed in sub- sub plots. The obtained results indicated that increasing sulphur application up to 400 kg/ha., significantly increased all studied yield, yield components and grain composition traits, i.e. number and weight of spikes/m^2, number of spikelets and grains/spike, 1000-kernel weight, biological, straw and grain yield/ha, harvest index, grain protein, nitrogen, phosphorus and potassium content in the two studied seasons. Also, ammonium sulphate (as nitrogen source) produced the highest values of the previous trait. However, inoculated barley grains with phosphorein biofertilizers showed the highest of the studied traits except phosphorus grains content in the two seasons, where inoculation with mycorrhizae showed the highest grains phosphorus content (0.273 and 0.287%) in the two successive seasons. Sulphur application at 400 kg/ha., combined with ammonium sulphate or phosphorein inoculation interaction produced the highest values of all studied traits, except P and K contents in grains, meanwhile 400 kg S/ha application combined with mycorrhizal inoculation had the highest P and K content in two seasons. Ammonium sulphate X phosphorein inoculation interaction had the same trend in the two seasons. Regarding the three factors of interaction effects, sowing inoculated grains with phosphorein under 400 kg S/ha and ammonium sulphate application produced the highest values of the studied traits, except P and K grains content in both seasons. Conversely, any of two and three factors of interaction did not reach significant level effect on 1000- grains weight in the two seasons.

Keywords: Sulphur rate, Nitrogen sources, Biofertilizers, Barley, Yield, attributes, Grain quality.

INTRODUCTION

Barley (*Hordeum vulgare*, L.) is grown as a commercial crop in one hundred countries around the world and it assumes the fourth rank in total cereal production in the world after wheat, rice and maize (FAO, 2004). Barley is considered as one of the most important cereal crops in Egypt. It is the major food source in many North African countries, because it tolerates the adverse environments compared to other cereal crops (Hayes *et al*., 2003). Nitrogen is the most important factor affecting crop morphology (Amanullah *et al*., 2008), increased grains yield with increasing nitrogen level (Singh and Uttam, 2000).

Plant growth is enhanced through conversion of nutritionally important elements as nitrogen and phosphorus by biological processes as nitrogen fixation and solubilization of rock phosphate (Mohammadi and Sohrabi, 2012).

Sulphur is considered as soil amendment. Oxidation of sulphur to H_2SO_4 is beneficial in alkaline soil to reduce pH, supply SO^-_4 to plants, makes

phosphorus and micronutrients more available in reclaim soils (Lindemann *et al*., 1991). Ghani *et al.* (1997) reported that microbial population in soil is not a limiting factor in elemental sulphur oxidation. Now days, biofertilizers inoculation is considered to limit the use of mineral fertilizers and supports an effective tool for desert development under less polluted environment, decreasing production costs, maximizing crop yield due to providing them with an available nutritive clement (Metin *et al*., 2012). Soil micro- organisms bind soil particles into stable aggregates, which improve soil structure and reduce erosion potential (Shetty *et al*., 1994).

Biofertilizer can be used as fertilizer or as soil amendment, depending on its effect on the plant nutrition.Hence, a fertilizer is a source of quickly available nutrients that have a direct and short-term effect on plant growth, while a soil amendment can influence plant growth indirectly by improving the physical and biological properties of the soil (Angelova *et al*., 2013).

A- Mycorrihzal fungi have been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by utilizing various mechanisms, such as enhancing nutrient uptake, producing plant growth hormones, improving rhizospheric and soil conditions, improvement in photosynthetic activity or water use efficiency, accumulation of compatible solutes, and production of higher antioxidant enzymes. As a result, AM fungi are considered suitable for bioamelioration of saline soils (Asghari *et al*. 2005, Hajiboland *et al.,* 2010, Manchanda and Garg 2011, and Evelin *et al*., 2012 and 2013).

The present investigation was carried out to study the effect of sulphur application rates, nitrogen sources and biofertilizers inoculation on growth, grains yield and its components of barley crop.

MATERIALS AND METHODS

Two filed experiments were carried out at the Experimental Farm, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt, during 2013/2014 and 2014/2015 growing seasons to study effect of sulphur application rates, nitrogen fertilizer sources and biofertilizers inoculation on growth, grain yield and components, besides grain chemical contents of six-rows barley cv. Giza 123. A split- split plot design with three replicates were used in both seasons. Three sulphur application rates (0, 200 and 400 kg/ha) were randomly assigned in the main plots, three nitrogen sources, i.e. urea (46% N), ammonium nitrate (33.5% N) and ammonium sulphate (20.6% N) were allocated in sub-plots and three biofertilizers treatments (uninoculation, mycorrhizae and phosphorein at 400 g/ha.) were randomly distributed in sub-sub plots. Barley was sown on 4^{th} and 8^{th} December in two growing seasons, respectively, after maize planting. Seeding rate was (70 kg/ha.) and plot size was 10.5 m^2 (1/400 fed.) with 3.5 m length and 3 m width. Sulphur applied during seed bed preparation, nitrogen fertilizer at 144 kg/ha., were applied in two equal doses before the first and second irrigations.

Phosphorene (*Bacillus megtherium phosphbacterium*) was performed by coating barely grains with each product individually using a sticking substance (Arabic gum 5%) just before sowing. A- mycorrhizal fungi (*Glomus macrocaripum*) was obtained from Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria Uinversity at the rate of 250 spores was mixed with grain. Recommended cultural practices for barley production were conducted Soil physical and analyses were carried out in the two growing seasons and showed in Table (1).

Table (1). Physical and chemical properties of experimental soil in 2013/2014 and 2014/2015 seasons.

Soil properties		
	2013/2014	**2014/2015**
A- Mechanical analysis		
Clay %	37	36
Sand %	33	34
Silt	30	30
Soil texture	Clay loam	Clay loam
B- Chemical properties		
pH (1:1)	8.30	8.41
EC (1:1) dS/m	3.70	3.65
1- Soluble cations (1:2)		
K^+	1.45	1.58
Ca^{++}	8.7	8.3
Mg^{++}	18.5	18.6
Na^{++}	13.8	13.8
2- Soluble anions (1:2)		
CO^-_3+ HCO^-_3	2.80	2.60
CL^-	19.80	18.80
SO^-_4	12.60	12.70
Calcium carbonate %	7.00	7.30
Total nitrogen %	0.91	0.81
Available Phosphorus (mg/kg)	3.55	3.41
Organic matter (%)	1.41	1.40

At harvest, one square meter was randomly taken in each sub- sub plot to determine number of spikes/m^2, ten random spikes were chosen in each sub-sub plot to calculate number of grains/spike and thousand kernel weight (g) was determined as an average of three samples. Biological and grain yield by harvesting all plants in each sub-sub and converted to tons/ha., harvest index besids protein N, P and K grain content were determined.

Protein percentage was determined by estimating the total nitrogen in the grains and multiplied by 6.25 to obtain the protein percentage according to grains protein percentage to AOAC (1990). NPK percentages were determined in the dry grains. Their dry weights were determined following drying in a drying chamber to a constant weight at 75^{o}C for 72 hour according to Tandon (1995). After dryness, the plant samples were milled and stored for analysis as reported. However, 0.5g of the grains powder was wet-digested with H_2SO_4–H_2O_2 mixture

according to (Lowther, 1980) and the following determinations were carried out in the digested solution to determine NPK. Total nitrogen was determined in digested plant material colorimetrically by Nessler`s method (Chapman and Pratt, 1978). Phosphorus was determined by the Vanadomolyate yellow method as given by Jackson (1973) and the intensity of colour developed was read in spectrophotometer at 405nm. Potassium was determined according to the method described by method Jackson (1973) using Beckman Flame photometer.

Collected data were statistically analyzed using Co stat (2005) statiscal program, and treatment mean were compared using the least significand differences method (L.S.D) at 5% probability level as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

A- Yield and yield attributes:

Data presented in Table (2) showed that studied yield components, i.e. spikes number and weight/m^2, number of spikelets and grains/spike and 1000-grain weight were, significantly, affected by sulphur application levels, nitrogen fertilizer sources and biofertilizer inoculation in the two studied seasons.

Increasing sulphur application from zero to 400 kg/ha., significantly increased the previous traits by 23.11% for number of spikes/m^2, 26.66 % for spikes weight/m^2, 17.90% for number of spikelets/spike, 14.48% for number of grains/spike and 7.21% for 1000- kernel weight as an average of the seasons, respectively. These increases in the studied yield components in barley crop might be reffered to the favorable effect of sulphur for decreeing soil pH and increasing phosphorus and micronutrients availability to plant (Lindemenn *et al*., 1991).

Results also, demonstrated that nitrogen application as ammonium sulphate produced the highest number of spikes/m^2 (394.14 and 388.96), heaviest spikes weight/m^2 (279.19 and 269.50g), highest number of spikelets/spike (51.74 and 52.51), highest number of grains/spike (38.66 and 39.70) and heaviest 1000- grain weight (46.46 and 49.55g) in the first and second growing seasons, respectively.

Concerning biofertilization treatments, results in Table (2) revealed that inoculated barley grains with mycorrhizae or phosphorein significantly increased all the studied yield attributes in the two seasons compared to uninoculated grains.

Barley grains inoculated with phosphorein biofertilizer showed the highest number of spikes/m^2 (402.20), spikes weight/m^2 (284.82g), number of spikelets/spike (52.96), number of grains/spikes (40.24) and 1000- grains weight (48.12g) as an average of the two seasons. These increase could be due to the stimulation effect of micro- organisms that produce plant

pgytohormons as IAA, Gas and SKs, which promote plant growth cell division, hence encouraging photosynthesis and assimilates accumulation (El- Khawas, 1990 and Hussein and Radwan 2001).

Concerning sulphur appliacation levels X N sources interaction effect, results in Table (3) showed that applied 400 kg S/ha to barley fertilized by ammonium sulphate shoed the highest number of spikes/m^2 (418.66) in the second season, weight of spikes/m^2 (335.17 and 300.77g), number of spikelets/spike (57.88 and 58.66) and number of grains/spike (42.0 and 43.44) in the first and second seasons, respectively.

Results presented in Table (3) indicated that biological, straw and grain yield besides harvest index were significantly affected with the three studied factors, where applied 400 kg S/ha produced the highest biological yield (18.03 and 17.87 ton/ha) straw yield (11.03 and 10.83 ton/ha), grain yield (6.97 and 7.03 ton/ha) and harvest index (38.51 and 39.19%) in the first and second seasons, respectively.

Data in Table (3) also, revealed that using ammonium sulphate as nitrogen source gave the highest values(17.24 and 16.99 ton/ha), (10.30 and 9.98 ton/ha), (6.99 and 6.78ton/ha) and (40.11 and 39.70%) for the respective traits in the two seasons, respectively. Also, inoculated barley grains with phosphorein showed the highest values (17.46 and 17.19 ton/ha), (10.61 and 10.52 ton/ha), (6.84 and 6.66 ton/ha) and (38.93 and 38.44%) for the previous characters in the two successive seasons.

On the other side, applied 400 kg S/ha combined with ammonium sulphate fertilization showed the highest biological, straw and grain yields besides H.I in the two seasons Table (4). However, sulphur application at 400 kg/ha inoculated grains with phosphorein produced the highest straw yield (12.02 ton/ha) in the first season, biological yield (19.99 and 19.77 ton/ha), grain yield (7.96 and 8.05 ton/ha) and H.I. (39.66 and 40.66%) in the first and second seasons, respectively. as reported in Table (5).

With respect to nitrogen sources X biofertilizers inoculation effect, results presented in Table (10) indicated that phosphorein inoculation combined with fertilization with ammonium sulphate produced that highest straw yield (10.81 ton/ha) in the first season, biological yield (18.72 and 18.78 ton/ha), grain yield (7.90 and 7.92 ton/ha) and harvest index (42.21 and 42.18%) in the two successive seasons, respectively..

Regarding three factors interaction effect, results presented in Table (7) showed that the highest straw yield in the first season (12.68 ton/ha), biological yield (21.77 and 21.78 ton/ha), grain yield (9.09 and 9.16 ton/ha) and HI (41.53 and 42.05%) in the first and second seasons, respectively. resulted from using 400 kg S/ha, ammonium sulphate as N source application to inoculated barley grains with phosphorein.

Table (2). Effect of sulphur application level, nitrogen fertilizer source and biofertilizers on barley yield components during 2013/2014 and 2014/2015 seasons.

Treatment	No. of number spikes/m^2		Spikes weight/m^2		No. of spikelets/spike		No. of grains/spike		1000- grain weight	
	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15
A) Sulphur rate (kg/ha)										
0	325.44c	332.48c	233.35c	238.62c	45.40c	45.81c	34.21c	35.88c	43.76c	46.66c
200	385.74b	392.29b	253.23b	252.84b	49.18b	49.14b	37.26	37.24b	45.57b	48.62b
400	410.59a	399.18a	303.96a	293.63a	52.29a	54.25a	39.66a	40.55a	46.87a	50.7a
L.S.D.at 0.05	7.07	4.56	3.78	2.35	0.29	0.55	0.56	0.46	0.82	1.22
B) N Sources										
Urea	358.48c	364.11c	249.32c	251.42c	47.11c	47.29c	35.10c	36.10c	44.51c	47.34c
Nitrate	369.14b	370.88a	262.03b	264.18b	49.03b	49.40b	49.03b	37.88b	45.23b	48.46b
Sulphate	394.14a	288.96a	279.19a	269.50a	51.74a	52.51a	38.66a	39.70a	46.46a	49.55a
L.S.D.at 0.05	2.08	2.66	1.26	1.60	1.03	0.55	0.51	0.46	0.70	0.61
C) Biofertilizer										
Control	343.44c	348.66c	242.75c	244.78c	45.92c	46.29c	34.81c	35.03c	44.25c	46.78c
Mycorrhizae	377.06b	372.14b	262.96b	255.51b	49.18a	49.85b	36.55b	37.95b	45.61b	48.68b
Phosphorein	401.25a	403.14a	284.83a	284.81a	52.85a	53.07a	39.77a	40.70a	46.35a	49.89a
L.S.D.at 0.05	2.79	2.71	1.40	1.98	0.79	0.60	0.83	0.31	0.61	0.76
Interactions										
A×B	ns	*	*	*	*	*	*	*	ns	ns
A×C	*	*	*	*	*	*	*	*	ns	ns
B×C	*	*	*	*	ns	ns	*	*	ns	ns
A×B×C	*	*	*	*	ns	*	ns	*	ns	ns

Means at the same column followed by the same letter are statistically equaled according to L.S.D. at 0.05 value, ns: not significant and *: significant difference at 0.05 level of probability.

Table (3). Effect of sulphur application level, nitrogen fertilizer source and biofertilizers on barley yield during 2013/2014 and 2014/2015 seasons.

Treatment	Biological yield (ton/ha)		Straw yield (ton/ha)		Grain yield (ton/ha)		Harvest index (H.I%)	
	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15
A) Sulphur level (kg/ha):								
0	14.25c	14.35c	8.88c	9.07c	5.58c	5.28c	39.16a	36.79c
200	15.56b	15.26b	9.66b	9.63b	5.89b	5.71b	37.55b	37.30b
400	18.03a	17.87a	11.06a	10.83a	6.97a	7.03a	38.85a	39.34a
L.S.D. at 0.05	0.06	0.02	0.01	0.21	0.23	0.02	0.18	0.11
B) Nitrogen fertilizer Source:								
Urea	14.61.c	14.97c	9.35c	9.48b	5.26c	5.49c	35.48c	36.53c
Nitrate	15.98b	15.51b	9.72b	9.76ab	6.26b	5.76b	37.57b	36.97b
Sulphate	17.24a	16.99a	10.30a	9.98a	6.94a	6.78a	40.26a	39.70a
L.S.D. at 0.05	0.06	0.02	0.02	0.39	0.20	0.02	0.20	0.11
C) Biofertilizer:								
Control	14.40c	14.48c	9.18c	8.93c	5.44c	5.33c	36.11c	36.77c
Mycorrhizae	15.97b	15.80b	9.978b	9.76b	6.18b	6.03b	38.49b	37.99b
Phosphorein	17.46a	17.19a	10.61a	10.52a	6.48a	6.66a	38.93a	38.44a
L.S.D. at 0.05	0.09	0.02	0.01	0.37	0.18	0.01	0.22	0.07
Interaction:								
A×B	*	*	*	ns	*	*	*	*
A×C	*	*	*	ns	*	*	*	*
B×C	*	*	*	ns	*	*	*	*
A×B×C	*	*	*	ns	*	*	*	*

Means at the same column followed by the same letter are statistically equaled according to L.S.D. at 0.05 value, ns: not significant and *: significant difference at 0.05 level of probability.

Table (4). The interaction between sulphur application levels and nitrogen fertilizer sources for biological yield, straw yield, grain yield (ton/ha) and harvest index (%) during 2013/2014 and 2014/2015 seasons.

Sulphur level (kg/ha)	N-source	Biological yield (ton/ha)		Straw yield (ton/ha)	Grain yield (ton/ha)		Harvest index (%) (H.I.)	
		2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14
0	Urea	13.61	14.06	8.90	4.70	4.93	34.58	35.10
	Nitrate	14.24	14.24	9.0	5.90	5.16	36.79	36.29
	Sulphate	14.90	145.76	8.75	6.14	5.74	39.01	38.75
200	Urea	13.83	14.29	9.94	4.89	5.17	35.36	36.22
	Nitrate	15.95	14.84	9.91	6.04	5.27	37.46	35.59
	Sulphate	16.89	16.64	10.13	6.76	6.70	39.84	40.08
400	Urea	16.38	16.56	10.15	6.17	6.36	37.58	38.26
	Nitrate	17.76	17.46	10.92	6.84	6.48	38.47	39.03
	Sulphate	19.94	19.57	12.02	7.91	7.91	40.49	40.27
L.S.D. 0.05		0.10	0.03	0.03	0.35	0.35	0.35	0.19

Table (5). Effect of sulphur application level and biofertilizers on biological yield, straw yield, grain yield (ton/ha) and harvest index (%) during 2013/2014 and 2014/2015 seasons.

Sulphur level (kg/ha)	Bio-fertilizer	Biological yield (ton/ha)		Straw yield (ton/ha)	Grain yield (ton/ha)		Harvest index (H.I %)	
		2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14
0	Control	13.0	13.39	8.26	4.99	4.93	35.25	36.76
	Mycorrhizae	14.30	14.16	8.85	5.45	5.12	37.87	36.17
	Phosphorein	15.45	15.51	9.54	5.90	5.79	38.12	37.21
200	Control	14.10	14.15	9.10	5.40	5.19	36.40	36.72
	Mycorrhizae	15.64	15.34	9.60	6.04	5.81	38.39	37.73
	Phosphorein	16.64	16.29	10.28	6.65	6.15	39.02	37.45
400	Control	16.11	15.91	10.18	5.92	5.87	36.67	36.82
	Mycorrhizae	17.98	17.91	10.88	7.05	7.18	39.20	40.07
	Phosphorein	19.99	19.77	12.02	7.96	8.05	39.66	40.66
L.S.D. 0.05		0.08	0.03	0.02	0.32	0.02	0.39	0.12

Table (6). Interaction between nitrogen fertilizer sources and biofertilizers for biological yield, straw yield, grain yield (ton/ha.) and harvest index (%) during 2013/2014 and 2014/2015 seasons.

N Source	Bio-fertilizer	Biological yield (ton/ha)		Straw yield (ton/ha)	Grain yield (ton/ha)		Harvest index (H.I%)	
		2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14
Urea	Control	13.54	13.88	8.78	4.76	4.99	35.13	35.97
	Mycorrhizae	14.47	14.92	9.16	5.26	5.60	36.22	37.37
	Phosphorein	15.82	16.11	10.06	5.75	5.87	36.16	36.24
Nitrate	Control	14.25	14.30	9.16	5.75	2.25	35.45	36.76
	Mycorrhizae	15.86	15.56	9.70	6.16	5.82	38.74	37.25
	Phosphorein	17.85	16.67	10.87	6.87	6.20	38.43	36.90
Sulphate	Control	15.41	15.27	9.61	5.80	5.75	37.64	37.57
	Mycorrhizae	17.59	16.93	10.48	7.11	6.68	40.49	39.35
	Phosphorein	18.72	18.78	10.81	7.90	7.92	42.21	42.18
L.S.D. 0.05		0.08	0.03	0.02	0.32	0.02	0.39	0.12

Table (7). The interaction effect among sulphur application levels, nitrogen sources and biofertilizers inoculation for biological yield, straw yield, grain yield (ton/ha) and harvest index (%) during 2013/2014 and 2014/2015 seasons.

Sulphur rate	N-Source	Bio-fertilizer	Biological yield (ton/ha)		Straw yield (ton/ha)	Grain yield (ton/ha)		Harvest index (H.I %)	
			2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14
		Control	12.68	13.21	8.22	4.71	4.78	34.56	34.55
	Urea	Mycorrhizae	13.50	13.89	8.78	5.02	4.86	34.93	34.58
		Phosphorein	14.67	14.95	9.64	6.72	5.17	34.26	37.57
		Control	12.94	13.34	8.29	6.19	4.96	36.49	36.57
0	Nitrate	Mycorrhizae	14.04	14.21	8.85	5.81	5.19	36.99	35.76
		Phosphorein	15.75	15.30	9.94	5.10	5.32	36.88	36.96
		Control	13.38	13.63	8.27	6.45	5.04	38.14	36.98
	Sulphate	Mycorrhizae	15.38	14.37	8.93	6.88	5.31	41.68	42.31
		Phosphorein	15.94	16.28	9.05	4.68	6.89	43.22	36.81
		Control	13.16	13.60	9.47	4.81	5.01	35.58	36.90
	Urea	Mycorrhizae	13.62	14.10	8.81	5.18	5.20	35.32	34.96
		Phosphorein	14.72	15.18	9.54	4.79	5.31	35.19	36.33
		Control	14.05	13.75	9.26	6.26	5.07	33.62	35.25
200	Nitrate	Mycorrhizae	16.15	15.16	9.89	7.06	5.34	38.76	35.18
		Phosphorein	17.65	15.40	10.59	5.51	5.42	40.0	37.01
		Control	15.09	14.89	9.57	7.04	5.51	36.54	41.05
	Sulphate	Mycorrhizae	17.14	16.75	10.09	7.72	6.88	41.09	42.20
		Phosphorein	18.44	18.29	10.72	5.21	7.72	41.88	35.35
		Control	14.79	14.71	9.57	6.26	5.20	35.26	40.25
	Urea	Mycorrhizae	16.29	16.77	9.88	6.26	6.75	38.43	39.19
		Phosphorein	18.07	18.21	10.99	7.06	7.14	39.05	36.93
		Control	15.75	15.74	10.0	5.75	5.73	36.53	36.93
400	Nitrate	Mycorrhizae	17.40	17.33	10.35	7.04	6.92	40.47	39.95
		Phosphorein	20.14	19.32	12.40	7.73	7.87	38.41	40.76
		Control	17.78	17.30	10.98	6.80	6.70	38.24	38.74
	Sulphate	Mycorrhizae	21.77	19.65	12.42	7.85	7.86	38.71	40.02
		Phosphorein	21.77	21.78	12.68	9.09	9.16	41.53	42.05
L.S.D. 0.05			0.13	0.05	0.04	0.55	0.04	0.67	0.21

B- Chemical composition of grains:

Data in Table (8) illustrated the three studied factors effect on crude protein, nitrogen, phosphorus and potassium content of grain in the two seasons. Increasing sulphur application up to 400 kg/ha produced the highest protein (9.03 and 7.27 %), nitrogen (1.44 and 1.163 %), phosphorus (0.273 and 0.299 %) and potassium (0.550 and 0.616 %) content in the first and second seasons, respectively.

Also, barely fertilized with ammonium sulphate produced the highest mean values of the studied traits (9.27 and 7.23 %) for protein (1.485 and 1.157 %) phosphorus, (0.266 and 0.285 %) and potassium (0.550 and 0.616 %) content in the two successive seasons, respectively.

Inoculation with phosphorein gave the highest protein content (9.14 and 7.27 %) nitrogen (1.433 and 1.163 %) and potassium (0.540 and 0.619 %) in the first and second seasons, respectively. However, mycorrhizae inoculation produced the highest phosphorus content (0.273 and 0.287 %) in the first and second seasons, respectively.

Concerning the three factors of interaction, results presented in Table (8) revealed that there were significant interactions among the traits under this study.

The previous results pointed out that interaction among the three studied factors had significant interaction for the yield, yield components and grain chemical composition.

Plant responses are deeply affected by the proportion of mineral N sources (Andrews *et al*., 2013). While NH^+_4 as sole nutrient can induce toxicity symptoms, its co-provision with NO_3^- generally promotes a synergistic effect leading to growth enhancement (Britto and Kronzucker, 2002). It is noteworthy that NH^+_4 tolerance was related to high root N metabolism sus- tained by high GS activities (Cruz *et al*., 2006), which in maize appear to be associated with the capacity to cope with the C skeleton demands (Schortemeyer *et al*., 1997).

Table (8). Effect of sulphur application level, nitrogen fertilizer source and biofertilizers on protein in grains %, N, P and K percentage during 2013/2014 and 2014/2015 seasons.

Treatment	Protein %		N %		P %		K %	
	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14	2013/14
A) Sulphur level (kg/ha):								
0	7.23c	6.92c	1.157c	1.108c	0.246c	0.255c	0.455c	0.463c
200	8.44b	7.20b	1.352b	1.153b	0.258b	0.268b	0.537b	0.268b
400	9.03a	7.27a	1.448a	1.163a	0.273a	0.299a	0.599a	0.697a
L.S.D. at 0.05	0.09	0.02	0.012	0.004	0.001	0.001	0.001	0.001
B) Nitrogen fertilizer source:								
Urea	7.18c	6.99c	1.148c	1.118c	0.251c	0.261c	0.512c	0.567c
Nitrate	8.26b	7.18b	1.324b	1.148b	0.259b	0.276b	0.529b	0.277b
Sulphate	9.27a	7.23a	1.485a	1.157a	0.266a	0.285a	0.550a	0.616a
L.S.D. at 0.05	0.08	0.01	0.014	0.002	0.001	0.002	0.001	0.002
C) Biofertilizer:								
Control	7.29c	6.97c	1.166c	1.115	0.250c	0.256c	0.503c	0.553c
Mycorrhizae	8.28b	7.16	1.358b	1.147	0.273a	0.287a	0.509	0.604b
Phosphorein	9.14a	7.27a	1.433a	1.163a	0.264b	0.278b	0.540a	0.619a
L.S.D. at 0.05	0.06	0.01	0.011	0.002	0.001	0.001	0.001	0.001
Interaction								
A×B	*	*	*	*	*	*	*	*
A×C	*	*	*	*	*	*	*	*
B×C	*	*	*	*	*	*	*	*
A×B×C	*	*	*	*	*	*	*	*

Means at the same column followed by the same letter are statistically equaled according to L.S.D. at 0.05 value., ns : not significant and *: significant difference at 0.05 level of probability.

CONCLUSION

In conclusion, applying 400 kg S/fed., and ammonium sulphate as nitrogen fertilizer source to inoculated barley grains of Giza 123 cultivar with phosphorein produced the highest grains yield, yield attributes and grains quality studied traits under Alexandria Governorate conditions.

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الملخص العربي

استجابة الشعير لمصادر السماد النتروجينى وإضافة الكبريت والتلقيح بالميكوريزا والبكتيريا المذيبة للفوسفور تحت ظروف التربة المتأثرة بالأملاح

فتحي إبراهيم رضوان ، محمود عبد العزيز جمعة ، ابراهيم فتح الله رحاب ، ذهب خليفة محمد جبريل

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أجريت تجربتان حقليتان بالمزرعة البحثية بكلية الزارعة سابا باشا بمنطقة أبيس جامعة الإسكندرية خلال الموسمين ٢٠١٣/٢٠١٤ ، و٢٠١٤/٢٠١٥،وذلك لدراسة تأثير مصادر السماد النتروجينى وإضافة الكبريت والتلقيح بالميكوريزا والبكتيريا المذيبة للفوسفور على الشعير تحت ظروف التربة المتأثرة بالأملاح . وتم استخدام تصميم القطع المنشقة مرتين في ثلاث مكررات في تنفيذ التجربتان ، حيث وزعت (معاملات إضافة الكبريت بمعدل ٠ ، ٢٠٠ ، ٤٠٠ كجم/هكتار) القطع الرئيسية ، ووزعت مصادر السماد النتروجينى (يوريا ، نترات امونيوم ،سلفات الأمونيوم) في القطع تحت الرئيسية ، التلقيح بالسماد الحيوي (بدون ، الميكوريزا ، الفوسفورين) كانت في القطع تحت الرئيسية.

ويمكن تلخيص أهم النتائج فيما يلي:

- أدت إضافة الكبريت بمعدل ٤٠٠ كجم لزيادة معنوية في جميع الصفات المدروسة في الموسمين.
- التسميد بسماد النتروجين في صورة سلفات الأمونيوم حقق أعلى قيم للصفات تحت الدراسة ، مقارنة بالصور الأخرى (يوريا ، نترات امونيوم) بالنسبة لارتفاع النبات ولصفات المحصول ومكوناته في موسمي الزراعة.
- حقق تلقيح حبوب الشعير بالميكوريزا أعلى قيم لمحصول ، يليه التلقيح بالفوسفورين ، مقارنة بمعاملة الكنترول (بدون تلقيح بالسماد الحيوي) الذى حقق أقل القيم لهذه الصفات خلال موسمي الدراسة.
- أدى زراعة الشعير تحت معدل إضافة الكبريت ٤٠٠ كجم + سماد النتروجين فى صورة سلفات الأمونيوم أعلى القيم للصفات المحصول المدروسة خلال موسمي الزراعة ، مقارنة بين بالمعاملات الأخرى حيث المعاملة بدون كبريت (الكنترول) سجلت أقل القيم.
- سجل إضافة الكبريت ٤٠٠ كجم + التلقيح بالفوسفورين أعلى استجابة للزيادة المحصولية ، حيث حقق أعلى قيم لمحصول الحبوب والقش والمحصول البيولوجي خلال موسمي الزراعة.
- سجل تسميد الشعير بالسماد النتروجين فى صورة سلفات الأمونيوم مع تلقيح الحبوب بالفوسفورين أعلى قيم لمحصول الحبوب ، والقش والبيولوجي ودليل الحصاد فى الموسمين ٢٠١٣/٢٠١٤ ، و٢٠١٤/٢٠١٥.
- أدت إضافة سماد الكبريت بمعدل ٤٠٠ كجم مع السماد النتروجين فى صورة سلفات الأمونيوم مع تلقيح الحبوب بالفوسفورين إلي الحصول علي أعلي القيم للمحصول (محصول الحبوب، والقش البيولوجي (طن/هكتار) وأيضاً دليل الحصاد (%) في كلا الموسمين.

Effect of Mineral, Organic and Bio-fertilization on Growth and Production of Moringa (*Moringa oleifera*, L.) Plants

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ABSTRACT: Two filed experiments were carried out at the Experimental Farm, Faculty of Agriculture (Saba Basha), Alexandria University at Abees region, Alexandria, Egypt during the two growing seasons of 2014 and 2015 to study the effect of mineral, organic and bio-fertilization on growth and productivity of moringa plants (*Moringa oleifera*, Lam).The experimental design was split plot were three replicates. The main plot were conducted for the five combination of organic manure plus mineral fertilizer of (100% organic, 75% organic manure + 25% mineral, 50% organic manure + 50% mineral, 25% organic manure + 75% mineral and 100% mineral), while, the four bio-fertilization treatments were uninoculation, phosphorein, A- mycorrhizal and cerealine were arranged in the sub-plot. The main results could be summarized as follows: (1) The application of 75% organic manure + 25% mineral; gave the highest mean values of all studied characters, (2) the application of 75% organic manure + 25% mineral with A- mycorrhizal inoculation was the best combination to obtain the highest mean values of plant height, stem length, stem diameter, number of branches /plant, fresh and dry weights/plant, K (%), total carbohydrate (%) and vitamin (C). However, all traits under study increased significantly due to inoculation treatments over the application 100% mineral with uninoculation treatments.

Keywords: *Moringa oleifera*, vegetative growth, inorganic, organic and bio-fertilization.

INTRODUCTION

Moringa (*Moringa oleifera*) is well known for its multi-purpose attributes, wide actaplability and case of establishment. Every part of the plant is of food value, moringa leaves contain seven times more vitamin-C than oranges, four times more calcium than milk, four times more vitamin-A than carrot, three times more potassium than banana and two times more milk. Hence, it is considered as a powerhouse of nutritional value (Morton, 1991). The seeds are also used for oil productions; this oil is used in art, cosmetics and medicine; and can be consumed as food. Bio-fertilizers are microbial inoculants used for application to either seeds or soil for increasing soil fertility with objective of increasing the number of such micro- organisms and to accelerate ecertain microbial processes (Mazher, *et al*. 2014).

Fertilization is one of the most important factors limiting the productivity of plants. The intensive use of expensive mineral fertilizers in recent gears results in environmental pollution problems. However, chemical fertilizers at extremely high rates for a long period decreased the potential activity of microflora. (Adeoye *et al*., 2005).

Additionally, organic manures in the form of compost, animal manure, farmyard manure (FYM) and green manure organic materials are generally added to soils to improve their physical and chemical properties. They enhance

the soil fertility by their composition of macro and micro-elements, amino acid, organic acids, sugars and organic matter (Abou El-Fadi, 1968). Furthermore, biofertilization is an important factor being used to produce products without some mineral fertilizer that cause environmental pollution problems and high rates of it leads in decrease the potential activity of microflora and the mobility of organic matters. Hence, the attention has been focused on the researches of biofertilization to safe alternative specific chemical fertilizers. Biofertilizers play vital role of increasing the number of microorganisms and accelerate certain of microbial process in the rhizosphere of inoculated soil of plants which can change the available form of some nutrients to be plants (Anjorin *et al*., 2010; Adebayo *et al*., 2011; Attia *et al*., 2014). This research, however, in an attempt to find out the best fertilization treatments, i.e. mineral fertilizer plus organic manure and biofertilizer on the vegetative growth and chemical composition of moringa (*Moringa oleifera*).

MATERIALS AND METHODS

The present investigation was carried out during both seasons of 2014 and 2015 at Abees Experimental Farm of the Faculty of Agricultural (Saba Basha), Alexandria University. A filed experimental was designed to study the effect of mineral, organic and bio-fertilization on growth and production of Moringa plants.

Some physical and chemical properties of the experimental field soil and organic matter during the two seasons were done and the data are shown in Tables (1 and 2).

Regarding the cultivated of *Moringa oleifera* plant look place the research and production station, Cairo (National Research Center). However, planted in 2.5 x 2.5 meter space. Mineral fertilizer was applied at 600 g/tree of ammonium nitrate (33.5%N), 250 g/tree of calcium super-phosphate (15.5% P_2O_5) and 300g/tree of potaium sulphate (50% K_2O). 1.5 kg sheep manure with 400 g biofertilizer (phosphorein and cerealine) and and cerealine) liter of A-mycorrhizal, and rate of calcium superphosphate were mixed with 0.15 m depth of top soil around the tree trunk at one dose at March, while nitrogen and potassium fertilizer were applied in three equal doses at April, May and June.

The applied treatments were a follow:
1.Fertilization
100 % organic
75% organic manure + 25% mineral
50% organic manure + 50% mineral
25% organic manure + 75% mineral
100% mineral

Table (1). Some physical and chemical properties of the experimental soil in 2014 and 2015 seasons

Soil properties	Season	
	2014	2015
A) Mechanical analyses :		
Clay %	42.50	43.00
Sand %	16.50	15.80
Silt %	41.00	41.20
Soil texture	**Clay loam soil**	
B) Chemical properties		
pH (1 : 1)	7.60	7.80
EC (dS/m)	2.20	2.30
1) Soluble cations (1:2) (cmol/kg soil)		
K^+	0.90	0.92
Ca^{++}	4.20	4.25
Mg^{++}	3.10	3.20
Na^{++}	8.20	8.15
2) Soluble anions (1 : 2) (cmol/kg soil)		
$CO_3^{--} + HCO_3^-$	2.80	2.70
Cl^-	11.30	11.50
SO_4^{--}	0.48	0.50
Calcium carbonate (%)	7.80	7.90
Total nitrogen (%)	0.48	0.49
Available phosphate (mg/kg)	3.60	3.70
Organic matter (%)	0.95	0.90

Table (2). Analysis of the applied organic manure (sheep manure).

Properties of organic manure	Value
pH	7.2
O.M %	35.5
O.C %	22.6
Total N%	2.05
Total P%	1.20
Total K%	1.50
C/N ratio	13.0:1

1.Biofertilizers treatments were randomly distributed in the sub plot a follows:

- Without inoculation (control)
- Inoculation with cerealine: An inoculate for all crops containing of *Azospirillum pp.* (10 cell/g), *Azotobacter chrooococum.*
- Inoculation with phosphorein: An inoculate for all crops containing of (*Bacillus megatherium*) soluble calcium phosphate. These inoculations are produced by the General Organization for Agriculture Equalization Ministry of Agriculture and land Reclamination Egypt (Ismali *et al*., 2009).

- Inoculation of A- mycorrhizal fungi: inoculants for Moringa with fungi (*Glomus mcrocarpium*) strain from plant production Dept. (Saba Basha) Alex. Univ., at a rate of 250ml of infected roots and was mixed with tress of Moringa plants.

The plants were harvested 3 times per seasons i.e. August 10th September 10th in the first and second seasons by cutting the vegetative parts.

The following data of vegetative growth were recorded:

Plant height (cm), stem length (cm), stem diameter (cm), number of branches /plant, shoot fresh weight (g) and shoot dry weight (g).

The chemical compositions were recorded as following:

For these analyses, the leaves were dried at 70℃ for 48hr., and ground. Leaves (0.5 g) were digested with sulphuric acid and hydrogen peroxide H_2SO_4+H_2O_2 according to the method of (Lowther, 1980) and the following determining were carried out in the digested solution to determine the following:

- Nitrogen content (N%)

Nitrogen was determined in digested plant material colorimetrically by Nessler`s method (Chapman and Pratt, 1978). Nessler solution (35 g KI/100 ml d.w. + 20g $HgCl_2$ / 500 ml d.w.) +120 g NaOH / 250 ml d.w. Reading was achieved using wave length of 420 nm and N was determined as percentage as follows:

% N = NH_4 % x 0.776485

- Phosphorus content (P %)

Phosphorus was determined by the Vanadomolyate yellow method as given by Jackson (1973) and the intensity of color developed was read in spectrophotometer at 405nm.

- Potassium content (K %)

Potassium was determined according to the method described by method Jackson (1973) using Beckman Flame photometer.

- Total soluble carbohydrates were determined, quantitatively, in the herb of sage by Anthron method according to Yemm and Willis (1954) as follows:

Extraction was carried out by grinding dry matter in Mahadavaine buffer (sodium citrate buffer, pH 6.8). Extracts were homogenized for 3 minutes and centrifuged at 4000 rpm for 15 min. the supernatant was then used to determine total soluble carbohydrates.

- Protein was determined by estimating the total nitrogen in the herbs and multiplied by 6.25 to obtain the percentage according to AOAC (1990).

- The ascorbic acid content of the juice was determined by titration with 4, 6 dichloro phenol-indo-phenol (AOAC, 1984) and calculated as milli-grams per 100 ml of juice.

The obtained data were statistically analyzed according to Gomez and Gomez (1984). The least significantly differences test (L.S.D.) at 0.05 was used in compare between means of the different treatments.

RESULTS AND DISCUSSIONS

A) Vegetative growth

The obtained results, given in Tables (3, 4 and 5) clearly show that combination of mineral plus organic manure fertilizer exhibited a significant effect on all estimated traits at the achieved three cuts during both seasons. Application of 75% organic manure + 25% mineral, significantly, increased plant height, stem length, stem diameter, number of branches /plant, shoot fresh and shoot dry weight/plant at the three cuts during both seasons. These results may be due to the nutritional benefits of organic manure which include improvement of soil fertility, water holding capacity and organic matter and response to organic manure attributed to increasing nitrogen nutrition as indicated by increased concentration in plant tissues (Dania *et al*., 2014).

Inoculation of A- mycorrhizal fungi, significantly, increased plant height, stem length, stem diameter, number of branches /plant, shoot fresh and shoot dry weight/plant at three cuts during both seasons in comparison to uninoculation treatments (control). It could be concluded that A- mycorrhizal fungi inoculation treatment promoted the production of moringa growth. However, these events could be attributed to more adsorption of nutrients which reflected more on growth, more cell division and enlargement more of tissue and organs and plant elongation. Also, the phosphate solubilizing bacteria and nitrogen fixing may increases. The synthesis of endogenous phytohormones, i.e. IAA, GAs and CKs which play an important role in formation of mass active root system which allow more nutrients uptake. The previous results agree, more or less, with the findings of Rajendrn *et al*. (2000) on *Cassuasin equisetifolia*, Manorama *et al*. (2007) on *Acaci mellifera* and Attia *et al*. (2014) on *Moringa oleifera*.

The interaction between organic manure + mineral and bio-fertilization was significant and affected all traits at the three cuts during both seasons (Tables 3, 4 and 5). Tables (6 and 7) decleard, the application of 75% organic manure+ 25% mineral, resulted in the highest shoot fresh and shoot dry weight mean values with inoculation with A- mycorrhizal.

Table (3). Plant height (cm) and stem length (cm) as affected by mineral-organic and biofertilization at the three cuts in 2014 and 2015 seasons.

Treatments	Plant height (cm)						Stem length (cm)					
	2014 Season			2015 Season			2014			2015		
	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut
A) Mineral + Organic												
100% Organic	100.24e	111.65d	124.10cd	111.65d	121.05d	134.83d	71.70b	89.30b	111.47b	79.14b	98.37b	123.69b
75% org. + 25% mineral	106.25a	118.06a	131.18a	118.04a	131.18a	115.75a	79.16a	98.95a	123.70a	87.95a	109.94a	137.42a
50 % org. + 50% mineral	102.30b	114.75b	127.12b	114.42b	127.13b	141.25b	64.09c	80.15c	100.19c	71.21c	89.05c	111.32c
25% org. +75% mineral	101.46c	112.75c	125.27c	112.88c	125.38c	139.16c	57.67d	72.09d	90.17d	64.13d	80.08d	100.09d
100% mineral	100.72d	111.92cd	123.54d	111.91d	125.18c	137.25c	52.23e	64.92e	81.15e	58.03e	73.13e	90.16e
LSD 0.05	**0.44**	**1.05**	**1.25**	**0.40**	**1.70**	**2.05**	**3.10**	**4.50**	**6.30**	**3.70**	**4.70**	**6.90**
B) Bio-fertilization												
Uninoculation	92.44d	102.73d	114.12d	102.71d	114.12d	126.73d	60.66d	75.85d	94.96d	67.43d	84.88c	105.10d
Phosphorein	101.43b	116.26b	125.89b	116.23b	129.85b	143.33b	66.27b	82.45b	103.08b	73.56b	93.61b	114.53b
Mycorrhizal	112.52a	125.03a	138.92a	124.96a	138.92a	154.33a	69.51a	86.71a	108.04a	76.89a	96.13a	120.14a
Cerealine	99.92e	111.29c	122.70c	111.15c	123.36c	136.33c	63.43c	79.29c	99.11c	70.49c	84.99c	110.06c
LSD 0.05	**1.02**	**2.10**	**2.30**	**1.50**	**2.20**	**2.50**	**2.40**	**3.10**	**3.90**	**2.20**	**3.30**	**4.10**
Interaction												
AxB	*	*	*	*	*	*	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference at 5% level using (L.S.D.) test

*: Significant at 0.05 level of probability.

Table (4). Stem diameter (cm) and number of branches/plant as affected by mineral-organic and biofertilization at the three cuts in 2014 and 2015 seasons.

Treatments	Stem diameter (cm)						Number of branches/plant					
	2014 Season			2015 son			2014			2015		
	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut
A)Mineral + Organic												
100% Organic	1.97b	2.20b	3.15b	2.20b	2.44b	3.52b	8.59c	9.70b	10.77b	9.68b	10.76b	11.96b
75% org. + 25% mineral	2.20a	2.45a	3.50a	2.44a	2.72a	3.88a	8.93a	9.93a	11.03a	9.93a	11.03a	12.26a
50 % org. + 50% mineral	1.99ab	2.02c	2.86c	2.21b	2.20c	3.16c	8.78b	9.75b	10.84b	9.75b	10.83b	12.04b
25% org. +75% mineral	1.61d	1.79d	2.57d	1.79d	1.99d	2.85d	7.89d	8.78d	9.75c	8.86c	9.75c	10.83c
100% mineral	1.66c	1.84d	2.31e	1.86c	1.88d	2.57e	7.24e	7.89c	8.69d	8.04d	8.77d	9.74d
LSD 0.05	**0.03**	**0.07**	**0.12**	**0.05**	**0.13**	**0.25**	**0.06**	**0.10**	**0.16**	**0.11**	**0.17**	**0.19**
B) Bio-fertilization												
Uninoculation	1.30d	1.44d	2.07d	1.44d	1.30d	2.29d	7.11d	7.90d	8.79d	7.91d	8.78d	9.76d
Phosphorein	2.01b	2.26b	3.21b	2.24b	2.49b	3.56b	8.60b	9.55b	10.61b	9.55b	10.61b	11.79b
Mycorrhizal	2.36a	2.63a	3.76a	2.62a	2.91a	4.17a	9.45a	10.46a	11.55a	10.51a	11.62a	12.91a
Cerealine	1.88c	1.90c	2.47c	2.08c	1.97c	2.74c	8.08e	8.90c	9.89c	9.05c	9.89c	10.99c
LSD 0.05	**0.04**	**0.11**	**0.20**	**0.14**	**0.23**	**0.30**	**0.11**	**0.13**	**0.18**	**0.20**	**0.25**	**0.40**
Interaction												
AxB	*	*	*	*	*	*	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference at 5% level using (L.S.D.) test
*: Significant at 0.05 level of probability.

Table (5). Fresh of shoot weight (g) and shoot dry weight (g) as affected by mineral-organic and biofertilization at the three cuts in 2014 and 2015 seasons.

Treatments	Shoot fresh weight (g)						Shoot dry weight (g)					
	2014 Season			2015 son			2014			2015		
	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut
A) Mineral + Organic												
100% Organic	81.27b	101.85b	127.35b	90.57b	113.35b	141.53b	16.30b	20.38b	25.48b	18.19b	22.64b	28.31b
75% org. + 25% mineral	90.58a	113.22a	136.41a	100.64a	125.63a	154.45a	18.11a	22.64a	29.06a	20.14a	25.16a	31.448a
50 % org. + 50% mineral	73.36c	91.70c	114.64c	86.51c	101.94c	127.31c	14.67c	18.34c	22.93c	16.30c	19.54c	25.48c
25% org. +75% mineral	66.72d	82.62d	103.18d	73.47d	91.54d	114.63d	13.20d	16.51d	20.72d	14.67d	18.34d	22.93d
100% mineral	59.43e	74.28e	93.61e	66.02	82.70e	103.17e	11.83e	14.85e	18.59e	13.19e	16.50e	21.48e
LSD 0.05	**3.10**	**5.10**	**6.20**	**3.60**	**5.50**	**7.20**	**0.85**	**1.10**	**1.18**	**1.03**	**1.15**	**1.30**
B) Bio-fertilization												
Uninoculation	66.04d	83.62d	103.20d	73.38d	91.70d	114.66d	13.29d	16.51d	20.64d	14.67d	17.67d	22.93d
Phosphorein	75.79b	94.74b	118.40b	84.21b	105.23b	131.59b	15.16b	18.94b	23.75b	16.76b	21.05b	26.35b
Mycorrhizal	83.81a	104.77a	130.94a	93.12a	116.40a	145.45a	16.76a	20.91a	26.20a	18.62a	23.18a	29.78a
Cerealine	71.07c	88.84c	111.05c	78.97c	98.41c	123.40c	14.34c	17.77c	22.22c	15.78c	19.74c	24.67c
LSD 0.05	**2.80**	**4.40**	**6.30**	**3.10**	**5.10**	**6.50**	**1.00**	**1.10**	**1.30**	**1.05**	**1.10**	**1.45**
Interaction												
AxB	*	*	*	*	*	*	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference at 5% level using (L.S.D.) test
*: Significant at 0.05 level of probability.

Table (6). Interaction between mineral+organic and biofertilization on shoots fresh weight/ plant (g) for moranga plant at three cuts during 2014 and 2015 seasons.

Treatments		Shoots fresh weight /plant (g)					
		2014 Season			2015 Season		
Org.+mineral	Biofertilization	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut
100% org.	Uninoculation	72.57	90.72	11.42	80.64	100.80	126.00
	Phosphorein	83.29	104.11	130.16	92.54	115.80	144.60
	Mycorrhizal	92.10	115.13	143.81	102.33	127.92	159.90
	Cerealine	78.10	97.63	121.98	86.78	108.48	185.60
75%org.+25%mineral	Uninoculation	80.64	100.30	126.00	89.60	114.66	140.00
	Phosphorein	92.84	115.68	144.44	102.88	128.20	160.66
	Mycorrhizal	102.34	127.92	159.90	113.70	142.13	177.66
	Cerealine	86.76	108.48	85.60	96.42	120.53	150.66
50%org.+50%mineral	Uninoculation	65.31	81.65	102.06	72.57	90.91	113.40
	Phosphorein	74.95	93.70	117.13	83.28	104.11	130.14
	Mycorrhizal	92.88	103.61	129.52	92.10	115.12	143.64
	Cerealine	70.29	87.83	109.84	78.10	97.63	122.04
25%org.+75%mineral	Uninoculation	58.78	73.82	91.86	65.31	81.65	102.06
	Phosphorein	67.46	84.32	105.42	74.95	93.65	117.12
	Mycorrhizal	74.59	93.25	116.57	88.88	103.60	129.51
	Cerealine	63.26	79.08	98.86	70.29	87.86	109.83
100%mineral	Uninoculation	52.90	66.13	82.66	58.78	73.48	91.85
	Phosphorein	60.73	75.89	94.87	67.46	84.99	105.41
	Mycorrhizal	67.14	83.92	104.91	74.59	93.25	116.56
	Cerealine	56.94	71.71	88.97	63.26	79.08	98.85
LSD 0.05		**3.30**	**5.40**	**6.50**	**3.50**	**5.70**	**7.40**

Table (7). Interaction between mineral+organic and biofertilization on shoots dry weight/ plant (g) for moranga plants at three cuts during 2014 and 2015 seasons.

Treatments		Shoots dry weight /plant					
		2014 Season			2015 Season		
Org. + mineral	Biofertilization	1st cut	2nd cut	3th cut	1st cut	2nd cut	3th cut
100%org.	Uninoculation	14.52	18.14	22.68	16.13	20.16	25.20
	Phosphorein	16.65	20.82	26.03	18.50	23.13	28.92
	Mycorrhizal	18.41	23.02	28.78	20.46	25.58	31.98
	Cerealine	15.61	19.52	24.41	17.35	21.69	27.12
75%org.+25%mineral	Uninoculation	16.12	20.16	25.20	17.91	22.40	28.00
	Phosphorein	18.51	23.13	28.92	20.56	25.70	32.26
	Mycorrhizal	20.46	25.58	31.98	22.74	28.42	35.53
	Cerealine	17.35	21.69	27.14	19.28	24.10	30.13
50 % org.+50%mineral	Uninoculation	13.06	16.33	20.41	14.51	14.80	22.67
	Phosphorein	14.99	18.73	23.43	16.65	20.81	26.02
	Mycorrhizal	16.57	20.72	25.90	18.41	23.02	28.78
	Cerealine	14.06	17.57	21.96	15.62	19.52	24.40
25%org.+75%mineral	Uninoculation	11.75	14.69	18.37	13.06	16.32	20.41
	Phosphorein	13.49	16.86	21.41	14.99	18.74	23.45
	Mycorrhizal	14.92	18.65	23.31	16.52	20.72	25.40
	Cerealine	12.65	15.82	19.79	14.05	17.57	21.90
100%mineral	Uninoculation	10.58	13.22	16.54	11.75	14.69	18.37
	Phosphorein	12.14	15.18	18.97	13.49	16.86	21.08
	Mycorrhizal	13.42	16.78	21.03	14.91	18.64	26.70
	Cerealine	11.38	14.23	17.79	12.62	15.81	19.76
LSD (0.05)		**1.08**	**1.15**	**1.28**	**1.06**	**1.20**	**1.47**

B) Chemical composition

Data presented in Tables (8 and 9) indicated that organic manure plus mineral fertilizers significantly affected nitrogen (%), phosphorus (%), potassium (%), protein (%), total carbohydrate (%) and vitamin (C) in both seasons. Application of 75% organic manure + 25% mineral; gave rise the highest mean values of all studied chemical composition parameters as compared with application 100 % mineral fertilizer in both seasons.

The increment in chemical composition of moringa leaves using the treatments of organic manure may be owing attributed to increase in the occupancy root zone of plant result of adding organic manure which reflected on N, P and K uptake by plant and confirm the pervious of vegetative growth. Similar results were obtained by Prabhakar and Hebbar (2007), Adebayo *et al*. (2011) on *Moringa oleifera*, Makinde (2013) on moringa plant and Attia *et al.* (2014) on moringa plant.

Concerning the bio-fertilization, treatments in Tables (8 and 9) revealed that inoculation moringa plants with bio-fertilization, increased all the studied of chemical composition in both seasons compared to uninoculated moringa (control).

It can, also, be suggested to use combined biofertilizer including phosphorein, A- mycorrhizal and cerealine biofertilizer including all biofertilizer to produce a high quality moringa trees. Several reports on biofertilizer utilization have emphasized that a single inoculation showed higher productivity than uninoculation treatment (control). Shah *et al*. (2006), Attia *et al.* (2014) and Mazher *et al.* (2014).

The interaction between combination organic manure and mineral and bio-fertilization were significant for N, P and K % in both seasons (Table 10). Application of 75% organic manure+ 25% mineral, gave the highest mean values of N% with cerealine, P% with phosphorein and K% with A- mycorrhizal inoculation as compared with was uninoculation treatment.

The significant differences for the interaction between combination organic manure plus mineral and bio-fertilization in both seasons due to application of 75% organic manure+ 25% mineral, brought about the greatest protein percentage with treatment of cerealine biofertilizer and total carbohydrate (%), vitamin (C) with A- mycorrhizal in both seasons (Table 11).

In conclusion, some organs of moringa are good source important minerals and these plants might be explored as a viable supplement and ready source of dietary minerals in animal and human food. There was a significant variation in macro and microelements in moringa leaves. Also, the application of 75% organic manure+ 25% mineral gave the highest vegetative growth and chemical composition with A- mycorrhizal inoculation.

Table (8). Nitrogen, phosphorus and potassium percentages as affected by mineral-organic and biofertilization in 2014 and 2015 seasons.

Treatments	2014 Season			2015 Season		
	N %	P %	K %	N %	P %	K %
A)Mineral + Organic						
100% Organic	2.91b	0.400b	2.35b	3.23b	0.448b	2.61b
75% org. + 25% mineral	3.22a	0.450a	2.61a	3.61a	0.498a	2.90a
50 % org. + 50% mineral	2.62c	0.360c	2.11c	2.91c	0.403c	2.33c
25% org. +75% mineral	2.35d	0.320d	1.91d	2.61d	0.355d	2.12d
100% mineral	2.12e	0.290e	1.96d	2.35e	0.318e	2.17d
LSD 0.05	**0.10**	**0.012**	**0.11**	**0.11**	**0.040**	**0.19**
B) Bio-fertilization						
Uninoculation	2.19c	0.250d	2.04d	2.43c	0.280d	2.16c
Phosphorein	2.57b	0.472a	2.15c	2.85b	0.520a	2.38b
Mycorrhizal	2.67b	0.410b	2.40a	2.92b	0.452b	2.66a
Cerealine	3.16a	0.326c	2.25b	3.51a	0.364c	2.48b
LSD 0.05	**0.11**	**0.015**	0.08	**0.12**	**0.050**	**0.17**
Interaction						
AxB	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference at 5% level using (L.S.D.) test

*: Significant at 0.05 level of probability.

Table (9). Protein (%), vitamin (C) and total carbohydrate (%) as affected by mineral-organic and biofertilization in 2014 and 2015 seasons.

Treatments	2014 Season			2015 Season		
	Protein %	Vitamin (C) mg/100 ml juice	Total carbohydrate %	Protein %	Vitamin (C) mg/100 ml juice	Total carbohydrate %
A) Mineral + Organic						
100% Organic	18.60b	0.526b	19.79b	20.20b	0.584b	22.06h
75% org.+25%	20.22a	0.584a	22.06a	22.47a	0.648a	24.51a
mineral50%org.+50%mineral	16.36c	0.467c	17.87c	18.18c	0.519c	19.85c
25% org.75% mineral	14.71d	0.420d	16.10d	16.34d	0.467d	17.87d
100% mineral	13.25e	0.409d	14.47e	14.70e	0.454d	16.08e
LSD 0.05	**1.10**	**0.35**	**1.30**	**1.20**	**0.052**	**1.45**
B) Bio-fertilization						
Uninoculation	14.02c	0.449d	14.27d	15.20c	0.497c	15.85d
Phosphorein	16.05b	0.497b	19.02b	17.83b	0.552a	21.14b
Mycorrhizal	16.67b	0.514a	22.03a	18.51b	0.570a	24.54a
Cerealine	19.70a	0.466c	16.90c	21.95a	0.518b	18.78c
LSD (0.05)	**1.20**	**0.015**	**1.50**	**1.30**	**0.040**	**1.90**
Interaction						
AxB	*	*	*	*	*	*

Means of each factor designated by the same letter not significantly different at 5% using least significant difference at 5% level using (L.S.D.) test

*: Significant at 0.05 level of probability.

Table (10). Interaction between mineral+organic and biofertilization on macronutrients (N, P and K %) for moranga plants in 2014 and 2015 seasons.

Treatments		N%		P%		K%	
Org. + mineral	Biofertilization	2014	2015	2014	2015	2014	2015
100% Org.	Uninoculation	2.41	2.67	0.28	0.31	2.13	2.36
	Phosphorein	2.83	3.13	0.52	0.58	2.36	2.62
	Mycorrhizal	2.93	3.25	0.45	0.50	2.64	2.93
	Cerealine	3.48	3.66	0.36	0.40	2.27	2.51
75% org. + 25% mineral	Uninoculation	2.68	2.97	0.30	0.34	2.87	2.63
	Phosphorein	3.14	3.49	0.59	0.65	2.62	2.91
	Mycorrhizal	3.26	3.62	0.50	0.55	2.93	3.26
	Cerealine	5.86	4.29	0.40	0.45	2.53	2.80
50 % org. + 50% mineral	Uninoculation	2.16	2.40	0.25	0.28	1.92	2.13
	Phosphorein	2.54	2.82	0.47	0.52	2.12	2.33
	Mycorrhizal	2.64	2.93	0.40	0.45	2.37	2.63
	Cerealine	3.13	3.47	0.32	0.36	2.04	2.21
25% org. +75% mineral	Uninoculation	1.94	2.16	0.22	0.25	1.73	1.92
	Phosphorein	2.28	2.54	0.41	0.45	1.91	2.12
	Mycorrhizal	2.37	2.63	0.57	0.40	2.13	2.37
	Cerealine	2.81	3.12	0.29	0.32	1.85	2.05
100% mineral	Uninoculation	1.75	1.94	0.20	0.22	1.57	1.74
	Phosphorein	2.06	2.28	0.37	0.40	1.75	1.94
	Mycorrhizal	2.13	2.37	0.33	0.36	1.92	2.13
	Cerealine	2.53	2.81	0.26	0.29	2.58	2.86
LSD (0.05)		**0.13**	**0.15**	**0.017**	**0.06**	**0.14**	**0.20**

Table (11). Interaction between mineral+organic and biofertilization on Protein %, Vitamin (C) and Total carbohydrate % for moranga plants in 2014 and 2015 seasons.

Treatments		Protein (%)		Vitamin (C) (mg/100 ml juice)		Total carbohydrate (%)	
Org. + mineral	Biofertilization	2014	2015	2014	2015	2014	2015
100% Org.	Uninoculation	16.72	16.72	0.488	0.543	15.68	17.42
	Phosphorein	17.64	19.60	0.543	0.604	20.89	23.22
	Mycorrhizal	18.32	20.35	0.562	0.624	24.00	26.97
	Cerealine	21.73	24.14	0.509	0.566	18.57	20.64
75% org. + 25% mineral	Uninoculation	16.74	18.60	0.542	0.603	17.42	19.36
	Phosphorein	19.63	21.81	0.604	0.671	23.22	25.80
	Mycorrhizal	20.36	22.62	0.624	0.688	26.47	29.96
	Cerealine	24.16	26.85	0.566	0.629	20.64	22.93
50 % org. +50% mineral	Uninoculation	13.53	15.03	0.434	0.483	14.11	15.68
	Phosphorein	15.88	17.64	0.482	0.536	18.80	20.89
	Mycorrhizal	16.49	18.31	0.494	0.554	21.84	24.27
	Cerealine	19.55	21.77	0.452	0.503	16.72	18.57
25% org. +75% mineral	Uninoculation	12.16	13.61	0.396	0.434	12.70	14.11
	Phosphorein	14.28	15.87	0.434	0.482	16.98	18.80
	Mycorrhizal	14.83	16.47	0.450	0.499	19.66	21.84
	Cerealine	17.57	19.52	0.407	0.452	15.04	16.71
100% mineral	Uninoculation	10.93	12.14	0.380	0.422	11.43	12.09
	Phosphorein	12.84	14.27	0.422	0.469	15.23	16.91
	Mycorrhizal	13.33	14.81	0.436	0.480	17.69	19.66
	Cerealine	15.80	17.56	0.396	0.440	13.54	15.04
LSD (0.05)		**1.22**	**1.33**	**0.040**	**0.055**	**1.35**	**1.96**

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الملخص العربي

تأثير التسميد المعدني والعضوي والحيوي علي نمو وإنتاج نباتات المورينجا

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أجريت تجربتان حقليتان في المزرعة البحثية بكلية الزراعة (سابا باشا) جامعة الأسكندرية–منطقة أبيس– حمهورية مصر أثناء موسمي الزراعة ٢٠١٤، ٢٠١٥ لدراسة تأثير التسميد المعدني والعضوي والحيوي علي نمو وإنتاج نباتات المورينجا.

صممت التجربة بتصميم القطع المنشقة مرة واحدة مع ثلاث مكررات وكانت القطع الريئسية عبارة عن خمس تداخلات من التسميد المعدني والعضوي وهي (١٠٠% سماد عضوي، ٧٥% سماد عضوي+٢٥% سماد معدني،٥٠% سماد عضوي+٥٠% سماد معدني،٢٥% سماد عضوي+٧٥% سماد معدني،١٠٠% سماد معدني)، بينما أربع معاملات تسميد حيوي (بدون تلقيح، فوسفورين، ميكوريزا، سيريالين) كانت موزعة في القطع المنشقة الأولي.

وكانت من أهم النتائج المتحصل عليها مايلي:

١. أدي إضافة ٧٥% سماد عضوي+٢٥% سماد معدني للحصول علي أعلي القيم للصفات المدروسة.
٢. كان التفاعل بين ٧٥% سماد عضوي+٢٥% سماد معدني مع التلقيح بالميكوريزا أفضل تفاعل للحصول علي أعلي متوسط قيم لإرتفاع النبات، طول الساق، قطر الساق، عدد الأفرع/نبات، الوزن الرطب والجاف للمجموع الخضري/نبات، النسبة المئوية للبوتاسيوم والكربوهيدرات وفيتامين سي، ولكن أدي التفاعل بين إضافة ٧٥% سماد عضوي+٢٥% سماد معدني مع التلقيح بالسيريالين إلي أعلي نسبة مئوية للنيتروجين ومحتوي البروتين بينما التلقيح بالفوسفورين إلي أعلي نسبة مئوية للفوسفور لنبات المورنيجا في الدراسة. مع هذا فإن جميع الصفات تحت الدراسة تأثرت معنوياً بمعاملات التلقيح وإضافة ١٠٠% سماد معدني مع المعاملة بدون تلقيح.

Sunflower Water Requirements Using Single and Dual Crop Coefficients

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ABSTRACT: A field experiment of drip-irrigated Sunflower (Helianthus annuus) was conducted at the Experimental Farm, Faculty of Agriculture (Saba-Basha), Alexandria University, Egypt during 2013 growing season to develop seasonal K_c values for drip irrigated sunflower. In this context the objectives were:
1. to analyze the ability of the FAO-56 single and dual crop coefficient models for assessment the regional evapotranspiration and water requirements, 2. to estimate an adequate water quantity needed for the sunflower.
The sunflower variety Sakha 53 was cultivated at 28th April and harvesting was done at 8 August, 2013. Seeds were sown at 4-5 seeds in each hill with a spacing of 0.3 m within each row and 0.6 spacing, then thinned to one plant after 2 weeks from sowing. After emergence, the plots were irrigated by the drip irrigation method. All field practices were done as usually recommended for sunflower cultivation. The irrigation treatments based on replenishment of soil water depletion according to reference evapotranspiration (ET_0). The irrigation treatments were; irrigation at 20, 40, 60, 80 and 100% of ET_0. The results indicated that seasonal sunflower evapotranspiration (mm) has higher value with field irrigation approach and the lower value was for standard FAO single approach. The seasonal evapotranspiration (single crop coefficient approach) was less than the seasonal evapotranspiration of dual crop coefficient approach. It appears that ETc estimation of sunflower crop is more accurate by dual crop coefficient approach than those produced by single crop coefficient approach because of using more parameters and taking the soil practices and crop characteristics in consideration. The basal crop coefficient values cannot be proposed for all climates and regions because of different climatic conditions and crop management practice under different regions. The present study recommended that for the present conditions and the same other conditions, the irrigation of sunflower crop must be done according to the dual crop coefficient approach because it is more accurate than single crop coefficient and close up to the field conditions.

Keywords: sunflower, water requirements, single crop coefficient, dual crop coefficient, FAO Penman- Monteith model

INTRODUCTION

Water scarcity in semi-arid or arid regions is one of the main factors limiting agricultural development. The impact of such water scarcity is amplified by inefficient irrigation practices. Therefore, the first step toward sound management of the scarce water resources in these regions requires an accurate estimation of the water needs and consumption of irrigated agriculture. Several models have been developed to simulate crop evapotranspiration (ET_c) and in some cases, its components (soil evaporation and plant transpiration). These models ranged from complex (Braud *et al*., 1995) to more simple and conceptual ones (Olioso *et al*., 1999). FAO-56 is based on the concepts of reference evapotranspiration ET_0 and

crop coefficients K_c, which have been introduced to separate the climatic demand from the plant response (Allen *et al*., 1998). There are two approaches to estimate crop evapotranspiration: the single and the dual crop coefficients. In the FAO-56 single crop coefficient approach, the effect of both crop transpiration and soil evaporation are integrated into a single crop coefficient, K_c, the FAO-56 dual crop coefficient approach describes the relationship between maximal evapotranspiration ET_c and reference evapotranspiration ET_0 by separating K_c into a basal crop (K_{cb}) and soil water evaporation (K_e) coefficients. In the semi-arid Mediterranean region of southern Morocco, Er-Raki *et al.* (2010) applied the single approach and found that the approach overestimates AET by about 18% when using the crop coefficient suggested by Allen (2000).

Knowledge of crop coefficient (K_c) is essential for the estimation of water use. It helps in determining the water requirement of the crops according to their growth stage and environmental factors. Studies have found that K_c for the same crop may vary from place to place based on factors such as climate and soil evaporation (Allen *et al*., 1998; Kang *et al*., 2003). Doorenboss and Pruitt (1977) and Kang *et al.* (2003) emphasized the need to develop regional K_c for accurate estimation of water use, under a specific climatic condition.

Numerous empirical methods have been developed to estimate evapotranspiration from different climatic variables. Examples of such methods include Penman-Monteith (Monteith, 1965) and Blaney-Criddle model (Blaney and Criddle, 1950). Blaney-Criddle model requires the temperature data while the FAO-Penman-Monteith requires additional parameters such as wind speed, humidity and solar radiation. The Blaney-Criddle method is used to calculate monthly K_c values as compared to daily and less data is needed for this method.

The Food and Agricultural Organization (FAO) recommended FAO-Penman Monteith (FAO-PM) method as the sole standard method for computation of ET_0 (Allen *et al*., 1998). FAO-PM can provide accurate ET_0 estimates for weekly or even hourly periods.

Accurate prediction of crop water use is the key to develop the efficient irrigation management practices making it imperative to develop K_c for a specific crop. Doorenbos and Pruitt (1977) prepared a comprehensive list of K_c for various crops under different climatic conditions by compiling results from different studies. A similar list of K_c was also given by Allen *et al.* (1998) and Doorenbos and Kassam (1979). However, K_c for a crop may vary from one place to another, depending on factors such as climate, soil, crop type, crop variety, irrigation methods (Kang *et al*., 2003). Thus, for an accurate estimation of the crop water use, it is imperative to use a regional K_c. Brouwe and Heibloem (1986) stated that the steps for development of K_c as: determination of the total growing period of the crop, identifying the length of different growth stages, and determination of K_c

values for each growth stage. However, K_c cannot be measured directly, but is estimated as a ratio (ET_C/ET_0). While ET_0 can be estimated using one of the several available methods, ET_c can be estimated by a lysimeter study as reported by Grattan *et al.* (1998). There are two approaches to estimate crop evapotranspiration: the single and the dual crop coefficients. The FAO-56 dual crop coefficient approach (Allen *et al*., 1998) describes the relationship between crop evapotranspiration, ET_c and reference evapotranspiration, ET_0 by separating the single K_c into the basal crop K_{cb} and soil water evaporation K_e coefficients, while in the FAO-56 single crop coefficient approach, the effect of both crop transpiration and soil evaporation are integrated into a single crop coefficient. Many studies have focused on the application of the single approach for determining olive water requirement within Mediterranean regions (Palomo *et al*., 2002; AbidKarray *et al*., 2008; Martinez-Cob and Faci, 2010). In the semi-arid Mediterranean region of southern Morocco, Er-Raki *et al*. (2008) applied also the single approach over the same study site of this work, and they found that the approach overestimates AET by about 18% when using the crop coefficient suggested by Allen *et al*. (1998). Recently, several studies used the FAO-56 dual crop coefficient for estimating water consumptions of different crops (Hunsaker *et al*., 2003, 2005; Allen *et al.*, 2005 a, b; Paço *et al*., 2006; Er-Raki *et al*., 2007). Some of these studies adopted the FAO-56 dual approach to use satellite-based vegetation index (Hunsaker *et al*., 2003, 2005; Er-Raki *et al*., 2007; González-Dugo and Mateo, 2008; Er-Raki *et al*., 2010). The results show that relating the basal crop coefficient K_{cb} to remotely sensed vegetation index greatly improves the performance of the FAO-56 method. However, Er-Raki *et al*. (2006) showed that the performance of the FAO-56 method has some limitations when there is high soil evaporation or when stress occurs. To overcome this problem and then enhance the FAO-56 performances, ET derived from thermal infrared (TIR) observations was assimilated into FAO-56 single source model (Er-Raki *et al*., 2008) in order to estimate accurately the water consumption of olive orchards in the semi-arid region of the Ten sift basin (central of Morocco).

The goal of this study was to develop seasonal and growth stages K_c values for drip irrigated sunflower. In this context the objectives of this study were:
1. to analyze the ability of the FAO-56 dual crop coefficient model for assessment the regional evapotranspiration and water requirements.
2. to estimate an adequate water quantity needed for the sunflower and to determine the best quantity of irrigation by using the FAO- single and dual crop coefficient approaches.

MATERIALS AND METHODS

1. Experimental site and conditions

This study was conducted during the 2013 summer season at the Experimental Farm, Faculty of Agriculture (Saba-Basha), Alexandria University,

Egypt. The farm is located at Abees region located at 31˚ 10.102′ N and 29˚ 58.085′ E with an altitude of (-5 m) under sea level. The site was planted with corn crop in the previous season. This area is characterized by a semi-arid climate, the weather is hot and dry from May to August where temperatures ranged from 25 to 30 ºC. On the other hand, the average values of rainfall were 186.2 mm per year. Wind speed average was 13.5 km/day and relative humidity average was about 69.5 %. Some climatologically data on the experimental site were taken from Nouzha Weather Station and are given in Table (1).

2. Soil of the experimental site

Soil samples were collected from the experimental soil for both surface (0-30 cm) and subsurface (30-60 cm) layers. Some physical and chemical properties of the experimental field soil are presented in Table (2). The soil properties were performed according to the methods outlined in Carter and Gregorich (2008). The soil of the experimental site is clayey texture with water table level of 1 m down the soil surface, the groundwater is moderately saline (2.5 dS/m) and the contribution of water table to plant water requirements is low in the site of experiment.

3. Sunflower cultivation

Sunflower (*Helianthus annuus*) variety Sakha 53 early variety (100 days' crop age) was selected for the study at 2013 summer season. Plant sowing date was at 28 April, 2013. Seeds were sown (4-5 seeds) in each hill with spacing of 0.3 m within each row. Thinning to one plant per hill was carried out after 15 days from sowing to obtain a final plant population of 55500 plants/ha. After emergence, the plots were irrigated by the drip irrigation method, Table (3) shows the chemical analysis of irrigation water. Irrigation was terminated at 5 August, complete canopy and initial blooming date was at 13 June, and harvesting data was at 9 August. All field practices were done as usually recommended for sunflower cultivation. Phosphorus fertilizer as calcium superphosphate (15.5% P_2O_5) was fully added to the soil during seed preparation at 336 kg P_2O_5 ha^{-1}. Ammonium Nitrate (33.5% N) at the rate of 168 kg ha^{-1} were applied at two equal doses, one after sowing and the second after one month later. Potassium Sulfate (48% K_2O) at the rate of 67 kg ha^{-1} were added at two equal doses, one after sowing and the second after one month later.

Table (1). Daily maximum, minimum and average temperature, wind speed, solar radiation for the experimental Site during the experimental period

Months	Average minimum daily temperature T_{min} (°C)	Average maximum daily temperature T_{max} (°C)	Average daily temperature T_m (°C)	Average daily wind speed U_2 (m/s)	Average relative humidity %	Average atmospheric pressure mb	Average precipitation mm/month	Average daily solar radiation (MJ/m^2/day)
April 2013	14.8	24.6	19.4	11.18	62.9	1014.8	0	34.12
May 2013	18.8	28.7	23.5	9.79	68.0	1012.4	3.1	35.90
June 2013	21.7	30.3	25.6	10.83	68.4	1011.1	0	37.41
July 2013	23.4	30.2	26.6	11.66	71.4	1008.1	0	36.64
August 2013	23.9	31.7	27.8	9.58	72.1	1008.9	0	34.99

Table (2). Some physical and chemical properties of the experimental site

Soil parameters	0-10cm depth	10-20cm depth	20-40cm depth	Unit
Particle size distribution(%)				
Sand	29.7	29.7	32.2	**%**
Silt	15.0	17.5	15.0	**%**
Clay	55.3	52.8	52.8	**%**
Textural class	Clay	Clay	Clay	**-**
Soil bulk density	1.240	1.245	1.248	Mg/
Soil moisture content at field capacity (θ_{fc})	0.3513	0.3613	0.3687	m^3m^{-3}
Soil moisture content at permanent wilting point (θ_{wp})	0.1221	0.1281	0.1295	m^3m^{-3}
Plant available water content (PAW)	0.2292	0.2332	0.2392	m^3m^{-3}
Organic matter content	2.87	2.87	2.15	%
Total calcium carbonate	18.12	18.12	15.78	%
Electrical Conductivity (EC_w), (1:1, soil: water extract) dS/m	6.98	6.29	5.94	ds/m
pH (1:1, soil : water suspension)	8.05	8.15	8.25	-
Soluble Cations				
Ca^{2+}	2.38	1.69	1.42	meq/
Mg^{2+}	7.85	6.05	4.50	meq/
Na^{+}	58.15	54.13	52.13	meq/
K^{+}	1.35	1.12	1.12	meq/
Soluble Anions				
$CO^{=}_{3+} HCO^{-3}$	10.20	9.92	2.12	meq/
Cl^{-}	44.00	44.39	41.00	meq/
$SO^{=}_{4}$	14.03	7.70	12.54	meq/

Table (3). Chemical analysis of irrigation water used in the field experiment

Parameters	Value	unit
pH	7.35	-
EC_{iw}	0.60	dSm^{-1}
Soluble Cations		
Ca^{+2}	1.89	$meql^{-1}$
Mg^{+2}	0.81	$meql^{-1}$
K^{+}	2.74	$meql^{-1}$
Na^{+}	0.46	$meql^{-1}$
Soluble Anions		
$CO^{=}_3 + HCO^{-}_3$	1.98	$meql^{-1}$
Cl^{-}	0.810	$meql^{-1}$
SO_4^{-2}	3.14	$meql^{-1}$

At harvest, the sample of plants (1 m of the row × 0.60 m width of the row = 0.60 m^2) of the two central ridge were chosen to determine the sunflower yield and the total yield per ha^{-1} was calculated.

4. Irrigation regime

The irrigation treatments were based on replenishment of soil water depletion according to the reference evapotranspiration (ET_0). The irrigation treatments were:

I1 irrigation at 20% of ET_0,
I2 irrigation at 40% of ET_0,
I3 irrigation at 60% of ET_0,
I4 irrigation at 80% of ET_0, and
I5 irrigation at 100% of ET_0

Irrigation water in drip irrigation system was taken by a water pump. Distribution lines consisted of PVC pipe manifolds for each plot. The diameter of the polyethylene laterals was 16 mm and each lateral irrigated one plant row. The inline emitter discharge rate was 4 l h^{-1} at 100 kPa operating pressure. The actual emitter discharge rate was calibrated before starting the experiment. The drip network calibration was performed and the actual rate of emitter was 3.43 l h^{-1}.

Soil water content was measured by sampling a soil from each row with soil tube 0.025 m diameter at three depths i.e. 0-10, 10-20 and 20-60 cm below soil surface then determined by gravimetric method. Soil water contents were monitored prior each irrigation and after irrigation at surface and subsurface depths through electronic pressure transducer (electronic tensimeter).

5. Crop Evapotranspiration

The irrigation requirements were calculated according to the Penman-Monteith equation (Allen *et al*., 1998) according the following equation:

$$ET_{crop} = \frac{ET_{drip}}{E_a (1\text{-}LR)} \quad (2)$$

Where:

ET_{crop} is the crop evapotranspiration, mm/day
ET_{drip} is the crop evapotranspiration under drip irrigation system, mm/day
E_a is the efficiency of irrigation system (assumed as 95 % for drip irrigation system under the present conditions).
LR is the Leaching Requirements required for salt leaching in the root zone depth (assumed as 15 %). and

$$ET_{drip} = K_r \times K_c \times ET_0 \quad (3)$$

K_r is the reduction factor that reflects the percent of soil covering by crop canopy and can be calculated by the equation described in Karmeli and Keller (1975):

$$K_r = \frac{GC}{0.85} \quad (4)$$

Where, GC is the ground cover fraction (plant canopy area divided by soil area occupied by one plant, assumed as 0.6).

K_c is the crop coefficient ranging from 0.35 (for initial stage) to 1.15 (for development stage) for sunflower (Allen *et al*., 1998). We need the length and crop coefficient (K_c) for each of the 4 growth stages: initial, crop development, mid-season and late season stages. The crop coefficients (K_c and K_{cb}) were collected from FAO (Allen *et al*., 1998) and are presented in Table (4).

Table (4). Crop coefficient (K_c) and development stages period for sunflower

Growth stages	K_c Single crop coefficient	K_{cb} Basal Crop Coefficient	Stage period, days
Initial	0.35	0.15	20
Crop development	0.35 - 1.15	0.15 - 1.05	25
Mid-season	1.15	1.05	38
Late-season	1.15 - 0.35	1.05 – 0.2	20

ET_0 is the reference evapotranspiration calculated with FAO Penman-Monteith equation (Allen *et al*., 1998) using the climatic data collected from the Nouzha Weather Station as follows:

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} U_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34U_2)} \quad (5)$$

Where:

ET_0 Reference evapotranspiration, mm day^{-1}
R_n Net radiation at the crop surface, MJ m^{-2} day^{-1},
G Soil heat flux density, MJ m^{-2} day^{-1}, Generally very small and assumed to be zero).
T Mean daily air temperature at 2.0 m height, ℃,
U_2 Wind speed at 2 m height, m s^{-1} ,
e_s Saturation vapor pressure at 1.5 to 2.5-m height, kPa,
e_a Actual vapor pressure at 1.5 to 2.5-m height, kPa,
$e_s - e_a$ Saturation vapor pressure deficit, KPa,
Δ Slope vapor pressure curve, kPa℃$^{-1}$,
γ Psychometric constant, kPa℃$^{-1}$.

The effect of soil water stress on crop ET is accounted by multiplying the crop coefficient by the water stress coefficient (K_s), which is given by the following equation:

$$K_s = \frac{TAW\text{-}D_r}{TAW\text{-}RAW} = \frac{TAW\text{-}D_r}{(1\text{-}p)TAW} \quad (6)$$

Where:
TAW is the total available water in the root zone depth (mm),
RAW is the readily available water in the root zone (mm), RAW=p*TAW,
p is the fraction of TAW that a crop can extract from the root zone without water stress (assumed as 0.45) and
D_r is the root zone depletion in the root zone (mm)

The total available water in the root zone is estimated as follows:

$$TAW = 1000(\theta_{FC} - \theta_{WP})Z_r \quad (7)$$

Where:
θ_{FC} is the field capacity (m^3/m^3),
θ_{WP} is the permanent wilting point (m^3/m^3) and
Z_r is the effective rooting depth (m)
The adjusted K_c due to water stress is:

$$K_{c\text{-}adj} = K_s \times K_c \quad \text{for single crop coefficient} \quad (8)$$

$$K_{c\text{-}adj} = K_s \times K_{cb} + K_e \quad \text{for dual crop coefficient} \quad (9)$$

The field crop evapotranspiration (ET_C) was calculated using the following equation (10):

$$ET_C = P + I - D - R \pm \Delta S \quad (10)$$

Where ET_C is the crop evapotranspiration (mm), P is precipitation (mm), I is irrigation (mm), D is the water drained (mm), R is the runoff (mm) and ΔS represents the changes in soil water storage during the growth period. D and R were considered as zero because of control irrigation. The changes in soil moisture were estimated with soil moisture measurements at different depths.

6. Development of Crop Coefficient

The K_c values were developed for sunflower crop using ET_0 estimates from FAO-PM method. To compute K_c based on crop development stage, it is important to establish the length of different crop growth stages (Table 4). Allen *et al.* (1998) divided the crop cycle into four stages: initial stage (marked with about 10% of plant cover), development stage (marked with the growth of plant 10% ground cover to effective cover i.e., flowering), mid-season stage (effective cover to start maturity) and late season stage (Start of maturity to harvest).

7. Sunflower crop coefficient (K_c)

The crop coefficients of the sunflower during the different growth stages according to the standard FAO methodology were presented in Table (5).

Table(5). Sunflower crop coefficient at growing periods (Doorenbose and Kassam, 1986)

Growth stages	Period length (days)	K_c value
Initial	20	0.35
Development	25	0.75
Midseason	38	1.10
Late season	20	0.35

Crop coefficient obtained for four growth stages of crop growing periods. The four growth stages of crop growing periods are as follows:

1. Initial period (planting to 10% ground cover)
2. Crop development (10% ground cover to effective cover i.e., flowering)
3. Mid-season (Effective cover to start maturity)
4. Late-season (Start of maturity to harvest)

The calculation procedure for crop evapotranspiration (ETc) consists of:

1. identifying the crop growth stages, determining their lengths, and selecting the corresponding K_c coefficients;
2. adjusting the selected K_c coefficients for frequency of wetting or climatic conditions during the stage;
3. constructing the crop coefficient curve (allowing one to determine K_c values for any period during the growing period); and
4. calculating ET_c as the product of ET_o and K_c.

8. Crop coefficient (field approach)

The single crop coefficient (K_c single) was defined as the ratio of the measured ET_c by field soil moisture measurement to the ET_0 estimated by the FAO Penman –Monteith equation (Allen *et al*., 1998) under standard condition as follows:

$$K_{c\text{-single}} = \frac{ET_c}{ET_0} \quad (11)$$

The dual crop coefficient under standard conditions can be presented as:

$$K_{c\text{-dual}} = \frac{ET_c}{ET_0} = K_{cb} + K_e \quad (12)$$

Where:
K_{cb} is the basal crop coefficient and K_e is the soil evaporation coefficient.

Therefore, crop development and its characteristics were recorded during the growing season to separate the individual growing stages of sunflower being the initial, development, mid and end stages.

9. Crop coefficient (FAO approach)

1. Single crop coefficient

The values for large number of crops are presented in Allen *et al.* (1998). They are based on average conditions in sub-humid climate. FAO has presented a correction equation to normalize the K_c value for other places with different climatological and soil conditions.

The value of $K_{c\ ini}$ can be estimated from Figures 29 and 30 (Allen *et al*., 1998) as follows:

$$K_{c\,ini} = K_{cini}(\text{Fig. 29}) + \frac{(\text{I-10})}{(40\text{-}10)}\left[K_{cini}(\text{Fig 30}) - K_{cini}(\text{Fig 29})\right] \quad (13)$$

Where:
$K_{c\ ini}$ is the value for $K_{c\ ini}$ from Figure 29 (Allen *et al*., 1998)
$K_{c\ ini}$ is the value for $K_{c\ ini}$ from Figure 30 (Allen *et al*., 1998)
I is the average infiltration depth (mm)

The values 10 and 40 in Equation are the average depths of infiltration(mm) upon which Figures 29 and 30 (Allen *et al*., 1998) are based.

Drip irrigation wet only a fraction of the soil surface, the fraction of the surface wetted, f_w ranged from 0.3 to 0.4. The Kc_{ini} can be calculated from the following equation:

$$K_{cini} = f_w \times K_{cini}(\text{Tab, Fig}) \quad (14)$$

Where:
f_w is the fraction of surface wetted by irrigation (0 – 1), 0.3 for drip irrigation
$K_{c\ ini}$ (Tab, Fig) is the value of $K_{c\ ini}$ from Table 12 or Figure 29 or 30 (Allen *et al*., 1998).

The value of $K_{c\ mid}$, specific adjustment in climate where RH_{min} differ from 45% or where U_2 is larger or smaller than 2.0 m/s was used. The value of $K_{c\ mid}$ is adjusted as:

$$K_{c\text{-}mid} = K_{c\text{-}mid}(\text{Tab}) + \left[0.04(U_2\text{-}2) - 0.004(RH_{min}\text{-}45)\right]\left[\frac{h}{3}\right]^{0.3} \quad (15)$$

Where:
$K_{c\ mid}$ (Tab) is the value of Kc mid taken from FAO Table (12), Allen *et al*. (1998)

U_2 is the mean value of daily wind speed at 2 m height over the soil surface during the mid- season growth stage (m/s) for 1 m/s <= U_2 <= 6 m/s.
RH_{min} is the mean value for daily minimum relative humidity during the mid-season growth stage 9%), for 20% <= RH_{min}<=80%.
h is the mean plant height during the mid-season growth stage (m) for 0.1 m<h< 10 m.

The value of $K_{c\ end}$ is adjusted as:

$$K_{c\text{-}end}=K_{c\text{-}end}\ (Tab)+\left[0.04(U_2\text{-}2)\text{-}0.004(RH_{min}\text{-}45)\right]\left[\frac{h}{3}\right]^{0.3} \quad (16)$$

2. Dual crop coefficient

The crop coefficient is divided into two parts (Equation). The first part is the basal crop coefficient (K_{cb}) that refers to the crop transpiration component of ET_c when the soil surface is dry but transpiration is occurring at a potential rate, i.e., water is not limiting transpiration (Allen *et al*., 1998). The second part is the soil evaporation coefficient K_e that describes the soil evaporation component of ET_c.

Similar to the single crop coefficient approach, a correction equation is used to determine K_{cb} in mid- and end-season stages of sunflower through the following equations:

$$K_{cb\text{-}mid}=K_{cb\text{-}mid}\ (Tab)+\left[0.04(U_2\text{-}2)\text{-}0.004(RH_{min}\text{-}45)\right]\left[\frac{h}{3}\right]^{0.3} \quad (17)$$

$$K_{cb\text{-}end}=K_{cb\text{-}end}\ (Tab)+\left[0.04(U_2\text{-}2)\text{-}0.004(RH_{min}\text{-}45)\right]\left[\frac{h}{3}\right]^{0.3} \quad (18)$$

The soil evaporation coefficient (K_e) depends on several parameters such as the irrigation period, irrigation depth, soil properties, wetting area, and crop development.

When the soil is wet, evaporation from the soil surface occurs at maximum rate. Therefore, the dual crop coefficient can never exceed a maximum value, $K_{c\ max}$. The K_e can be determined as:

$$K_e = K_r(K_{c\ max}\text{-}K_{cb}) <= f_{ew}\ K_{c\ max} \quad (19)$$

Where:
K_e is the soil evaporation coefficient (-)
K_{cb} is the basal crop coefficient,
$K_{c\ max}$ is the maximum value of Kc following irrigation,
K_r is the evaporation reduction coefficient depends on the cumulative depth of water depleted from the topsoil,
f_{ew} is the fraction of the soil that is both exposed and wetted
The $K_{c\ max}$ range from 1.05 to 1.30 and can be expressed as:

$$K_{c\,max} = \max\left\langle \left[1.2+(0.04(U_2-2)-0.004(RH_{min}-45))\left(\frac{h}{3}\right)^{0.3} \right] \right\rangle, \{K_{cb}+0.05)\} \quad (20)$$

$$K_r = \frac{TEW-Di}{TEW-REW} \quad (21)$$

Where:

TEW is the maximum cumulative depth of evaporation (depletion) from the soil surface layer

REW is the readily evaporable water (mm)

Di is the cumulative depth of evaporation (depletion) from the soil surface layer.

$$f_{ew} = \min(1-f_c, f_w) \quad (22)$$

$1-f_c$ is the average exposed soil fraction not covered by vegetation (0.01-1), Table (6)

f_{ew} is the average fraction of soil surface wetted by irrigation (0.01-1)

Table (6). Common values of fractions covered by vegetation (fc) and exposed sunlight($1-f_c$), Allen *et al*. (1998).

Crop growth stage	f_c	$1-f_c$
Initial stage (I)	0.0-0.1	1.0-0.9
Crop development stage (II)	0.1-0.8	0.9-0.2
Mid-season stage (III)	0.8-1.0	0.2-0.0
Late (end) season stage (IV)	0.8-0.2	0.2-0.8

10. Experimental design

A randomized complete block design (RCBD) with five treatments. Irrigation treatments were conducted using a drip irrigation system. The drip irrigation system was divided into three plots (replicates), and each plot had one valve.

11. Statistical analysis

Seed and oil yields were analyzed using a single-factor analysis of variance (ANOVA), and multiple comparisons were done for significant effects among treatment with the least significant difference (LSD) test by SPSS (Windows V18). The analysis was performed at 0.05 probability level of significant. The Duncan's Multiple Range Test was used for comparisons among different sources of variance.

RESULTS AND DISCUSSION

The sunflower growing periods were divided to four stages; initial, development, mid- and late growing stages. The sunflower planting period started on 28 April and was finished on 8 August. Table (7) illustrates the length of growing stages, crop coefficient (K_c) and reference evapotranspiration (ET_0).

Table (7). Growth period, crop coefficient and reference evapotranspiration of sunflower

Growth stage	Period length (days)	K_c value	ET_0 (mm)
Initial stage (I)	20	0.35	87.2
Crop development stage (II)	25	0.75	142.7
Mid-season stage (III)	38	1.15	182.0
Late (end) season stage (IV)	20	0.35	93.5
Total	103		505.4

1. Reference Evapotranspiration (ET_0)

The daily ET_0 was calculated according to the FAO Penman-Monteith equation (Allen *et al*., 1998). During the sunflower growing season, the daily ET_0 varied from 3.21 to 9.97 mm/day with an average of 4.91 mm/day and total value of 505.4 mm/season. The variation of ET_0 during the growing period is illustrated in Figure (1).

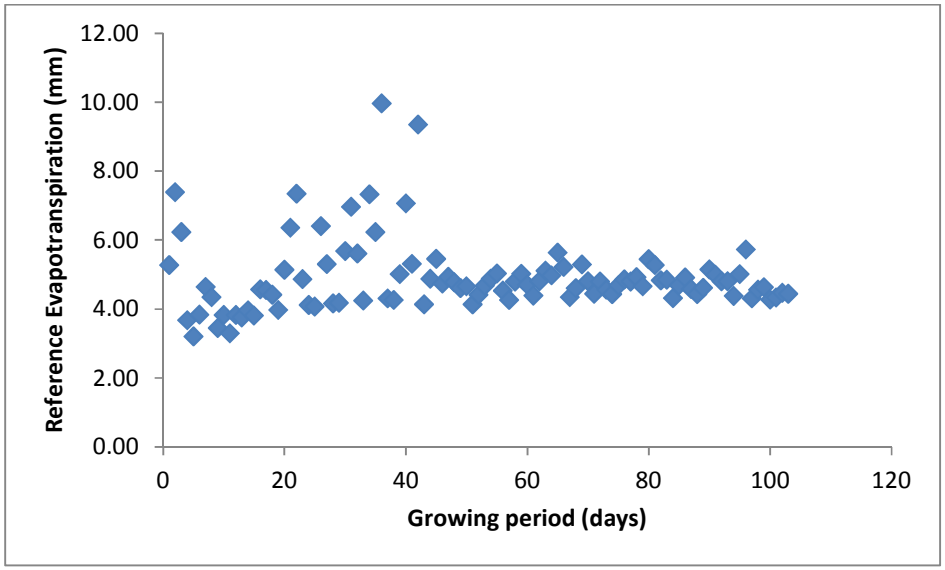

Fig. (1). Daily variation of reference evapotranspiration during growing period of sunflower

2. Crop evapotranspiration (ET_c) of sunflower

1. FAO single crop coefficient (K_c)

The daily sunflower evapotranspiration (ET_c) using standard single crop coefficient is illustrated in Figure (2).

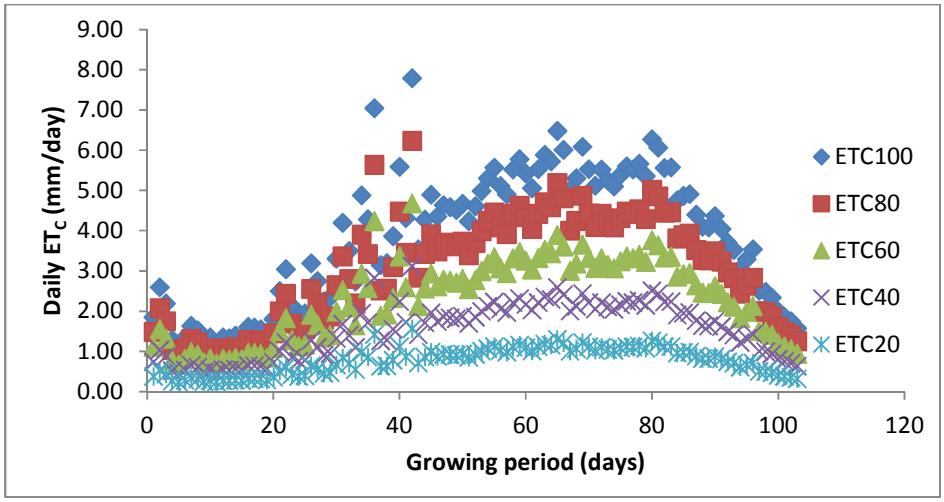

Figure (2). Daily variation of sunflower crop evapotranspiration (ET_c) with irrigation regimes using single crop coefficient.

Table (8) shows the sunflower crop evapotranspiration during initial, development, mid- and late growth stages according FAO standard approach. The crop evapotranspiration was decreased as water regime (% of ET_0) decreased. The crop coefficient was 0.35, 1.15 and 0.35 for initial, mid- and late growth stages as mentioned by Allen *et al*. (1998).

Table (8). Sunflower crop evapotranspiration(mm/ha) of growth stages with irrigation regimes (Single crop coefficient standard FAO approach)

Growth stage	K_c value (standard FAO K_c)	ET_0 (mm)	100% ET_0 (mm)	80% ET_0 (mm)	60% ET_0 (mm)	40% ET_0 (mm)	20% ET_0 (mm)
Initial stage (I)	0.35	87.2	30.5	24.4	18.3	12.2	6.1
Crop development stage (II)	0.75	142.7	110.2	88.2	66.1	44.1	22.0
Mid-season stage (III)	1.15	182.0	209.3	167.4	125.6	83.7	41.9
Late (end) season stage (IV)	0.35	93.5	68.5	54.8	41.1	27.4	13.7
Total (mm)		505.4	418.5	334.8	251.1	167.4	83.7

The seasonal sunflower crop evapotranspiration (ET_c) according to standard FAO methodology were 418.5, 344.8, 251.1, 167.4 and 83.7 mm for 100, 80, 60, 40 and 20% ET_0 irrigation regimes, respectively (Table 8).

The seasonal crop evapotranspiration (ET_c) of sunflower according to single crop coefficient field approach were 466.2, 372.9, 279.9, 186.5 and 93.2 mm for 100, 80, 60, 40 and 20% ET_0 irrigation regimes, respectively (Table 9). The single crop coefficient was 0.58, 0.89, 1.19 and 0.35 for initial, development, mid- and late growth stages under field conditions.

There are little differences between standard and field approach of crop coefficient, but the initial crop coefficient ($K_{C\ ini}$) is larger in field approach because of field conditions of the present experiment. Generally, crop coefficient depends on weather conditions, growth characteristics and ground cover of sunflower under field conditions.

Table (9). Sunflower actual crop evapotranspiration (mm) of growth stages with irrigation regimes (single crop coefficient field approach)

Growth stage	K_c value (Field approach)	ET_0 (mm)	100% ET_0 (mm)	80% ET_0 (mm)	60% ET_0 (mm)	40% ET_0 (mm)	20% ET_0 (mm)
Initial stage (I)	0.58	87.2	50.6	40.5	30.4	20.2	10.1
Crop development stage (II)	0.89	142.7	128.7	103.0	77.2	51.5	25.7
Mid-season stage (III)	1.19	182.0	216.6	173.3	129.9	86.6	43.3
Late (end) season stage (IV)	0.35	93.5	70.3	56.3	42.2	28.1	14.1
Total (mm)		505.4	466.2	372.9	279.7	186.5	93.2

2. Basal crop coefficient (K_{cb})

The crop coefficient, soil evaporation and dual daily crop coefficient of sunflower crop were obtained during the growing period. The values of basal crop coefficient during sunflower growing period are shown in Table (10). The values were 0.32, 0.69, 1.05 and 0.25 for initial, development, mid- and late growth stages according to FAO standard approach (Table 10).The basal crop coefficient (i.e., transpiration component) gradually increased as the highest value was obtained in the development growth stage. Thus, the transpiration value was decreased during late growing stage. The soil evaporation was differed according to the water regime, it reached 0.61, 0.55, 0.51, 0.43 and 0.34 for 100, 80, 60, 40 and 20% of ET_0. The seasonal crop evapotranspiration (ET_c) of sunflower according to dual crop coefficient standard approach were 484.7, 377.7, 291.0, 184.4 and 99.0 mm for 100, 80, 60, 40 and 20% ET_0 irrigation regimes, respectively (Table 10).

Table (10). Sunflower crop evapotranspiration (mm) of growth stages with irrigation regimes (dual crop coefficient standard FAO approach)

Growth stage	K_{cb} value (standard approach)	ET_0 (mm)	100% ET_0 (mm)	80% ET_0 (mm)	60% ET_0 (mm)	40% ET_0 (mm)	20% ET_0 (mm)
Initial stage (I)	0.32	87.2	58.4	45.7	36.7	24.3	14.1
Crop development stage (II)	0.69	142.7	135.1	103.3	79.9	50.8	27.5
Mid-season stage (III)	1.05	182.0	217.5	164.4	124.5	77.3	40.0
Late (end) season stage (IV)	0.25	93.5	83.7	64.2	49.9	31.9	17.4
Total (mm)		505.4	484.7	377.7	291.0	184.4	99.0

The seasonal crop evapotranspiration (ET_c) of sunflower according to dual crop coefficient field approach were 496.1, 388.9, 288.8, 188.7 and 100.8 mm for 100, 80, 60, 40 and 20% ET_0 irrigation regimes, respectively (Table 10). The dual crop coefficient (field approach) was 0.35, 0.69, 1.09 and 0.35 for initial, development, mid- and late growth stages (Table 11).

Table (11). Sunflower crop evapotranspiration (mm) of growth stages with irrigation regimes (dual crop coefficient field approach)

Growth stage	K_{cb} value (field approach)	ET_0 (mm)	100% ET_0 (mm)	80% ET_0 (mm)	60% ET_0 (mm)	40% ET_0 (mm)	20% ET_0 (mm)
Initial stage (I)	0.35	87.2	58.6	47.1	37.7	24.9	14.3
Crop development stage (II)	0.69	142.7	134.9	106.0	81.7	51.8	27.9
Mid-season stage (III)	1.09	182.0	216.6	168.2	127.1	78.8	40.6
Late (end) season stage (IV)	0.35	93.5	86.0	67.7	52.3	33.3	18.0
Total (mm)		505.4	496.1	388.9	298.8	188.7	100.8

The seasonal crop evapotranspiration (ET_c) of sunflower according to Irrigation field approach was 496.0, 398.1, 299.6, 200.1 and 103.3 mm for 100, 80, 60, 40 and 20% ET_0 irrigation regimes, respectively (Table 12). The crop coefficient was 0.51, 0.90, 1.50 and 0.54 for initial, development, mid- and late growth stages under field conditions.

Table (12). Sunflower crop evapotranspiration (mm) of growth stages with irrigation regimes (Field Irrigation approach)

Growth stage	K_c value (field approach)	ET_0 (mm)	100% ET_0 (mm)	80% ET_0 (mm)	60% ET_0 (mm)	40% ET_0 (mm)	20% ET_0 (mm)
Initial stage (I)	0.51	87.2	44.2	38.4	35.7	28.4	19.5
Crop development stage (II)	0.90	142.7	128.4	104.0	75.3	50.3	22.5
Mid-season stage (III)	1.50	182.0	272.5	217.0	162.8	106.3	53.2
Late (end) season stage (IV)	0.54	93.5	50.9	38.7	25.9	15.1	8.1
Total		505.4	496.0	398.1	299.6	200.1	103.3

The daily sunflower crop coefficient can be calculated by the best fitted polynomial equation (Table 13):

K_c-single(standard) =7.0E-08DAP^4-2.0E-05DAP^3+0.0015DAP^2-0.0184DAP+0.3783 (R^2=0.9700)

K_c-single(field) =3.0E-08DAP^4-1.0E-05DAP^3+0.001DAP^2-0.0113DAP+0.5913 (R^2=0.9688)

K_{cb}-dual(standard)=5.0E-08DAP^4-2.0E-05DAP^3+0.0013DAP^2-0.0159DAP+0.3427 (R^2=0.9695)

K_{cb}-dual(field)=6.0E-08DAP^4-2.0E-05DAP^3+0.0014DAP^2-0.017DAP+0.3762 (R^2=0.9695)

K_c(field irrigation)=8.0E-08DAP^4-2.0E-05DAP^3+0.0017DAP^2-0.0185DAP+0.5215 (R^2=0.9671)

Where DAP is the days after planting

Table (13). Crop coefficient during growth stages according to different approaches

Methods	Initial	Mid-	Late
Single crop coefficient standard approach(K_c)	0.35	1.15	0.35
Single crop coefficient field approach ($K_{c\ adj}$)	0.58	1.19	0.35
Basal crop coefficient standard approach (K_{cb})	0.32	1.05	0.25
Basal crop coefficient field($K_{cb\ adj}$)	0.35	1.09	0.35
Field irrigation approach (K_c)	0.51	1.50	0.54

The results indicated that seasonal sunflower evapotranspiration (mm) has higher value with field irrigation approach and the lower value was for standard FAO single approach. The seasonal evapotranspiration (single crop coefficient approach) was less value than the seasonal evapotranspiration of dual crop coefficient approach.

The seasonal water requirements for sunflower crop with considering the irrigation and soil practices are illustrated in Table (14). The results indicated that water requirements of sunflower growing season were higher with irrigation approach and lower with single crop coefficient approach.

Comparison of the measured single crop coefficient with standard values of FAO showed that, the measured K_c value at the initial stage was higher than the FAO standard value (by about 74.3% higher). The $K_{c\text{-}ini}$ greatly depends on the evaporating power of the atmosphere (ET_0), the water supply during a wetting event and the time interval between wetting events. Consequently, the $K_{c\text{-}ini}$ is influenced by the different irrigation strategies and soil practices.

Therefore, field management in the present study may not similar to the FAO-56 conditions. The FAO's predicted K_c may not predict the evapotranspiration that occurs in the initial growing stage. The measured value of late stage ($K_{c\text{-}end}$) is larger than proposed value of FAO-56 by about 11.42% (Table 15).

Table (14). Sunflower water requirements (m^3/ha) with irrigation regimes

Growth stage	100% ET_0(m^3/ha)	80% ET_0(m^3/ha)	60% ET_0(m^3/ha)	40% ET_0(m^3/ha)	20% ET_0(m^3/ha)
single crop coefficient standard FAO approach					
Initial stage (I)	378.1	302.5	226.9	151.2	75.6
Crop development stage (II)	1364.7	1091.8	818.8	545.9	272.9
Mid-season stage (III)	2591.8	2073.4	1555.1	1036.7	518.4
Late (end) season stage (IV)	848.6	678.9	509.2	339.5	169.7
Total water requirements	5183.3	4146.6	3110.0	2073.3	1036.7
single crop coefficient field approach					
Initial stage (I)	626.6	501.3	376.0	250.6	125.3
Crop development stage (II)	1593.8	1275.1	956.3	637.5	318.8
Mid-season stage (III)	2682.0	2145.6	1609.2	1072.8	536.4
Late (end) season stage (IV)	870.8	696.7	522.5	348.3	174.2
Total water requirements	5773.2	4618.6	3463.9	2309.3	1154.6
dual crop coefficient standard approach					
Initial stage (I)	723.4	566.0	455.0	301.4	174.6
Crop development stage (II)	1673.4	1279.8	989.3	629.1	340.0
Mid-season stage (III)	2693.7	2036.3	1541.6	957.8	495.8
Late (end) season stage (IV)	1036.1	795.0	618.1	395.5	216.1
Total water requirements	6126.6	4677.0	3604.0	2283.9	1226.5
(dual crop coefficient field approach					
Initial stage (I)	726.0	582.9	466.7	307.9	177.2
Crop development stage (II)	1670.5	1312.2	1011.8	641.6	345.0
Mid-season stage (III)	2682.0	2083.1	1574.0	975.9	503.0
Late (end) season stage (IV)	1065.1	837.9	647.8	412.0	222.7
Total water requirements	6143.6	4816.0	3700.3	2337.4	1247.9
field irrigation approach					
Initial stage (I)	547.7	475.2	441.9	351.7	241.5
Crop development stage (II)	1590.1	1287.9	932.5	622.9	278.6
Mid-season stage (III)	3374.6	2687.3	2016.1	1316.4	658.8
Late (end) season stage (IV)	630.3	479.6	320.2	187.2	100.3
Total water requirements	6142.7	4930.1	3710.7	2478.3	1279.3

Table (15). Seasonal water requirements of sunflower (%) as related to single crop coefficient (FAO standard)

Methods	100%ET_0	80%ET_0	60%ET_0	40%ET_0	20%ET_0
Single standard	100.00	100.00	100.00	100.00	100.00
Single field	111.38	111.38	111.38	111.38	111.38
Dual standard	118.20	112.79	115.89	110.16	118.31
Dual field	115.80	112.80	115.88	110.14	118.27
Irrigation	118.51	118.89	119.32	119.53	123.40

According to FAO-56 method corrected by equation (16 and 17), the sunflower K_{cb} values were 0.32, 1.05 and 0.25 for initial, mid- and late-season stages, respectively. Actually, the measured values of K_{cb} (0.35, 1.09 and 0.35, respectively) were similar to the standard FAO method values. The K_{cb} values are correlated with crop variety, cultivation pattern, crop coverage, soil practices and also the final crop yield. Different field treatments especially short irrigation intervals may keep the soil water content at optimum or higher value may lead to more or less evaporation occurring that affect the K_e and K_{cb} values. The field measurement to predict soil evaporation needs some practices to be more accurate to reduce the measured error.

The soil evaporation, K_c and K_{cb} coefficients are greatly affected by irrigation strategy, canopy coverage, local weather conditions, soil practices and irrigation system, therefore more investigation must be considering in determination of these parameters.

The higher values of sunflower water requirements for dual than single crop coefficients by about 3.1% may be due to more parameters affected the determination of dual K_{cb} than single K_c. Therefore, the values of K_c must be determined for different regions and different agricultural parameters, then local determination of crop coefficient has been recommended. The water requirements of sunflower with field irrigation approach were more than the dual crop coefficient approach by about 3.48% as mean of all water regimes.

The use of crop coefficients presented by FAO-56 (Allen *et al*., 1998) is common for use with crop water requirements estimation around the world. The present study showed that dual crop coefficient approach is located between the single crop coefficient and field irrigation approaches (∓3.2%). Therefore, dual crop coefficient is the more precise estimation of crop water requirements of sunflower than single coefficient and field irrigation approaches. The presented values of single and dual K_c will be useful in estimating sunflower water requirements of different crop growth stages and irrigation scheduling under semi-arid regions such as the present experimental conditions.

It appears that ET_c estimation of sunflower crop is more accurate by dual crop coefficient approach than those produced by single crop coefficient approach because of using more parameters and taking the soil practices and crop characteristics in consideration. The basal crop coefficient values cannot be proposed for all climates and regions because of different climatic conditions and crop management practice under different regions.

The present study recommended that under the same conditions, the irrigation of sunflower crop must be done according to the dual crop coefficient approach because it is more accurate than single crop coefficient and close up to the field irrigation conditions. Also, the field measurement to predict soil evaporation needs some practices to be more accurate to reduce the measured error.

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الملخص العربي

الاحتياجات المائية لزهرة الشمس باستخدام معاملات المحصول المفرد والمزدوج

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أجريت تجربة حقلية لمحصول زهرة الشمس تحت نظام الري بالتنقيط فى المزرعة البحثية لمحطة التجارب الزراعية لكلية الزراعة (سابا باشا) – جامعة الاسكندرية في منطقة أبيس – مصر خلال موسم الصيف ٢٠١٣م . وكان الهدف من الدراسة هو ايجاد معامل المحصول لمحصول زهرة الشمس تحت نظام الرى بالتنقيط – لتحقيق هذا الهدف تم ما يلي: ١– تحليل قدرة معامل المحصول المفرد والمزدوج المقترح من قبل الفاو لتقييم البخر–نتح والاحتياجات المائية المحلية. ٢– تقدير كمية مياه الرى المطلوبة لمحصول زهرة الشمس تحت نظم الحساب المختلفة. وقد تم زراعة محصول زهرة الشمس صنف سخا ٥٣ فى ٢٨ ابريل وتم الحصاد فى ٨ اغسطس ٢٠١٣ – وقد تم زراعة بذور زهرة الشمس (٥–٤ بذور) فى جور على ابعاد ٠.٣ متر داخل الخط و٠.٦ متر بين الخطوط وقد تم خفها بعد اسبوعين من الزراعة الى نبات واحد فى الجورة. بعد الانبات تم رى التجربة بخمس معاملات للري هى ٢٠، ٤٠، ٦٠، ٨٠ و ١٠٠% من البخر نتح المرجعي٠ وتوضح النتائج ان البخر–نتح الموسمي كان اعلى قيمة مع برنامج الرى الحقلي طبقا لحالة الرطوبة فى التربة وأقل قيمة مع برنامج معامل المحصول المفرد القياسي تبعا لمقترح الفاو. وقد كانت قيم الاستهلاك المائي الموسمي بطريقة معامل المحصول المفرد القياسي أقل من قيمة الاستهلاك المائي الموسمى مع معامل المحصول المزدوج القياسي. ويظهر من النتائج ان البخر–نتح من محصول زهرة الشمس أكثر دقة باستخدام معامل المحصول

المزدوج عن استخدام معامل المحصول المفرد وذلك بسبب ارتباطه مع عوامل متعددة تأخذ فى الاعتبار خدمة التربة وخواص المحصول. قيم معامل المحصول الاساسي (K_{cb}) لايمكن تعميمها لكل المناطق والنطاقات المناخية لاختلاف الظروف المناخية وادارة المحصول تحت ظروف المناطق المختلفة. وتوصى هذه الدراسة بانه فى مثل الظروف الحالية او الظروف المشابهة فان رى محصول زهرة الشمس يجب ان يتم باستخدام معامل المحصول المزدوج لانه اكثر دقة عن معامل المحصول المفرد وقريب من الظروف الحقلية.

Effect of *Schinus Terebinthifolius* Extracted Oil, 8-Hydroxyquinoline Sulphate and Citric Acid on The Longevity and Quality of Calla Lily Cut Flowers

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ABSTRACT: This investigation was carried out at Antoniades Research Branch, Horticulture Research Institute, Ministry of Agriculture, Alexandria, Egypt during 2014 and 2015 seasons. The study was a trial to investigate the effects of using *Schinus terebinthifolius* extracted (essential oil) at (0.3, 0.6 and 0.9 ml oil/L) as an environmentally safer treatment, Citric acid at (0.1,0.2 and 0.3g/L) and 8-hydroxyquinoline sulphate [8-HQS] at (100,150 and 200 mg/L) on the cut flowers of *Zantedeschia aethiopica L.* to define the best concentrations treatments to enhance the postharvest quality and the vase life. All treatments used caused a significant increase in the calla–lily vase life compared to control. The *S. terebinthifolius* essential oil treatments gave the lowest loss of flower fresh weight percentage (LFFW) in both seasons. The highest significant amount of water absorbed by calla- lily flowers were obtained from *S. terebinthifolius* essential oil treatment at 0.9 ml/L in both seasons which resulted in more freshness upon the cut flowers. The reducing sugar content obtained from all treatments was better than control. Both the treatment of 8-HQS at 200 mg/L and of 0.9 ml/L *S. terebinthifolius* essential oil prolonged the vase life of cut flowers. However the cut flowers resulted from the alternative environmentally safer treatment 0.9 ml/L of *S. terebinthifolius* essential oil at the end of the experiment, was more fresh compared with those of 200 mg/L of 8-HQS treatment.

Key words: vase life **-** *Zantedeschia aethiopica* – 8-hydroxyquinoline sulphate [8-HQS] – Citric acid -*Schinus terebinthifolius* extracted oil

INTRODUCTION

Vase life of cut flowers is an important factor in consumer preference. Short vase life is one of the most limiting factors related to cut flowers (Kader, 2003; Ahsan *et al.*, 2012). Under normal conditions, cut flowers could last only for a few days maintaining their beauty and attractiveness. However, most of the people like to enjoy cut flowers in their natural beauty and appearances for a longer period of time having the socioeconomic value of flowers intact (Tsegaw *et al.,* 2011; Zamani *et al.,* 2011). Hence there is a dire need to explore possibilities of extending vase life by using different biocides (Chapman and Austinbrown, 2007).

The effectiveness of hydroxyquinoline (HQ) as an apparent biocide in cut flower handling solutions has been known for decades (Van Doorn, 1997). Sulphate (HQS) and citrate (HQC) are the most commonly used HQ compounds in flower handling (Loubaud and Van Doorn, 2004; Van Doorn, 1997).Citric acid (CA) is a widespread organic acid in the plant kingdom and makes a weak acid in water. Citric acid is commercially advised for a number of cut flowers like chrysanthemum (Dole *et al.,* 1999). Also, CA reduces the risk of vascular blockage in cut flowers through its anti- embolism trait (Bhattacharjee *et al.*, 1993).

Recently, natural compounds such as plant essential oils are used as a new idea for controlling bacterial and fungal contamination and reducing postharvest losses of horticultural crops such as fruits, vegetables and flowers. Researches and commercial applications have revealed that natural compounds can be a suitable replacement for common chemical compounds (Solgi *et al.*, 2009). Hegazi and El-Kot (2009) showed that the essential oils of clove hindi, cinnamon, ginger, marjoram and fennel are used for gladiolus to reduce microbes accumulation in containers and increase the vase life.

Schinus terebinthifolius plant is known as Brazilian pepper, Aroeira, Florida holly, Rose pepper, or Christmas berry belongs to the Anacardiaceae family (Manrique *et al.,* 2008). The plant has a very long history of use and appears in ancient religious artifacts and on idols among some of the ancient Chilean Amerindians. In South and Central America, Brazilian pepper tree is reported to have astringent, antibacterial, antiviral and wound healing properties. (Molina-Salinas *et al.*, 2006).

Zantedeschia aethiopica (known as calla lily and arum lily) is a species in the family Araceae, native to Southern Africa in Lesotho, South Africa, and Swaziland. It is a rhizomatous herbaceous perennial plant (Courtier and Clarke ,1997) It is produced and marketed as a cut flower and a flowering potted plant for its attractive spathes, commonly referred to as flowers (Brian and Richard , 1991).

The aim of this study was to investigate the effects of *Schinus terebinthifolius* extracted essential oil, Citric acid and 8-hydroxyquinoline sulphate (8-HQS) on the vase life and quality of cut *Zantedeschia aethiopica* and find the best concentrations treatments to enhance the vase life of cut calla lily.

MATERIALS AND METHODS

The present study was carried-out at Antoniades Research Branch, Horticulture Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons of 2014 and 2015.

A-Source of the cut flowers:

Cut flowers were obtained from a well-known commercial nursery in Alexandria.

B-Cut flowers preparations:

The harvest point of cut flowers was determined when the inflorescences were completely open, before the spathe tip curled down and without pollen on the spadix (Nowak & Runicki , 1990). On the 7th of May 2014 and 2015 (in the first and second seasons, respectively), cut flowers were transported to the laboratory under dry conditions, they were recut before treatments to the length of 75 cm and put in a conditioning treatment (5% sucrose solution in distilled water for 12 hours) .

C-Preparation of essential oil from *Schinus terebinthifolius*:

Fruits of *Schinus terebinthifolius* were cut into small pieces (100 grams) and hydro-distillated for 3 h, in a Clevenger apparatus 48 (Salem *et al*., 2013). The oil was dried over anhydrous Na_2SO_4, and measured with respect to the mass of fresh weight of fruits (6.65 mL/100 grams fresh weight). The oil was kept dry in sealed Eppendorf tubes and stored at 4℃.

D-Chemicals used in the experiment:

1- 8-hydroxyquinoline sulphate (8-HQS) at concentrations of (100-150 and 200 mg/ L) in distilled water.
2- Citric acid (CA) at concentrations of (0.1, 0.2 and 0.3 g/L) in distilled water 3- *Schinus terebinthifolius* essential oil at concentrations of (0.3, 0.6 and 0.9 ml of extracted oil /L) .

E-Cut flower treatments:

On the 8th of May 2014 and 2015 (in the first and second seasons, respectively) conditioned flowers were recut to 70 cm and put in glass jars containing the three previous chemicals and distilled water (control) and the flowers were remained in the lab at the average temperature of (23.5^o-25^o) and average humidity (55%-57%).

F-Experimental layout and statistical analysis:

The experimental layout was a randomized complete block design (RCBD). It consists of ten treatments with three replicates each treatment contains three cut flowers. The means of the individual factors and their interactions were compared by L.S.D test at 5% level of probability. The data were statistically analyzed according to the method described by Snedecor and Cochran (1989).

G-Data were recorded as the following:

1-The postharvest characters

a- Vase life (days)

It was determined as the number of days from starting the experiment to the fading stage. The fading stage was set at the point when the cut flower turned green or when the tops of the spathes had dried. (Beata and Anna, 2011).

b- Loss of flower fresh weight percentage (L.F.F.W):

It was determined at the fading stage as the flowing formula (Tarek *et al* ., 2013)

$$\textbf{L.F.F.W. (\%)} = \frac{Initial\ fresh\ weight - Final\ fresh\ weight}{Initial\ fresh\ weight} \times 100$$

c- Final water uptake (g):

It was calculated at the end of the experiment as the following formula

Water uptake (g)= The amount of solution at the beginning of the experiment – the amount of the solution remaining at the end of the experiment

d- Flower fresh weight / flower dry weight ratio (FWR) :

At the fading stage the flowers were oven dried at 75°c for 48 hours to get the flower dry weight (F.D.W.) Then the fresh weight was divided by the dry weight as below (Mahmoud ,2013).

$$\text{FWR}=\frac{\text{Fresh weight per flower (g)}}{\text{Dry weight per flower (g)}}$$

e- Relative fresh weight (RFW)

Fresh weight of the flowers was determined just before the immersion of the flowers into the solutions and collected every two days until the vase life of the flowers was terminated. The fresh weight of each flower was expressed relative to the initial weight to represent the water status of the flower (He *et al.,* 2006)

$$\text{Relative fresh weight (RFW)} = \frac{Wt}{W0} \times 100$$

Where *Wt* is the weight of stem (g) at *11th May ,13th May and 15th May* and *W0* is the initial fresh weight of the same stem (g)

f- Vase Solution Uptake Rate:

The VSU rate was measured according to the formula below (Damunupola, 2009)

$$\text{VSU rate} = \frac{(St-1) - St}{\text{IFW of stem}} \times 100$$

Where (St) is weight of vase solution (g) at *11th May ,13th May and 15th May* ,(St-1) is weight of the vase solution (g) on the previous day and (IFW) is the initial fresh weight (g) .

2- Chemical analysis

Reducing sugar content (%)

Reducing sugar content was determined in dried spathes according to the method described by Malik and Singh (1980) .

RESULTS

1-The postharvest characters

a-Vase life (days)

Data presented in Table (1) showed that the 8-HQS treatments, *S. terebinthifolius* essential oil at (0.3ml/L and 0.9 ml/L) and Citric acid at 0.2 g/L gave the highest vase life in the first season .However the treatments of 8- HQS at 200 mg/L , schinus extracted oil at 0.9 m/L and Citric acid at 0.3 g/L gave the highest significant vase life in the second season . On the other hand, the lowest vase life was noted in the control treatment (7 and 8 days) in the first and second season, respectively .

b- Loss of flower fresh weight percentage LFFW (%)

Table (1) cleared that the highest LFFW was obtained from control treatment (35.97 and 31.51%) in the first and second season, respectively. On the other side, all *S. terebinthifolius* essential oil treatments gave the lowest LFFW in both seasons.

Table (1). Average of vase life (days) and loss of flower fresh weight (LFFW) (%) of calla –lily cut flowers as affected by 8HQS, *Schinus terebinthifolius* essential oil and citric acid in the seasons of 2014 and 2015.

Treatment	Vase life (days)		Loss of flower fresh weight LFFW (%)	
	2014	2015	2014	2015
Control (distilled water)	7.00^{c}	8.00^{d}	35.97^{a}	31.51^{a}
8HQS 100 mg/ L	10.33^{a}	10.00^{b}	27.43^{c}	29.33^{abc}
8HQS 150 mg/L	10.00^{a}	9.67^{bc}	29.35^{c}	28.15^{cd}
8HQS 200 mg/L	11.00^{a}	11.33^{a}	28.03^{c}	28.84^{bcd}
Essential oil 0.3 m/L	10.00^{a}	9.67^{bc}	23.46^{ef}	23.76^{f}
Essential oil 0.6 m/L	9.67^{b}	10.00^{b}	22.47^{ef}	24.83^{ef}
Essential oil 0.9m/L	10.00^{a}	10.33^{a}	21.08^{f}	23.69^{f}
Citric acid 0.1 g./L	8.33^{bc}	8.67^{cd}	32.61^{b}	30.91^{ab}
Citric acid 0.2 g./L	10.00^{a}	8.33^{cd}	26.91^{cd}	26.40^{de}
Citric acid 0.3 g./L	9.33^{b}	10.33^{a}	24.44^{de}	25.01^{ef}
L.S.D.$_{at\ 0.05}$	1.55	1.16	2.76	2.57

Means of treatments in the column have the same letters , are not significantly different at 5% level .

c- Final water uptake (g)

Table (2) showed that the highest significant amount of water absorbed by calla- lily flowers were recorded from the treatments of *S. terebinthifolius* essential oil at 0.9 m/L (24.79 and 23.83 g) in the first and second seasons respectively. On the other hand, the control treatment gave the lowest significant water uptake (14.28 and 16.65g) in the first and second season, respectively.

d-Fresh weight /dry weight ratio (FWR)

Results in Table (2) cleared that the highest FWR (33.17) was obtained from Citric acid treatment at (0.3 g/L) in the first season and both Citric acid at (0.3 g/ L) and *S. terebinthifolius* essential oil at (0.9 ml /L) in the second season . However the lowest FWR was obtained from the control treatment (21.46 and 21.83) in the two seasons, respectively .

Table (2). Average of final water uptake (g) and fresh weight/ dry weight ratio (FWR) of calla –lily cut flowers as affected by 8HQS, *Schinus terebinthifolius* essential oil and citric acid in the seasons of 2014 and 2015.

Treatment	Final water uptake (g)		Flower fresh weight/flower dry weight ratio (FWR)	
	2014	2015	2014	2015
Control (distilled water)	14.28^{f}	16.65^{e}	21.46^{d}	21.83^{c}
8HQS 100 mg/L	20.09^{c}	23.21^{ab}	26.25^{bcd}	28.60^{ab}
8HQS 150 mg/L	17.89^{de}	21.80^{bc}	24.77^{cd}	25.36^{abc}
8HQS 200 mg/L	19.13^{cd}	20.81^{c}	27.15^{abcd}	27.74^{ab}
Essential oil 0.3 m/L	24.63^{ab}	22.19^{bc}	29.67^{abc}	27.60^{ab}
Essential oil 0.6 m/L	22.76^{b}	21.48^{c}	30.21^{abc}	28.51^{ab}
Essential oil 0.9m/L	24.79^{a}	23.83^{a}	32.19^{ab}	28.80^{a}
Citric acid 0.1 g./L	18.07^{de}	17.64^{de}	26.28^{bcd}	24.15^{bc}
Citric acid 0.2 g./L	16.37^{e}	18.26^{d}	28.01^{abc}	27.42^{ab}
Citric acid 0.3 g./L	19.44^{cd}	18.54^{d}	33.17^{a}	29.58^{a}
L.S.D.$_{at\ 0.05}$	1.71	1.42	6.28	4.47

Means of treatments in the column have the same letters , are not significantly different at 5% level .

e-Relative fresh weight (RFW)

Data presented in Table (3) showed that all *S. terebinthifolius* essential oil treatments gave a high significant increase in RFW along the experiment period as and the lowest significant RFW was obtained in the control treatment in both seasons.

Table (3). Average of relative fresh weight (RFW) of calla –lily cut flowers as affected by 8HQS, *Schinus terebinthifolius* essential oil and citric acid in the seasons of 2014 and 2015.

Treatment	Relative fresh weight (RFW)					
	2014			2015		
	11th May	13th May	15th May	11th May	13th May	15th May
Control(distilled water)	88.98^{e}	87.68	63.82^{d}	88.69^{d}	79.20^{e}	46.11^{e}
8HQS100 mg/L	91.63^{cde}	90.56	72.57^{cb}	91.01^{bcd}	81.88^{bced}	70.66^{bc}
8HQS150 mg/L	90.84^{ed}	89.14	70.65^{c}	91.76^{bcd}	82.83^{abcd}	66.85^{d}
8HQS200 mg/L	90.30^{ed}	88.77	71.97^{cb}	90.17^{cd}	80.63^{ed}	71.16^{cb}
Essential oil 0.3 m/L	94.06^{abc}	90.10	76.54^{ab}	93.46^{ab}	84.38^{ab}	75.75^{a}
Essential oil 0.6 m/L	95.17^{ab}	90.67	73.86^{abc}	95.29^{a}	85.59^{a}	75.69^{a}
Essential oil 0.9m/L	96.35^{a}	90.11	78.93^{a}	95.20^{a}	83.97^{abc}	74.97^{a}
Citric acid 0.1 g./L	92.75^{bcd}	89.05	69.38^{cd}	91.34^{bcd}	80.72^{de}	70.15^{cd}
Citric acid 0.2 g./L	92.20^{cd}	89.86	73.08^{cb}	92.48^{abc}	81.02^{bcde}	73.60^{ab}
Citric acid 0.3 g./L	91.73^{cde}	88.60	73.87^{abc}	92.37^{abc}	81.01^{cde}	74.96^{a}
L.S.D.$_{at\ 0.05}$	2.96	N.S.	5.7	3.27	3.18	3.31

Means of treatments in the column have the same letters, are not significantly different at 5% level.

f-Vase solution uptake rate (VSU)

Data on Table (4) showed that the highest significant VSU was recorded after the treatment 0.9 ml/L *S. terebinthifolius* essential oil treatment (16.38) on the 11th of May; this value was dropped sharply and recorded 5.34 on the 13th of May and 7.53 on the 15th of May. For 8HQS treatments the VSU of the treatment 150 ppm recorded 10.43 on the 11th of May; this value dropped moderately and recorded 6.33 on the 13th of May and 10.02 on the 15th of May in 2014 season. The same trend was obtained on 2015 season. The treatment 0.9 m/L *S. terebinthifolius* essential oil recorded (16.25) on the 11th of May; this value decreased sharply and recorded 5.78 on the 13th of May and 7.46 on the 15th of May. For 8-HQS treatments the VSU of the treatment 150 ppm was 10.23 on The 11th of May this value dropped moderately and recorded 7.37 on the 13th of May and 10.30 on the 15th of May and the lowest VSU was obtained from the control treatment all over the two seasons .

Table (4). Average of vase solution uptake rate (VSU) of calla –lily cut flowers as affected by 8HQS, *Schinus terebinthifolius* essential oil and citric acid in the seasons of 2014 and 2015.

Treatment	Vase solution uptake rate (VSU), %					
	2014			2015		
	11th May	13th May	15th May	11th May	13th May	15th May
Control (distilled water)	8.41^{f}	5.65^{bc}	5.99^{e}	11.72^{d}	5.48^{d}	3.61^{g}
8HQS 100 mg/L	11.78^{cd}	6.69^{a}	9.41^{ab}	10.66^{d}	6.79^{bc}	8.42^{b}
8HQS 150 mg/L	10.43^{de}	6.33^{ab}	10.02^{a}	10.23^{d}	7.37^{ab}	10.30^{a}
8HQS 200 mg/L	9.89^{ef}	6.70^{a}	8.88^{bc}	12.02^{cd}	8.07^{a}	7.42^{bcd}
Essential oil 0.3 m/L	14.53^{b}	5.18^{c}	8.08^{d}	15.74^{ab}	7.22^{b}	6.96^{cde}
Essential oil 0.6 m/L	15.16^{ab}	5.42^{bc}	5.01^{e}	14.09^{c}	6.74^{c}	6.57^{de}
Essential oil 0.9m/L	16.38^{a}	5.34^{bc}	7.53^{de}	16.25^{a}	5.78^{c}	7.64^{bc}
Citric acid 0.1 g./L	9.54^{ef}	5.64^{bc}	5.56^{e}	10.96^{d}	5.54^{cd}	5.92^{ef}
Citric acid 0.2 g./L	12.32^{c}	4.73^{c}	5.91^{e}	9.44^{d}	4.79^{d}	5.11^{f}
Citric acid 0.3 g./L	11.10^{cde}	5.21^{c}	6.75^{e}	10.30^{d}	4.40^{d}	5.68^{ef}
L.S.D.$_{at\ 0.05}$	1.82	1.01	1.33	2.39	1.18	1.04

Means of treatments in the column have the same letters, are not significantly different at 5% level.

2-Chemical analysis

Reducing sugar content

Data in Table (5) showed that for both seasons the lowest significant reducing sugar content was obtained after distilled water treatment as compared to the other treatments.

Table (5). Average reducing sugar content of calla –lily cut flowers as affected by 8HQS, *Schinus terebinthifolius* essential oil and citric acid in the seasons of 2014 and 2015.

Treatment	Reducing sugar contents	
	2014	2015
Control (distilled water)	2.30^{b}	2.12^{b}
8HQS 100 ppm	3.21^{ab}	3.10^{a}
8HQS 150 ppm	3.33^{a}	3.10^{a}
8HQS 200 ppm	3.33^{a}	3.27^{a}
Essential oil 0.3 m/L	2.99^{ab}	3.26^{a}
Essential oil 0.6 m/L	3.03^{ab}	3.37^{a}
Essential oil 0.9m/L	3.03^{ab}	3.47^{a}
Citric acid 0.1 g./L	2.68^{ab}	3.08^{a}
Citric acid 0.2 g./L	3.19^{ab}	2.91^{a}
Citric acid 0.3 g./L	3.33^{a}	3.13^{a}
L.S.D.$_{at\ 0.05}$	0.91	0.66

Means of treatments in the column have the same letters , are not significantly different at 5% level .

DISCUSSION

From the previous results it was noticed that all treatments led to a significant increase in calla – lily vase life .The positive effect of 8-hydroxyquinoline sulphate (8-HQS) may act as an antimicrobial agent and hence, reduce stem plugging and preventing the accumulation of microorganism in xylem vessels (Larsen and Cromarty, 1967). Also it is clear from the results that *S. terebinthifolius* essential oil treatments prolonged the vase life of calla-lily flowers and these results could be due to the antibacterial and antifungal activities of the oil (Gundidza *et al.* 2009). Citric acid treatments

led to caused increment in vase life this may be due to its act in reducing the pH of water and, consequently, the proliferation of bacteria, which block the xylem vessels in the cut region and interfere with the normal flux of water through the stem (Nowak & Rudnicki, 1990). The lowest significant final water uptake, FWR values and highest significant LFFW was obtained from the distilled water treatment which may explain the lowest vase life of the control flowers. Also all the treatments led to a significant increase in reducing sugar content and this increment may increase the osmotic potential of the flowers , thus improving their ability to absorb nutrients and maintain their turgidity, which may explain the increase of flower longevity in different treatments in this study (Prathamesh and John 2013).

When flowers are detached from the plant, water loss continues through transpiration. The ideal flower preservative is that which allows water absorption in flower tissues or water absorption from the preservative solution maintains a better water balance and flower freshness and saves from early wilting resulting in enhancing vase-life. (Salunkhe *et al.,* 1990). Our study cleared that for RFW values of the schinus oil treatments had high value along the experiment period which may illustrate the freshness and flower longevity of flowers after this treatments. Also, it has been noticed that the high value of VSU on the 11th of May explains the freshness and flower longevity of calla flower from *S. terebinthifolius* oil treatments. Moreover, the moderate decrease of the VSU along the experiment period may clarify the increase of the flower longevity after 8-HQS treatments. Although the study demonstrated that the treatment of 8-HQS at the rate of 200 ppm and the treatments of 0.9 ml/L schinus oil had the same significant level in the vase life . The cut flowers resulted from the treatment 0.9 ml/ L *S. terebinthifolius* essential oil at the end of the experiment was more fresh than the cut flowers of 200 ppm of 8-HQS this may be due to the lowest significant LFFW value in both seasons from *S. terebinthifolius* essential oil treatments and also due to the highest final water uptake from 0.9 m/L *S. terebinthifolius* essential oil.

From the obtained results it could be recommended to use the *S.terebinthifolius* essential oil at the rate of 0.9 m/L as natural environmentally alternative flower biocide as compared to8-HQS treatments or the citric acid treatments.

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الملخص العربي

تأثير إستخدام مستخلص زيت نبات الفلفل عريض الأوراق و٨-هيدروكسي كانولين سلفات والستريك أسيد على الجودة والقدرة الحفظية لأزهار نبات الكلا بعد القطف

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أجريت هذه الدراسة خلال موسمي (٢٠١٤ ، ٢٠١٥) في فرع بحوث أنطونيادس ، معهد بحوث البساتين، وزارة الزراعة، الاسكندرية،جمهورية مصر العربية . تهدف هذه الدراسة إلى تقييم استخدام كل من المستخلص الزيتي لنبات الفلفل العريض بتركيزات (٠.٣ ، ٠.٦ ، ٠.٩ مل / لتر) ، حامض الستريك بتركيز (٠.١ ، ٠.٢ ، ٠.٣ جم / لتر) و٨ هيدروكسي كينولين سلفات (8-HQS) بتركيز (١٠٠ ، ١٥٠ ، ٢٠٠ مجم/ لتر) للحصول على

أفضل المعاملات لتحسين جودة أزهار الكلا و طول بقائها في الفازة . أظهرت النتائج أن جميع المعاملات المستخدمة أدت إلى زيادة معنوية في عمر أزهار القطف مقارنة بالكنترول . أظهرت النتائج أن المعاملة بتركيزات الزيت المختلفة أعطت أقل نسبة مئوية للفقد في الوزن الخضري في كلا الموسمين . بينما أعطت نتائج المعاملة بمستخلص الزيت بتركيز (٠.٩ مل/ لتر) أعلى معدل امتصاص للماء في كلا الموسمين مما أدى إلى بقاء الأزهار أكثر نضارة وكان محتوي السكريات المختزلة المتحصل عليه من جميع المعاملات أفضل من الكنترول ، على الرغم من أن كلا المعاملين ٨ هيدروكسي كينولين سلفات بتركيز ٢٠٠ ملجم / لتر والمعاملة مستخلص الزيت (٠.٩ مل/ لتر) أدتا إلى زيادة عمر الأزهار إلا أن المعاملة بمستخلص الزيت (٠.٩ مل/ لتر) أعطت ازهار أكثر نضارة .

الكلمات الدالة : عمر الأزهار– أزهار الكلا – ٨ هيدروكسي كينولين سلفات– حامض الستريك – الزيت المستخلص من نبات الفلفل عريض الأوراق .

Response of *Jatropha curcas* Plants to Foliar Applied Ascorbic Acid for Decreasing the Harmful Effect of Cadmium Pollution in The Irrigation Water

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ABSTRACT: The present study was carried-out at Antoniades Research Branch, Horticultural Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons of 2014 and 2015. The aim of this study was to evaluate the effects of irrigation water contaminated with cadmium on *Jatropha curcas* plants grown in sandy soil and the possibility of using ascorbic acid spray treatments to overcome the effects of cadmium pollution. Seedlings of *Jatropha curcas* were planted individually in plastic pots (30 cm diameter) filled with 8 kg of sandy soil. The contaminated irrigation water treatments were four concentrations of cadmium 0,100, 200 and 300 mg/l were applied. The plants were also sprayed with ascorbic acid at concentrations of 0, 250 and 500 mg/l by monthly spraying in both seasons.

The results showed that for vegetative growth parameters there was no significant difference in the interaction between cadmium concentrations and foliar spray by ascorbic acid. While a significant reduction was observed in all parameters after irrigation with cadmium contaminated water and a significant increase in vegetative growth parameters was observed after 500 mg/l ascorbic acid application. For chlorophyll and carbohydrate content, the highest significant value was obtained from plants irrigated with tap water and sprayed with 500 mg/l ascorbic acid while the highest significant amount of cadmium content in leaves, stem and roots was obtained from the treatment 300 mg/l cadmium without application of ascorbic acid.
Key wards: *Jatropha curcas*, cadmium, ascorbic acid.

INTRODUCTION

Planting Jatropha in Egypt started in 2004 (SWERI, 2009). It is still in the experimental stage, but it has been proved that the potential to plant this tree is high in the marginal areas and desert. The planting of this tree has succeeded in Upper Egypt. Nevertheless, the growth and blooming periods in this area is shorter than that in other countries; it produces flowers after 18 months while in other countries it needs three years. The industrial effluents often contain large quantity of toxic heavy metals (Ghavri and Singh, 2010). These metals are non bio-degradable and persistent and can be differentially toxic to microbes (Giller *et al*., 2009), plants (Ghavri *et al*., 2010; Sharma *et al*., 2010), animals (Rainbow, 2007) and human being (Lim and Schoenung, 2010). *Jatropha curcas* L. (Family: Euphorbiaceae) is a potential biodiesel plant, which (Gunaseelan, 2009), can survive harsh environments of semi-arid agro-climatic conditions, wastelands (Mangkoedihardjo and Sunahmadia, 2008) and grows fast with little maintenance. It can reach a height of 3-8 m. Genus Jatropha has 172 species having significant economic importance is native to Central America and distributed in Africa and Asia (Cano- Asseleih *et al*., 1989 and Fairless, 2007). Among the various Jatropha species, *J. curcas, J. glandulifera, J. gossypifolia* (Achten *et al*., 2008), identified as the most suitable oil bearing plant, and has been recommended for plantation on waste land. *Jatropha curcas* L., is a perennial crop with potential such as medicinal and biodiesel

crop recently and is recognized as potential oil seed (Effendi *et al*., 2010; Rafii *et al*., 2012; Shabanimofrad *et al*., 2011). This plant has a great importance as a medicinal plant in treating tropical diseases of dermatological origin (Igbinosa *et al*., 2009). Also the attention on this crop has increased due to high rate of ozone layer depletion and global warming effect caused by increased usage of fossil fuel resulting in environmental pollution. Renewable biofuel feed stocks are perceived to be essential contributors to the energy supply portfolio as they contribute to the world energy supply security, reducing dependency on fossil fuel resources and provide opportunity for mitigating greenhouse gases (Sudhakar and Nalini, 2011). This newly introduced crop, which grows abundantly in wild and abandoned land, has its seed and oil yield unpredictable especially in tropical climate. Favourable environmental conditions that affect its production are yet to be known (Ovando-Medina *et al*., 2011 and Divakara *et al*., 2010). Ginwal *et al*. (2005) reported that Jatropha has adapted itself to wide range of environmental and ecological conditions which suggests that, there exists considerable amount of genetic diversity yet to be detected for potential realization (Rao *et al*., 2008).

Plants need trace amount of heavy metal but their excessive availability may cause plant toxicity (Sharma *et al*., 2006). Phytotoxic concentration of the heavy metals referred in the literature does not always specify the levels (Wua *et al*., 2010). Cadmium is a toxic heavy metal that has an environmental concern (Mahler *et al.,* 1981). There are many sources of environmental cadmium pollution, including fuel combustion, industrial sludges, phosphate fertilizers, and mine tailings (Unhalekhana and Kositanont, 2008). Cadmium can be absorbed by the human body through respiration and consumption, and cadmium then accumulates in the liver and kidney, causing acute and chronic symptoms such as nausea, abdominal pain, diarrhea, kidney dysfunction, and osteomalacia (Simmons *et al*., 2005).

Ascorbic acid is an essential antioxidant in the ascorbate-glutathione pathway, but it also protects enzymes that have prosthetic transition metal ions. Furthermore, it is a cofactor for many enzymes, including those involved in the cell wall synthesis, most notably in the hydroxylation of proline residues (Ishikawa *et al.* 2006). Moreover, alternative oxidase can be induced by H_2O_2 accumulation and, as ascorbate is involved in controlling the intracellular H_2O_2 level, this might provide the means for a concerted interaction to protect the cell against uncontrolled oxidation (Bartoli *et al.* 2006).

In this study *Jatropha curcas* was selected due to its characteristics as non-edible plant which can grow in tropical areas and its commercial viability for the production of biodiesel, therefore the objective of this study is to determine the potential of *Jatropha curcas* in removing heavy metals from the soil affected contaminated irrigation water and to investigate on the ability of Jatropha in removing heavy metals.

MATERIALS AND METHODS

The present study was carried-out at Antoniades Research Branch, Horticultural Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons of 2014 and 2015. The aim of this study was to evaluate the effects of irrigation water contaminated with cadmium on *Jatropha curcas* plants grown in sandy soil, the possibility of using ascorbic acid spray treatments to overcome the effects of cadmium pollution.

On the 15th of February, 2014 and 2015 (in the first and second seasons, respectively) identical seedlings of *Jatropha curcas* (70-80 cm height and 20-25 leaves per plant in average) were planted individually in plastic pots (30 cm diameter) filled with 8 kg of sandy soil. The chemical constituents of the soil were measured as described by Jackson (1958) in Table (1).

Table (1). Chemical analyses of the used sandy soil for the two successive seasons 2014 and 2015.

Season	pH	EC (dSm^{-1})	Soluble cations (meq/l)				Soluble anions (meq/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_2^{--}
2014	7.94	1.57	3.4	3.4	6.5	1.2	3.6	6.7	2.4
2015	7.91	1.52	3.2	3.0	6.3	1.1	3.3	6.5	2.2

On the 1st of March (in both seasons), the contaminated irrigation water treatments were initiated. Four concentrations of cadmium acetate [$(CH_3COO)_2$ $Cd.2H_2O$] 0,100, 200 and 300 mg/l were applied. The plants were irrigated three times per week; at the end of the experiment every plant received about 250 liters per pot of contaminated water in Table (2). In both seasons, the plants were received by monthly spraying from 15th May till 15th August in both seasons. The plants were also sprayed with ascorbic acid at concentrations of 0, 250 and 500 mg/l. Control plants were sprayed with tap water. On 30th of September in the both seasons, the plants were harvested.

Table (2). Total amount of the water used for each plant (l/pot) in each treatment during the growing two season of 2014 and 2015.

Field Capacity (%)	Months of first and second seasons							
	March	**April**	**May**	**June**	**July**	**August**	**September**	**Total**
90 %	28.00	30.00	32.00	35.00	40.00	45.00	40.00	**250.00**

In the two seasons, all plants received NPK chemical fertilization using soluble fertilizer (Kristalon 19-19-19) at the rate of 3 g/ pot. Fertilization was repeated every 30 days throughout the growing season (from the 1th of March till the 30th of September). In addition, weeds were removed manually upon emergence.

Data recorded :

(1) Vegetative growth parameters:

Plant height (cm), number of leaves per plant, leaves dry weight per plant (g), leaves area (cm^2) according to Koller (1972), stem diameter (cm), stem dry weight (g), root length (cm) and root dry weight (g).

(2) Chemical analysis determination:

- Total chlorophylls content was determined as a SPAD from the fresh leaves of plants for the different treatments under the experiment at the end of the season using Minolta (chlorophyll meter) SPAD 502 according to Yadava (1986).
- Total carbohydrates percentage in the leaves was determined according to Dubios *et al.*(1956).
- Determination of Cadmium content. Plant samples were divided into leaves stem and roots. They were then dried at 72˚C in an oven until completely dried. The dried plant samples were ground to powder. The dried samples were then digested for extraction of cadmium, using the method described by Piper (1947) method and the concentration of heavy metal was determined using an atomic absorption spectrophotometer.
- Available heavy metal, i.e. (Cadmium) in soil samples were extracted by DPTA solution according to Lindsay and Norvell (1978) and determined by Inductively Coupled Plasma Spectrometry.
- Transfer factor (TF) is given by the relation: the ratio of the concentration of metal in the shoots to the concentration of metal in the soil (Chen *et al.*, 2004). The transfer factor is a value used in evaluation studies on the impact of routine or accidental releases of pollutant into the environment.

The layout of the experimental design was split plot design with three replicates. Each replicate contained three plants. The main plots were the contaminated irrigation water levels while the sup plots were the concentrations of ascorbic acid. The means of the individual factors and their interactions were compared by L.S.D test at 5% level of probability according to Snedecor and Cochran (1974).

RESULTS

Vegetative growth:

Plant height (cm)

Data presented in Table (3) Showed that, in both seasons, irrigation water contaminated with cadmium decreased the height of *Jatropha curcas* plants, compared to plants irrigated with tap water (control). Plants irrigated with tap water had the highest mean values of plant height 43.49 and 47.33 cm in the first and second seasons, respectively. Moreover, raising the cadmium concentration caused steady significant reductions in plant height, with the highest concentration (300 mg/l) giving significantly the shortest plants (with mean heights of 33.36 and 36.49 cm in the two seasons, respectively) than those receiving any other cadmium concentration.

Plant height was also significantly affected by spraying the plants with ascorbic acid. In both seasons, plant height increased gradually when the ascorbic acid concentration was raised from 0 mg/l (control) to 500 mg/l. Accordingly, it can be seen from the data in Table (3) that *Jatropha curcas* plants sprayed with 500 mg/l ascorbic acid were significantly taller (with mean plant heights of 39.70 and 42.60 cm in the first and second seasons, respectively) than plants sprayed with any other ascorbic acid concentrations.

Regarding the interaction between the effects of irrigation with contaminated cadmium water and ascorbic acid treatments on growth rate of the plant height of *Jatropha curcas* plants, the results recorded in the two seasons show that, the highest values were obtained in the plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l (with mean heights of 44.91 and 48.50 cm in the first and second seasons, respectively). On the other hand, the shortest plants (with mean heights of 30.25 and 33.83 cm in the first and second seasons, respectively) were resulted in when the plants were irrigated using the highest cadmium concentration (300 mg/l) without ascorbic acid treatment. It can also been seen from the data presented in Table (3) that in many cases, spraying the plants with ascorbic acid reduced the undesirable effect of contaminated water with cadmium.

Number of leaves per plant

The data presented in Table (3) show the effect of contaminated water with cadmium on number of leaves formed on *Jatropha curcas* plants. In both seasons, plants irrigated with tap water had the highest number of leaves 21.71 and 19.99 leaves per plant in the first and second seasons, respectively. While, the lowest number of leaves (16.83 and 15.27 leaves per plant in the first and second seasons, respectively), was formed by plants that were irrigated using the highest cadmium concentration (300 mg/l).

Concerning the effect of ascorbic acid treatments on the number of leaves, the data recorded in the two seasons (Table 3) show that only one ascorbic acid treatment 500 mg/l caused a significant increase in the number of leaves giving mean values of 19.95 and 17.91 leaves per plant in the first and second seasons, respectively, compared to that of control plants (17.62 and 16.62 leaves per plant in the two seasons, respectively).

Data in Table (3) show that, significant interaction was detected in both seasons between the effects of irrigation with contaminated cadmium water and ascorbic acid treatments on the number of leaves formed by *Jatropha curcas* plants. Combining irrigation using tap water with spraying the plants with ascorbic acid at 500 mg/l gave the highest number of leaves of 22.33 and 20.50 leaves per plant in the first and second seasons, respectively. On the other hand, the lowest number of leaves of 15.33 and 14.33 leaves per plant in the first and second seasons, respectively, were obtained on plants irrigated using the highest cadmium concentration 300 mg/l and sprayed without any ascorbic acid concentration.

Leaves dry weight (g) per plant

The results recorded in the two seasons Table (3) show that the highest dry weight values of leaves (20.22 and 22.00 g in the first and second seasons, respectively), were obtained from plants irrigated with tap water. Moreover, the recorded values were decreased steadily with raising the cadmium concentration. Accordingly, the lowest values 15.50 and 16.96 g per plant in the first and second seasons, respectively, were obtained from plants irrigated with the highest cadmium concentration 300 mg/l.

Data presented In Table (3) also show that spraying *Jatropha curcas* plants with ascorbic acid at 500 mg/l significantly increased the dry weight of leaves giving values of 18.46 and 19.80 g per plant in the first and second seasons, respectively, compared to the control (16.28 and 18.33 g per plant in the first and second seasons, respectively).

Regarding the interaction between the effects of irrigation with contaminated cadmium water and ascorbic acid treatments on the dry weight of leaves of *Jatropha curcas*, the data presented in Table (3) showed that the highest dry weight values of leaves of (20.88 and 22.55 g in the first and second seasons, respectively), were obtained in plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l, whereas the lowest dry weights of leaves of 14.06 and 15.73 g in the first and second seasons, respectively, were obtained when the plants were irrigated using the highest cadmium concentration 300 mg/l without any ascorbic acid treatment.

Leaves area (cm^2)

The results recorded for both seasons Table (3) show that irrigation with contaminated cadmium water decreased the leaf area of *Jatropha curcas* plants, compared to plants irrigated with tap water (control). In both seasons, plants irrigated with tap water (control) had the largest leaves with mean areas of 1172.80 and 1284.13 cm^2 in the first and second seasons, respectively. The leaf area was decreased steadily with raising the cadmium concentration. Accordingly, the smallest leaves with mean areas of 699.96 and 763.92 cm^2 in the first and second seasons, respectively, were those formed on plants that were irrigated using the highest cadmium concentration 300 mg/l.

Data presented in Table (3) show that, in most cases, the different ascorbic acid treatments had no significant effect on leaf area of *Jatropha curcas* plants. The only exception to this common trend was recorded in the first season, with plants sprayed using ascorbic acid at 500 mg/l forming significantly larger leaves with a mean area of (1021.99 and 1095.17 cm^2 in the first and second seasons, respectively), than those formed by control plants (731.25 and 825.93 cm^2).

Data presented in Table (3) also show that significant interaction was detected between the effects of irrigation with contaminated cadmium water and ascorbic acid treatments on the area of *Jatropha curcas* leaves. The largest leaves with mean areas of 1375.95 and 1499.90 cm2 in the first and second

seasons, respectively, was formed by plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l. On the other hand, the smallest leaves (with areas of 584.20 and 654.73 cm^2 in the first and second seasons, respectively) were obtained on plants irrigated using the highest cadmium concentration 300 mg/l at the lowest concentration 0 mg/l ascorbic acid treatment.

Table (3). Averages of plant height (cm), number of leaves per plant, leaves dry weight (g) and leaves area (cm^2) of *Jatropha curcas* plants as influenced by Cadmium (Cd), Ascorbic acid (AA) and their combinations (Cd× AA) in the two seasons of 2014 and 2015.

Treatments		Plant height (cm)		Number of leaves per plant		Leaves dry weight (g)		Leaves area (cm^2)	
Cd (mg/l)	Ascorbic acid (mg/l)	2014	2015	2014	2015	2014	2015	2014	2015
0	0	42.33	46.50	21.16	19.66	19.68	21.62	941.91	1040.20
	250	43.25	47.00	21.66	19.83	20.10	21.85	1200.54	1312.31
	500	44.91	48.50	22.33	20.50	20.88	22.55	1375.95	1499.90
Average		**43.49**	**47.33**	**21.71**	**19.99**	**20.22**	**22.00**	**1172.80**	**1284.13**
100	0	33.66	40.50	16.83	17.00	15.65	18.83	710.53	859.89
	250	37.16	42.08	18.83	17.50	17.28	19.56	860.86	961.16
	500	39.83	43.83	20.16	18.33	18.52	20.37	994.41	1077.91
Average		**36.88**	**42.13**	**18.60**	**17.61**	**17.15**	**19.58**	**855.26**	**966.32**
200	0	33.91	36.91	17.16	15.50	15.76	17.16	688.38	748.90
	250	36.33	39.75	18.33	16.83	16.89	18.48	803.18	884.10
	500	38.25	40.08	19.33	17.00	17.78	18.63	893.20	932.70
Average		**36.16**	**38.91**	**18.27**	**16.44**	**16.81**	**18.09**	**794.92**	**855.23**
300	0	30.25	33.83	15.33	14.33	14.06	15.73	584.20	654.73
	250	34.00	37.66	17.16	15.66	15.80	17.51	691.30	766.84
	500	35.83	38.00	18.00	15.83	16.66	17.66	824.40	870.20
Average		**33.36**	**36.49**	**16.83**	**15.27**	**15.50**	**16.96**	**699.96**	**763.92**
Mean (AA)	0	35.03	39.43	17.62	16.62	16.28	18.33	731.25	825.93
	250	37.68	41.62	18.99	17.45	17.51	19.35	888.97	981.10
	500	39.70	42.60	19.95	17.91	18.46	19.80	1021.99	1095.17
L.S.D. at 0.05	**Cd**	**1.77**	**1.36**	**0.95**	**1.60**	**0.82**	**0.63**	**44.10**	**36.23**
	AA	**0.81**	**0.86**	**0.41**	**0.45**	**0.37**	**0.40**	**18.25**	**22.40**
	Cd * AA	**1.86**	**1.98**	**0.95**	**1.03**	**0.86**	**0.92**	**42.02**	**51.57**

Stem diameter (cm)

The data recorded for the stem diameter of *Jatropha curcas* plants in the two seasons Table (4) show that irrigation with contaminated cadmium water decreased stem thickness, compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the thickest stems, with mean diameters of 5.28 and 5.75 cm in the first and second seasons, respectively. Raising the cadmium concentration in irrigation water caused a

steady reduction in stem diameter. This reduction in stem diameter was significant (compared to the control), even at the lowest cadmium concentration (300 mg/l), which gave stem diameters of 4.05 and 4.43 cm in the first and second seasons, respectively.

In contrast to the effect of cadmium treatments, ascorbic acid treatments improved stem diameter of *Jatropha curcas* plants, compared to the control. Moreover, plants sprayed with 500 mg/l ascorbic acid had significantly thickest stems (with mean diameters of 4.82 and 5.18 cm in the first and second seasons, respectively), compared to the those of control plants, or plants sprayed with any other ascorbic acid concentration.

Regarding the interaction between the effects of irrigation with contaminated cadmium water and ascorbic acid treatments on growth rate of the stem diameter of *Jatropha curcas* plants, the results recorded for the two seasons (Table 4) show that significant differences were detected between the values obtained from plants receiving the different treatment combinations. The highest values (5.46 and 5.90 cm in the first and second seasons, respectively) were obtained in the plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l. On the other hand, the thinnest stems (with diameters of 3.68 and 4.11 cm in the first and second seasons, respectively) were obtained in the plants irrigated using the highest cadmium concentration 300 mg/l without ascorbic acid treatment. It can also be seen that in some cases, the ascorbic acid treatments helped to overcome the adverse effect of the cadmium treatments on stem thickening.

Stem dry weight (g)

Data presented in Table (4) show that, in both seasons, irrigation using contaminated water with cadmium significantly decreased dry weights of stem of *Jatropha curcas* plants, compared to plants irrigated with tap water (control). Plants irrigated with tap water had the heaviest mean dry weight of stems 37.25 and 41.34 g per plant in the first and second seasons, respectively. The dry weight of stems showed a gradual reduction as the cadmium concentration was increased. Accordingly, the lowest dry weights of stem 30.16 and 33.57 g per plant in the first and second seasons, respectively, were recorded in plants receiving the highest cadmium concentration 300 mg/l.

The results recorded in the two seasons (Table 4) show that, in both seasons, spraying the plants with ascorbic acid increased the dry weight of stem. In both seasons, spraying plants with 500 mg/l ascorbic acid gave the heaviest dry weight of stem 34.09 and 37.47 g per plant in the first and second seasons, respectively. These values were significantly higher than those of control plants, or plants receiving any other ascorbic acid concentration.

Regarding the interaction between the effects of irrigation contaminated water with cadmium and ascorbic acid treatments, the results recorded in the two seasons show that the heaviest stems dry weights of 38.55 and 42.01 g per plant in the first and second seasons, respectively, were those of plants irrigated with tap water and sprayed without ascorbic acid. On the other hand,

the lowest stem dry weights (29.28 and 32.52 g per plant in the first and second seasons, respectively) were obtained in plants irrigated using the highest cadmium concentrations 300 mg/l without ascorbic acid treatment.

Root length (cm)

Data presented in Table (4) show that all the tested treatments of irrigation water contaminated with cadmium significantly decreased the root length (cm) of *Jatropha curcas,* compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the highest mean root length 50.02 and 49.60 cm in the first and second seasons, respectively. Raising the cadmium concentration caused a steady reduction in the root length, which reached its lowest values 38.35 and 40.37 cm in the first and second seasons, respectively, in plants irrigated using the highest cadmium concentration 300 mg/l.

The data in Table (4) also indicate that ascorbic acid treatments had a significant effect on the root length. In both seasons, *Jatropha curcas* plants sprayed with ascorbic acid, compared to the control plants. As with the other vegetative growth parameters, spraying the plants with ascorbic acid at 500 mg/l gave the heaviest root length 45.65 and 45.87 cm in the first and second seasons, respectively.

Regarding the interaction between the effects of irrigation using water contaminated with cadmium and ascorbic acid treatments on root length of *Jatropha curcas* plants, the results recorded in the two seasons showed that, the highest values were obtained in plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l (with mean length of 51.65 and 50.69 cm in the first and second seasons, respectively). On the other hand, the shortest roots (with mean length of 31.78 and 39.57 cm in the first and second seasons, respectively) were those irrigated using the highest cadmium concentration 300 mg/l without ascorbic acid treatment. It can also been seen from the data presented in Table (4) that in many cases, spraying the plants with ascorbic acid reduced the undesirable effect of cadmium.

Root dry weight (g)

Data presented in Table (4) show that irrigation of *Jatropha curcas* plants using water contaminated with cadmium significantly decreased the dry weights of roots, compared to plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the heaviest dry weight of roots 28.73 and 31.67 g per plant in the first and second seasons, respectively. Steady significant reductions in the dry weight of roots were recorded as the cadmium concentration in the irrigation water was increased, with the highest cadmium concentration 300 mg/l giving the lowest mean values in both seasons 22.83 and 25.26 g per plant in the first and second seasons, respectively.

Regarding the effect of ascorbic acid treatments on the dry weight of roots, data in Table (4) show that spraying *Jatropha curcas* plants with ascorbic

acid at 500 mg/l significantly increased the recorded values, compared to the control. The highest weight dry roots 25.59 and 28.13 g per plant in the first and second seasons, respectively, were those of plants sprayed with ascorbic acid at 500 mg/l.

Regarding the interaction between the effects of irrigation using water contaminated with cadmium and ascorbic acid treatments, the data presented in Table (4) show that the highest values (29.71 and 32.28 g per plant in the first and second seasons, respectively) were obtained in plants irrigated with tap water and sprayed without ascorbic acid. On the other hand, the lowest dry weight of roots (21.67 and 24.12 g per plant in the first and second seasons, respectively) were obtained from plants irrigated using the highest cadmium concentration 300 mg/l, with no ascorbic acid treatment.

Table (4). Averages of stem diameter (cm), stem dry weight (g), root length (cm) and root dry weight (g) of *Jatropha curcas* plants as influenced by Cadmium (Cd), Ascorbic acid (AA) and their combinations (Cd × AA) in the two seasons of 2014 and 2015.

reatments		Stem diameter (cm)		Stem dry weight (g)		Root length (cm)		Root dry weight (g)	
Cd (mg/l)	Ascorbic acid(mg/l)	2014	2015	2014	2015	2014	2015	2014	2015
000	0	5.14	5.65	38.55	42.01	48.68	48.77	29.71	32.28
	250	5.26	5.72	36.35	41.08	49.73	49.35	28.23	31.46
	500	5.46	5.90	36.85	40.93	51.65	50.69	28.26	31.27
Average		**5.28**	**5.75**	**37.25**	**41.34**	**50.02**	**49.60**	**28.73**	**31.67**
100	0	4.09	4.92	29.32	32.20	38.71	38.81	23.29	25.88
	250	4.52	5.12	32.10	34.66	42.73	42.73	24.57	27.52
	500	4.84	5.33	33.50	37.27	45.80	44.94	25.31	28.05
Average		**4.48**	**5.12**	**31.64**	**34.71**	**42.41**	**42.16**	**24.39**	**27.15**
200	0	4.12	4.49	26.00	29.30	39.05	35.36	21.89	23.96
	250	4.42	4.83	33.50	35.97	41.77	42.26	25.19	27.21
	500	4.65	4.87	34.77	37.25	43.98	46.09	25.26	27.23
Average		**4.39**	**4.73**	**31.42**	**34.17**	**41.60**	**41.23**	**24.11**	**26.13**
300	0	3.68	4.11	29.28	32.52	34.78	39.57	21.67	24.12
	250	4.13	4.58	29.93	33.75	39.09	39.76	23.29	25.70
	500	4.36	4.62	31.27	34.45	41.20	41.78	23.54	25.98
Average		**4.05**	**4.43**	**30.16**	**33.57**	**38.35**	**40.37**	**22.83**	**25.26**
Mean (AA)	0	4.25	4.79	30.78	34.00	40.30	40.62	24.14	26.56
	250	4.58	5.06	32.97	36.36	43.33	43.52	25.32	27.97
	500	4.82	5.18	34.09	37.47	45.65	45.87	25.59	28.13
L.S.D. at 0.05	**Cd**	**0.21**	**0.16**	**1.45**	**1.61**	**2.05**	**1.56**	**1.10**	**1.08**
	AA	**0.09**	**0.10**	**0.55**	**0.58**	**0.94**	**0.74**	**0.21**	**0.34**
	Cd * AA	**0.21**	**0.23**	**1.28**	**1.34**	**2.17**	**1.72**	**0.50**	**0.78**

Chemical constituents
Total chlorophyll content (SPAD)

The results presented in Table (5) show that the highest content of total chlorophyll was obtained in plant irrigation with tap water 54.52 and 54.73 SPAD in the first and second seasons, respectively. Raising the cadmium concentration in irrigation water resulted in steady significant reductions in the total chlorophyll content, which reached its lowest value 49.46 and 49.89 SPAD in the first and second seasons, respectively, in plants receiving the highest cadmium concentration 300 mg/l.

The results of leaf chemical analysis Table (5) also show that ascorbic acid treatments had clear effect on the total chlorophyll content. The recorded mean values ranged from 53.79 and 53.96 SPAD in the first and second seasons, respectively, in plants sprayed with ascorbic acid at 250 mg/l to 48.92 and 49.43 SPAD in the first and second seasons, respectively, in plants sprayed with ascorbic acid at 0 mg/l.

Regarding to the interaction between the effects of irrigation using water contaminated with cadmium and ascorbic acid treatments, the data presented in Table (5) showed that the highest total chlorophyll contents of 56.19 and 56.29 in the first and second seasons, respectively, were found in leaves of plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l, the lowest values of 46.37 and 47.13 in the first and second seasons, respectively, were obtained in plants irrigated with cadmium water at 100 mg/l and sprayed with tap water.

Carbohydrates content (%)

Data resulting from chemical analysis Table (5) show that, the total carbohydrates % in the dried leaves of *Jatropha curcas* plants was decreased steadily with raising the cadmium concentration in the irrigation contaminated water with cadmium. The highest mean carbohydrates content 20.28 and 20.27 % in the first and second seasons, respectively, was found in the leaves of control plants, whereas the lowest mean value 18.15 and 18.48 % in the first and second seasons, respectively, was found in plants irrigated with water containing the highest cadmium concentration 300 mg/l.

The results in Table (5) also show that most of the tested ascorbic acid concentrations increased the mean total carbohydrates % in the leaves of *Jatropha curcas* plants, compared to the control. Among the plants receiving the different ascorbic acid treatments, plants sprayed with 250 mg/l ascorbic acid had the highest carbohydrate % in leaves 19.55 and 19.83 % in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation using water contaminated with cadmium and ascorbic acid treatments on the carbohydrates content % of leaves. The results presented in Table (5) show that the highest mean values of 20.82 and 20.85 % in the first and second seasons,

respectively, were obtained in the leaves of plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l. On the other hand, the lowest carbohydrates content was obtained in the leaves of plants irrigated with cadmium water at 100 mg/l and receiving no ascorbic acid treatment.

Cadmium content in leaves (mg/kg)

The data resulting from leaves chemical analysis Table (5) show that, the cadmium content (mg/kg) in the dried leaves of *Jatropha curcas* plants was raised steadily with raising the cadmium concentration in the irrigation water. The lowest mean cadmium content 0.002 and 0.003 mg/kg in the first and second seasons, respectively, was found in the leaves of control plants, whereas the highest mean value 0.009 and 0.010 mg/kg in the first and second seasons, respectively, was found in plants irrigated with water containing the highest cadmium concentration 300 mg/l.

Concerning the effect of ascorbic acid treatments on the cadmium content in leaves, the data recorded in the two seasons Table (5) show that only one ascorbic acid treatment 500 mg/l caused a significant decrease in the cadmium content in leaves giving mean values of 0.003 and 0.004 mg/kg in the first and second seasons, respectively, compared to that of control plants had the highest cadmium content in leaves 0.008 and 0.009 mg/kg in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation using water contaminated with cadmium and ascorbic acid treatments on the cadmium content in leaves. The results in Table (5) show that the lowest mean values of 0.002 and 0.002 mg/kg in the first and second seasons, respectively, were obtained in the leaves of plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l. On the other hand, the highest cadmium content was obtained in the leaves of irrigated with cadmium water at 300 mg/l and receiving no ascorbic acid treatment (0.014 and 0.015 mg/kg in the first and second seasons, respectively).

Cadmium content in stem (mg/kg)

The data resulting from stem chemical analysis Table (5) show that, the cadmium content (mg/kg) in the dried stem of *Jatropha curcas* plants was raised steadily with raising the cadmium concentration in the irrigation water. The lowest mean cadmium content 0.007 and 0.009 mg/kg in the first and second seasons, respectively, was found in the stem of control plants, whereas the highest mean value 0.023 and 0.029 mg/kg in the first and second seasons, respectively, was found in plants irrigated with water containing the highest cadmium concentration 300 mg/l.

Concerning the effect of ascorbic acid treatments on the cadmium content in stem, the data recorded in the two seasons Table (5) show that only one ascorbic acid treatment 500 mg/l caused a significant decrease in the cadmium content in stem giving mean values of 0.011 and 0.012 mg/kg in the first and second seasons, respectively, compared to that of control plants had

the highest cadmium content in stem 0.026 and 0.028 mg/kg in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation using water contaminated with cadmium and ascorbic acid treatments on the cadmium content in stem. The results in Table (5) show that the lowest mean values of 0.005 and 0.006 mg/kg in the first and second seasons, respectively, were obtained in the stem of plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l. On the other hand, the highest cadmium content was obtained in the stem of irrigated with cadmium water at 300 mg/l and receiving no ascorbic acid treatment (0.041 and 0.045 mg/kg in the first and second seasons, respectively).

Cadmium content in roots (mg/kg)

The data resulting from roots chemical analysis Table (5) show that, the cadmium content (mg/kg) in the dried roots of *Jatropha curcas* plants was raised steadily with raising the cadmium concentration in the irrigation water. The lowest mean cadmium content 0.011 and 0.013 mg/kg in the first and second seasons, respectively, was found in the roots of control plants, whereas the highest mean value 0.039 and 0.042 mg/kg in the first and second seasons, respectively, was found in plants irrigated with water containing the highest cadmium concentration 300 mg/l.

Concerning the effect of ascorbic acid treatments on the cadmium content in roots, the data recorded in the two seasons Table (5) show that only one ascorbic acid treatment 500 mg/l caused a significant decrease in the cadmium content in roots giving mean values of 0.016 and 0.018 mg/kg in the first and second seasons, respectively, compared to that of control plants had the highest cadmium content in roots 0.038 and 0.041 mg/kg in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation using water contaminated with cadmium and ascorbic acid treatments on the cadmium content in roots. The results in Table (5) show that the lowest mean values of 0.008 and 0.009 mg/kg in the first and second seasons, respectively, were obtained in the roots of plants irrigated with tap water and sprayed with ascorbic acid at 500 mg/l. On the other hand, the highest cadmium content was obtained in the roots of irrigated with cadmium water at 300 mg/l and receiving no ascorbic acid treatment (0.059 and 0.065 mg/kg in the first and second seasons, respectively).

Table (5). Averages of chemical constituents characteristics of *Jatropha curcas* plants as influenced by cadmium (Cd), Ascorbic acid (AA) and their combinations (Cd×AA) in the two seasons of 2014 and 2015.

Treatments		Chlorophyll content (SPAD)		Carbohydrates content (%)		Cadmium content in leaves (mg/kg)		Cadmium content in stem (mg/kg)		Cadmium content in roots (mg/kg)	
Cd (mg/l)	Ascorbic acid (mg/l)	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
000	0	52.87	53.29	19.84	19.75	0.003	0.004	0.010	0.012	0.014	0.017
	250	54.52	54.61	20.20	20.23	0.003	0.003	0.008	0.009	0.012	0.013
	500	56.19	56.29	20.82	20.85	0.002	0.002	0.005	0.006	0.008	0.009
Average		**54.52**	**54.73**	**20.28**	**20.27**	**0.002**	**0.003**	**0.007**	**0.009**	**0.011**	**0.013**
100	0	46.37	47.13	17.18	17.46	0.007	0.007	0.022	0.022	0.032	0.032
	250	55.77	55.86	20.18	20.43	0.007	0.006	0.020	0.020	0.029	0.028
	500	54.00	54.42	19.73	20.14	0.003	0.004	0.010	0.012	0.015	0.017
Average		**52.04**	**52.47**	**19.03**	**19.34**	**0.005**	**0.005**	**0.017**	**0.018**	**0.025**	**0.025**
200	0	49.04	49.13	17.90	18.20	0.011	0.012	0.033	0.035	0.048	0.050
	250	53.36	53.45	19.26	19.45	0.008	0.008	0.024	0.025	0.035	0.036
	500	52.44	52.53	18.67	18.78	0.005	0.006	0.016	0.018	0.023	0.026
Average		**51.61**	**51.70**	**18.61**	**18.81**	**0.008**	**0.008**	**0.024**	**0.026**	**0.035**	**0.037**
300	0	47.42	48.17	17.56	17.84	0.014	0.015	0.041	0.045	0.059	0.065
	250	51.51	51.95	18.57	19.24	0.009	0.010	0.027	0.029	0.038	0.041
	500	49.47	49.57	18.33	18.36	0.005	0.005	0.014	0.015	0.020	0.021
Average		**49.46**	**49.89**	**18.15**	**18.48**	**0.009**	**0.010**	**0.027**	**0.029**	**0.039**	**0.042**
Mean (AA)	0	48.92	49.43	18.12	18.31	0.008	0.009	0.026	0.028	0.038	0.041
	250	53.79	53.96	19.55	19.83	0.006	0.006	0.019	0.020	0.028	0.029
	500	53.02	53.20	19.38	19.53	0.003	0.004	0.011	0.012	0.016	0.018
L.S.D. at 0.05	**Cd**	**0.71**	**0.82**	**0.79**	**0.28**	**0.003**	**0.004**	**0.009**	**0.013**	**0.012**	**0.019**
	AA	**0.84**	**0.81**	**0.43**	**0.35**	**0.002**	**0.003**	**0.005**	**0.008**	**0.007**	**0.012**
	Cd * AA	**1.94**	**1.86**	**0.98**	**0.82**	**0.004**	**0.006**	**0.012**	**0.020**	**0.015**	**0.027**

Transfer factor (TF) of heavy metals

Transfer factor (TF) indicates the efficiency of plants to transfer metals from root to the aerial parts.

Cadmium content in soil samples (mg/kg)

Data in Table (6) showed that the lowest average of cadmium content was observed in soil cultured by untreated plants, while the highest average of cadmium content was observed in soil after the treatment 300 mg/l cadmium and 0 mg/l ascorbic acid.

Table (6). Average of cadmium content in soil samples as influenced by cadmium concentrations in irrigation water and foliar application of citric acid on *Jatropha curcas* leaves at the end of second season (2015).

Treatments		Cadmium content in soil (mg/kg)
Cd (mg/l)	Ascorbic acid (mg/l)	
0	0	0.018
	250	0.014
	500	0.011
100	0	0.024
	250	0.022
	500	0.021
200	0	0.050
	250	0.048
	500	0.046
300	0	0.072
	250	0.068
	500	0.065

Transfer factor to leaves (TFL)

From the data presented in Table (7), it can be seen that the transfer factor in the dried leaves of *Jatropha curcas* plants was decreased steadily with raising the cadmium concentration in the irrigation water. Accordingly, the lowest cadmium value (0.143 in the second season) was found in plants irrigated with water containing cadmium concentration 300 mg/l, whereas the highest value (0.251 in the second season) was found in plants irrigated with water containing cadmium concentration 100 mg/l.

The results in Table (7) also show that the transfer factor in the dried leaves was reduced steadily with raising ascorbic acid concentration. Accordingly, the highest cadmium value (0.240 in the second season) was recorded in the leaves of control plants, whereas plants sprayed with the highest ascorbic acid concentration 500 mg/l had the lowest cadmium value (0.144 in the second season).

Regarding the interaction between effect of irrigation using water contaminated with cadmium and ascorbic acid concentrations on the transfer factor in the dried leaves, the data in Table (7) show that the highest mean values 0.291 in the second season, was obtained in plants irrigated with cadmium water at 100 mg/l and sprayed with tap water, while the lowest mean values 0.320 in the second season, was recorded in plants irrigated with cadmium water at 300 mg/l and sprayed with ascorbic acid at 500 mg/l.

Transfer factor to stem (TFS)

From the data presented in Table (7), it can be seen that the transfer factor in the dried stem of *Jatropha curcas* plants was decreased steadily with raising the cadmium concentration in the irrigation water. Accordingly, the lowest cadmium value (0.726 in the second season) was found in plants irrigated with water containing cadmium concentration 300 mg/l, whereas the highest value (0.798 in the second season) was found in plants irrigated with water containing cadmium concentration 100 mg/l.

The results in Table (7) also show that the transfer factor in the dried stem was reduced steadily with raising ascorbic acid concentration. Accordingly, the highest cadmium value (0.726 in the second season) was recorded in the stem of control plants, whereas plants sprayed with the highest ascorbic acid concentration 500 mg/l had the lowest cadmium value (0.434 in the second season).

Regarding the interaction between effect of irrigation using water contaminated with cadmium and ascorbic acid concentrations on the transfer factor in the dried stem, the data in Table (7) show that the highest mean values 0.916 in the first and second season, was obtained in plants irrigated with cadmium water at 100 mg/l and sprayed with tap water, while the lowest mean values 0.230 in the second season, was recorded in plants irrigated with cadmium water at 300 mg/l and sprayed with ascorbic acid at 500 mg/l.

Transfer factor to roots (TFR)

From the data presented in Table (7), it can be seen that the transfer factor in the dried roots of *Jatropha curcas* plants was decreased steadily with raising the cadmium concentration in the irrigation water. Accordingly, the lowest cadmium value (0.609 in the second season) was found in plants irrigated with water containing cadmium concentration 300 mg/l, whereas the highest value (1.137 in the second season) was found in plants irrigated with water containing cadmium concentration 100 mg/l.

The results in Table (7) also show that the transfer factor in the dried roots was reduced steadily with raising ascorbic acid concentration. Accordingly, the highest cadmium value (1.044 in the second season) was recorded in the roots of control plants, whereas plants sprayed with the highest ascorbic acid concentration 500 mg/l had the lowest cadmium value (0.628 in the second season).

Regarding the interaction between effect of irrigation using water contaminated with cadmium and ascorbic acid concentrations on the transfer factor in the dried roots, the data in Table (7) show that the highest mean values 1.333 in the first and second season, was obtained in plants irrigated with cadmium water at 100 mg/l and sprayed with tap water, while the lowest mean values 0.323 in the second season, was recorded in plants irrigated with cadmium water at 300 mg/l and sprayed with ascorbic acid at 500 mg/l.

Table (7). Averages of transfer factor to leaves, stem and roots of *Jatropha curcas* plants as influenced by cadmium (Cd), ascorbic acid (AA) and their combinations (Cd ×AA) in the two seasons of 2014 and 2015.

Treatments		Transfer factor to leaves (TFL)	Transfer factor to stem (TFS)	Transfer factor to roots (TFR)
Cd (mg/l)	Ascorbic acid (mg/l)	2015	2015	2015
000	0	0.222	0.666	0.944
	250	0.214	0.642	0.928
	500	0.181	0.545	0.818
Average		**0.205**	**0.617**	**0.896**
100	0	0.291	0.916	1.333
	250	0.272	0.909	1.271
	500	0.190	0.571	0.809
Average		**0.251**	**0.798**	**1.137**
200	0	0.240	0.700	1.000
	250	0.166	0.520	0.750
	500	0.130	0.391	0.565
Average		**0.178**	**0.537**	**0.771**
300	0	0.208	0.625	0.902
	250	0.147	0.426	0.602
	500	0.076	0.230	0.323
Average		**0.143**	**0.427**	**0.609**
Mean (AA)	0	0.240	0.726	1.044
	250	0.199	0.624	0.887
	500	0.144	0.434	0.628
L.S.D. at 0.05	**Cd**	**0.029**	**0.085**	**0.121**
	AA	**0.022**	**0.066**	**0.095**
	Cd * AA	**0.051**	**0.152**	**0.218**

DISCUSSION

This study revealed that at high heavy-metal concentrations, the plant height was significantly reduced, and the biomass was decreased. The root growth was more sensitive than other parameters, as roots rapidly absorbed water and had higher accumulations of heavy metal elements. The results presented by this study were in agreement with earlier reports on other plants, such as aquatic plant *wolffia arrhiza* (Piotrowska *et al.*, 2010), barley *Hordeum vulgare* (Tiryakioglu *et al.*, 2006) and *typha angustifolia* (Bah *et al.*, 2011). Other

studies with woody plant reported a higher inhibition of root elongation (Dominguez *et al*., 2009). In particular, Jatropha plants could bioaccumulate and bioconcentrate toxic heavy metals from an aqueous solution (Mohammad *et al*., 2010) and could be used as phytoremediation candidates in some countries (Juwarkar *et al*., 2008; Kumar *et al*., 2008; Jamil *et al*., 2009). Additionally, the plant seedling exhibited a high root/shoot ratio throughout the experiment. An alternative explanation might relate to a strong root system with many roots spread out over the entire soil for survival because root/shoot ratio could reflect plant's response to various environment factors (Otieno *et al.,* 2005; Lukacova Kulikova and Lux, 2010; Li *et al.,* 2010).

The physiological responses, such as the gas exchange rate and photosynthetic function, can be ascribed to the different effects of physico-chemical properties of heavy metals on the integrity and function of the photochemical apparatus of plant seedling fronds, as well as the impact on the chlorophyll concentrations in the leaves. The photosynthesis rate, CO2 assimilation rate, and stomatal conductance in response to cadmium heavy metal have been well documented (Chen *et al*., 2012). The maintenance of an intercellular CO2 concentration is concomitant with the leaf CO2 assimilation rate and reflected photosynthesis function of seedling in the different heavy metal-spiked soils. The chlorophyll and carotenoid contents played a central role in the energy manifestation of green plant. Any significant alteration of their contents possibly resulted in a marked effect on the entire metabolism of the plant (Piotrowska *et al*., 2010). In this study, cadmium resulted in a significant reduction in the chlorophyll contents, possibly due to the inhibition of chlorophyll biosynthesis or a breakdown of pigments and their precursors (Agrawal and Mishra, 2009). cadmium might replace the central Mg from chlorophyll molecules and thereby reduce the photosynthetic light-harvesting ability of plant (Agrawal and Mishra, 2009). In contrast, Car were less sensitive than Chl a and Chl b in response to both cadmium heavy metals, which probably facilitated the maintenance of photosynthetic apparatus against heavy metal stress (Piotrowska *et al.,* 2010). Car stabilized and protected the lipid phase of the thylakoid membrane by serving as the antioxidant to scavenge the free radicals (Polle *et al.,* 1992; Piotrowska *et al*., 2010).

Concerning treatments and the control sample, at a preliminary stage, one should note that the transfer factor of most treatments is lower than one for cadmium; which means that the physiological need of the plant for these elements is rather limited.

Trace elements translocation from roots to shoots via a number of physiological processes, including metal unloading into root xylem cells, long-distance carrying from the xylem to the shoots and metal reabsorption, by leaf mesophyll cells, from the xylem stream. Once the trace metals have been unloaded into the xylem vessels, the metals are carried to the shoots by the transpiration stream (Blaylock and Huang, 2000).

Ascorbic acid is the widely known compound used as an antioxidant and the most effective compound increasing the tolerance of plants to oxidative

stresses. Confirmed the role of ascorbic acid in oxidative stress or scavenging freeoxy-radicals (Smith *et al.,* 1989). In addition, ascorbic acid affects the physiological activities of the plants. Also, there is evidence that the tolerance of plants is correlated with the increased amount of ascorbic acid. The antioxidant defense system in the plant cells includes both enzymatic antioxidants such as Superoxide Dismutase, Catalase, Ascorbate Peroxidase and non-enzymatic antioxidants like ascorbic acid, Glutathione and tocopherol. When plants are subjected to environmental stresses, oxidative damage may result because the balance between the production of Reactive Oxygen Substances and their detoxification by the antioxidative system is altered (Gomez *et al*., 1999). Tolerance to damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify Reactive Oxygen Substances (Foyer *et al*., 1994). Taking all these observations together, it may be suggested as a hypothetical framework that Cd induces a transient loss in antioxidative capacity perhaps accompanied by a stimulation of oxidant producing enzymes, which results in intrinsic ascorbic acid accumulation. ascorbic acid then would act as a signalling molecule triggering secondary defences.

CONCLUSIONS

The concentrations of heavy metals increase in the environment from year to year**.** Therefore decontamination of heavy metal-contaminated water and soils is very important for maintenance of environmental health and ecological restoration. Phytoremediation is a new cleanup concept that involves the use of plants to clean or stabilize contaminated environments. Phytoremediation of metals is the most effective plant-based method to remove pollutants from contaminated areas. This green technology can be applied to remediate the polluted soils without creating any destructive effect of soil structure. Some specific plants, such as woody species, have been proven to have noticeable potential to absorb toxic heavy metals.

Phytoremediation of contaminated water and soil with heavy metals using non-edible plant like *Jatropha curcas* offers an environmental friendly and cost-effective method for remediating the polluted soil. The *Jatropha curcas* was found to be able to efficiently remove the heavy metals such as cadmium.

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الملخص العربي

إستجابة نباتات الجاتروفا للرش بحمض الأسكوربيك لتخفيض الأثر الضار للتلوث بالكادميوم في ماء الري

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أجريت هذه الدراسة في فرع البحوث بأنطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية – الإسكندرية ، مصر خلال الموسمين المتتاليين ٢٠١٤ و٢٠١٥. وكان الهدف من هذه الدراسة تقييم آثار مياه الري الملوثة بالكادميوم على نباتات الجاتروفا المزروعة في تربة رملية ، كذلك إمكانية استخدام الرش بحمض الاسكوربيك للتغلب على الآثار الضارة للكادميوم. زرعت شتلات الجاتروفا بشكل فردي في أوعية بلاستيكية (قطرها٣٠ سم) مملوءة ٨ كجم من التربة الرملية. وكانت معاملات مياه الري الملوثة بأربعة تراكيزات من الكادميوم وهى صفر ، ١٠٠، ٢٠٠ ، ٣٠٠ مجم/لتر. تم رش النباتات أيضا بحامض الاسكوربيك في ثلاث تركيزات هى صفر،٢٥٠و٥٠٠ مجم/لتر عن طريق الرش شهريا في كلا الموسمين.

أظهرت النتائج أن هناك اختلاف كبير في التفاعل بين تركيزات الكادميوم ورش النباتات بحامض الاسكوربيك. وقد لوحظ انخفاض كبير في كافة معاملات الري بالماء الملوث بالكادميوم وكذلك لوحظ زيادة كبيرة فى معدلات النموالخضرى بعد الرش ٥٠٠ مجم/لتر حمض الاسكوربيك. تم الحصول على أعلى قيمة من محتوى الكلوروفيل والكربوهيدرات من النباتات المروية بماء الصنبور والرش بتركيز ٥٠٠ مجم/لتر حامض الاسكوربيك في حين أن أعلى كمية كبيرة من محتوى الكادميوم في الأوراق والساق والجذور من خلال الرى بماء ملوث بتركيز ٣٠٠ مجم/لتر الكادميوم دون الرش حمض الاسكوربيك.

المـــراجـــع الأجنبية :

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Analysis of the Agricultural Economic Policy of Sunflower Crop in Egypt

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ABSTRACT: The objective of this study is to examine and analyze the agricultural economic policy adopted by the Government of Egypt to producing and marketing the sunflower crop during the period 2000-2013. The study utilized the policy analysis matrix as the method of analysis. The results indicated to: (1) Farmers are found to receive subsidies, for both of the inputs and final output. This is shown by the nominal protection coefficient of the inputs (NPCI) and outputs (NPCO) of 0.94 and 1.03, respectively. The effective protection coefficient (EPC) of 1.04 ascertains the existence of price subsidization to the sunflower seed in favor of the producers. (2) Egypt possesses a comparative advantage in the production of sunflower seeds. This is indicated to by the value of the domestic resources cost coefficient (DRC) of 0.66, which reflects the comparative advantage of Egypt enjoys in the utilization of local inputs to produce and export sunflower. (3) The profitability coefficient (PC) per feddan of 0.95 reveals that the profits realized from producing one feddan of the sunflower seeds under existing policies represents about 5% Less than the profits realized of its importation; i.e., it points to the failure of the adopted sunflower-seed production policies. Accordingly, the study recommends seeking a significant motivation to farmers to grow sunflower to benefit from its comparative advantage which Egypt enjoys.

Keywords: Policy Analysis Matrix, Nominal Protection Coefficients, Domestic Resources Cost Coefficient, Profitability Coefficient, Sunflower Seed.

الـتـــوصـيـــات:

إتساقاً لما تم التوصل إليه من نتائج مصفوفة تحليل السياسة لمحصول عباد الشمس في مصر خلال متوسط الفترة ٢٠٠٠ – ٢٠١٣ يوصي البحث بضرورة الاستمرار في تقديم الدعم للمزارعين في صورة عينية بشرط قيامهم بإتباع السياسات والتشريعات والقوانين والممارسات الزراعية الجيدة **Good Agricultural Practices (GAP)** كإستخدام التقاوي المحسنة عالية الإنتاجية والجودة واستخدام الميكنة الزراعية والمكافحة المتكاملة ومعاملات ما بعد الحصـاد ، الزراعـة التعاقديـة لـربط المـزارعين بالأسـواق المحليـة والخارجيـة وجمعيـاتهم التعاونيـة، المصـنعين، المصدرين، التجار بما يزيد من القدرة التنافسية الزراعية ويرفع من نسب التصنيع والتصدير مع خلق قيمة مضافة وفرص عمل منتجة، وبما ينعكس في زيادة الإنتاجية والجودة الزراعية وتحسين دخول ومستوى معيشة المزارعين وتحقيق التنمية الزراعية المستدامة والحفاظ على الأراضي الزراعية والبيئة وتقليل الهجرة من الريف.

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٢- معامل الحماية الإسمي للمنتجات: بلغ نحو ١,٠٣ خلال متوسط الفترة ٢٠٠٠-٢٠١٣ ، أي قيمته أكبر من الواحد الصحيح بما يعني أن السعر المحلي لطن بذور عباد الشمس أعلى من نظيره العالمي، وهذا يعني أن الدولة تقدم دعماً يتمتع به المزارع يقدر بنحو ٣% من قيمة الناتج بالسعر العالمي.

٣ - معامل الحماية الفعال: بلغ نحو ١,٠٤ خلال متوسط الفترة ٢٠٠٠-٢٠١٣ ، أي قيمته أكبر من الواحد الصحيح بما يشير إلى تحقق حماية موجبة لصالح المنتجين، بما يعني أن القيمة المضافة للمحصول بالأسعار المحلية تزيد عن نظيرتها المقيمة بالأسعار العالمية مما يؤكد على أن هناك دعماً لأسعار بذرة عباد الشمس بلغ نحو ٤% لصالح المنتجيين الزراعيين بما يعكس أثر صافي موجب للسياسة الاقتصادية الزراعية لمحصول عباد الشمس في مصر على كلاً من أسواق مستلزمات الإنتاج والمنتج النهائي.

٤- معامل تكلفة الموارد المحلية (معامل الميزة النسبية): بلغ نحو ٠,٦٦ خلال متوسط الفترة ٢٠٠٠-٢٠١٣ ، وأن قيمته أقل من الواحد الصحيح مما يعني تمتع الدولة بميزة نسبية في إنتاج بذور عباد الشمس ، وأن التكاليف اللازمة لإضافة ما قيمته جنيه واحد تمثل حوالي ٠,٦٦ جنيه /فدان خلال فترة الدراسة، أي وجود كفاءة في إستخدام الموارد الإنتاجية الطبيعية المتاحة لإنتاج بذور عباد الشمس مما يدعم **أثر التحويل Diversion Effect** للموارد في إنتاج هذا المحصول، وهذه الميزة تؤكد أهمية التوسع في زراعة هذا المحصول لزيادة الإنتاج الكلي منه لتغطية حاجة الاستهلاك المحلي من الزيوت وذلك مع الإستمرار في تقديم الدعم للمنتجين. وفي نفس الوقت تؤدي إلى تقلص حجم وقيمة الواردات من بذور عباد الشمس، ثم بعد ذلك سيكون هناك فائض من الإنتاج بعد الاستهلاك المحلي يتم توجيهه للتصدير للإستفادة من الميزة النسبية التي يتمتع بها محصول عباد الشمس في التجارة الخارجية.

٥- معامل الربحية: بلغ نحو ٠.٩٥ خلال متوسط الفترة ٢٠٠٠-٢٠١٣ ، أي أقل من الواحد الصحيح أي أن صافي عائد الفدان (الربح) من عباد الشمس بالسعر المحلي أقل من نظيره بالسعر العالمي ، حيث أن الربح المتحقق لإنتاج فدان من بذور عباد الشمس في ظل السياسة المتبعة حالياً يمثل نحو ٥% أقل عن الربح المتحقق من إستيراده، أي أنه يشير إلى فشل السياسة الزراعية المتبعة في إنتاج بذور عباد الشمس.

جدول (٧). نتائج معاملات مصفوفة تحليل السياسات لمحصول عباد الشمس خلال الفترة (٢٠٠٠-٢٠١٣).

م	معاملات مصفوفة تحليل السياسة	القيمة
١	**معامل الحماية الأسمي للمدخلات (NPCI)** Nominal Protection Coefficient of the Inputs	٠,٩٤
٢	**معامل الحماية الآسمي للمخرجات (NPCO)** Nominal Protection Coefficient of Outputs	١,٠٣
٣	**معامل الحماية الفعال(القيمة المضافة) (EPC)** Effective Protection Coefficient	١,٠٤
٤	**معامل تكلفة الموارد المحلية (الميزة النسبية) (DRC)** Domestic Resources Cost Coefficient	٠٦٦
٥	**معامل الربحية (PC)** Profitability Coefficient	٠.٩٥

المصدر: جُمعت وحُسبت من البيانات الواردة بالجدول رقم (٦).

ثالثاً: نتائج مصفوفة تحليل السياسة لمحصول عباد الشمس في مصر

تبين من خلال النتائج المتحصل عليها من مصفوفة تحليل السياسة لمحصول عباد الشمس خلال متوسط الفترة ٢٠٠٠-٢٠١٣ ،والواردة بالجدول رقم(٦) أن الأربحية الخاصة لمزارعي محصول عباد الشمس باستخدام الأسعار المحلية بلغت حوالي ٥٦٣,٢٦ جنيه/فدان، كما بلغت الأربحية الاجتماعية باستخدام الأسعار العالمية حوالي ٥٩٥,٨٤ جنيه/فدان، مما يعطي دلالة على فشل السياسة الاقتصادية الزراعية المتبعة وعدم تحقيق المنتجين مكاسب إضافية واستخدام الموارد المتاحة غير كفء.

كما تبين من النتائج المتحصل عليها من مصفوفة تحليل السياسة لمحصول عباد الشمس خلال متوسط الفترة ٢٠٠٠-٢٠١٣ ، والواردة بالجدول رقم(٦) مدى التدخل الحكومي حيث يتضح أن هناك أثر إيجاباً للسياسة الاقتصادية الزراعية المتبعة علي إجمالي العوائد إذ بلغ حوالي ٥٥,٠٩ جنيه / فدان ، في حين تبين أن هناك تأثيراً سلبياً للمدخلات التجارية بلغ حوالي ٢٠,٦٥ جنيه / فدان مما يعني أن الحكومة تقدم دعم للمنتجين كحافز للاستمرار في الإنتاج ، كما تبين وجود تأثير إيجابياً على الموارد المحلية الطبيعية إذ بلغ حوالي ١٠٨,٣١ جنيه / فدان ، وهذا يعني أن السياسة الزراعية المتبعة تتسم بالكفاءة في استخدام الموارد المحلية في إنتاج محصول عباد الشمس.

وأخيراً بلغ مؤشر الخسارة حوالي **٣٢,٥٩** جنيه / فدان مما يعطي دلالة على فشل السياسة المتبعة في إنتاج وتسويق محصول عباد الشمس في مصر خلال متوسط الفترة ٢٠٠٠-٢٠١٣.

جدول (٦). نتائج مكونات مصفوفة تحليل السياسة للإيرادات والتكاليف الفدانية لمحصول عباد الشمس بالجنيه خلال متوسط الفترة (٢٠٠٠-٢٠١٣)

	إجمالي العائد	التكاليف			صافي العائد
		المستلزمات	الموارد المحلية		
			العمل	الأرض	
الأسعار المحلية	٢١٣٩,٢٥	٣٣١,٩٣	٥٩٣,٦٤	٦٥٠,٤٢	٥٦٣,٢٦
الأسعار العالمية	٢٠٨٤,١٦	٣٥٢,٥٨	٤٨٥,٣٣	٦٥٠,٤٢	٥٩٥,٨٤
أثر السياسة	٥٥,٠٩	(٢٠,٦٥)	١٠٨,٣١	صفر	(٣٢,٥٩)

( ): القيم بين القوسين تُعبر عن قيمة سالبة. المصدر: جُمعت وحُسبت من البيانات الواردة بالجداول أرقام (٤)، (٥).

رابعاً: معاملات مصفوفة تحليل السياسة لمحصول عباد الشمس في مصر

تبين من خلال إشتقاق عدد من المعاملات الهامة من خلال مصفوفة تحليل السياسة لمحصول عباد الشمس في مصر خلال الفترة ٢٠٠٠-٢٠١٣، والواردة بالجدول رقم(٧) ما يلي:

١- **معامل الحماية الإسمي للمستلزمات:** بلغ نحو ٠,٩٤ خلال متوسط الفترة ٢٠٠٠ -٢٠١٣ ، أي أن قيمته أقل من الواحد الصحيح ، مما يعني أن تكلفة المدخلات بالأسعار المحلية والتي يدفعها منتجي عباد الشمس في مصر أقل من نظيرتها بالأسعار العالمية ، مما يؤكد على أن الدولة تقدم دعم للمزارع على مستلزمات إنتاج بذور عباد الشمس، وقدرت نسبة هذا الدعم بنحو٦%من قيمة المستلزمات بالسعر العالمي.

جدول(٥). الأسعار العالمية لبنود التكاليف الإنتاجية لمحصول عباد الشمس بالجنيه /فدان خلال الفترة (٢٠٠٠-٢٠١٣)

البيان	مستلزمات الإنتاج							الموارد المحلية			جملة التكاليف بالإيجار	سعر الحدود جنيه/طن	إجمالي العائد عند سعر الحدود	صافي العائد
	التقاوي	السماد البلدي	السماد الكيماوي	مصاريف عمومية	إجمالي مستلزمات الإنتاج	قيمة العمل البشري	قيمة العمل الآلي	جملة عنصر العمل	إيجار الأرض					
٢٠٠٠	٤٢	-	١٠٤,٩	٤٨,٣	١٩٥,٢٤	١٦٥,١٥٥	١٧١,١	٣٣٦,٢٠٥	٣٨٧,٦		٩١٩,٠٤٥	٤٤٨,٧٩	٤٣٩,٨١	(٤٧٩,٢٣)
٢٠٠١	٤٢	-	٩٣,١٧	٤٦,٦	١٨١,٧٧	١٦٩,٣٧٦	١٥٤,٢	٣٢٣,٥٩٦	٣٨٩,٣		٨٩٤,٦٧	٧٥١,٢٧	٧١٣,٧١	(١٨٠,٩٦)
٢٠٠٢	٣٧,٨	٣	١٠١,٢	٤٠	١٨٢	١٥٠,٠٨	١٠٢,٣	٢٥٢,٣٨	٣٣٧		٧٧١,٣٨	٣٧٠,٤٠	٣٥١,٨٨	(٤١٩,٥٠)
٢٠٠٣	٣١,٥	٣٩	١١٢,٢	٥١	٢٣٣,٧	١٧٦,٨٨	١٤١,٩	٣١٨,٧٨	٣٤٦		٨٩٨,٤٨	٥٦٧,٤١	٥٥٦,٠٦	(٣٤٢,٤٢)
٢٠٠٤	٥٣,٥٥	٢٧	١٥١,٨	٧٣	٣٠٥,٣٥	٢٠١	١٧٨,٢	٣٧٩,٢	٣٤٩		١٠٣٣,٥٥	٤٤٢,٢٦	٤٢٨,٩٩	(٦٠٤,٥٦)
٢٠٠٥	٥٤,٦	٣١	١٥٩,٥	٦٦	٣١١,١	٢٢٦,٤٦	١٨٩,٢	٤١٥,٦٦	٣٤٠		١٠٦٦,٧٦	٦٩٤,٦٢	٦٧٣,٧٨	(٣٩٢,٩٨)
٢٠٠٦	٦١,٩٥	٥	١٧٦	٦٢	٣٠٤,٩٥	٢٥٧,٩٥	١٨٢,٦	٤٤٠,٥٥	٣٦٢		١١٠٧,٥	٣٧٥,٩٤	٣٧٥,٩٤	(٧٣١,٥٦)
٢٠٠٧	٧١,٤	٢	٢٤٣,١	٦٠	٣٧٦,٥	٢٥٩,٩٦	٢٠٠,٢	٤٦٠,١٦	٣٠٤		١١٤٠,٦٦	١٣٤٥,٥٦	١٣٧٢,٤٧	٢٣١,٨١
٢٠٠٨	٧٣,٥	-	٢٥٤,١	٧٩	٤٠٦,٦	٢٧٣,٣٦	٢٥١,٩	٥٢٥,٢٦	١٠١٩		١٩٥٠,٨٦	٢٥١٤,٣٥	٢٦٦٥,٢١	٧١٤,٣٥
٢٠٠٩	٧٧,٧	-	٢٢١,١	٧٥	٣٧٣,٨	٢٨٥,٤٢	٢٥٣	٥٣٨,٤٢	١٠١٧		١٩٢٩,٢٢	٧١٣,٥٣	٧١٣,٥٣	(١٢١٥,٦٩)
٢٠١٠	٨٦,١	٣٦	٢٣٨,٧	٩٠	٤٥٠,٨	٣٤٣,٠٤	٢٧٧,٢	٦٢٠,٢٤	١٠٢٥		٢٠٩٦,٠٤	١٩٦٧,٩٦	٢٠٤٦,٦٨	(٤٩,٣٦)
٢٠١١	١٠٣,٩٥	٤٩	٢٤٤,٢	٩١	٤٨٨,١٥	٣٥٥,١	٢٩٠,٤	٦٤٥,٥	١٠٢٢		٢١٥٥,٦٥	٥٣٩٦,٩٤	٥٦٦٦,٧٩	٣٥١١,١٤
٢٠١٢	١٢٠,٧٥	٢٦	٢٦٩,٥	١١٥	٥٣١,٢٥	٣٨٧,٢٦	٣٤٦,٥	٧٣٣,٧٦	١١١٠		٢٣٧٥,٠١	٥١٠٤,٠٥	٥٧٦٧,٥٨	٣٣٩٢,٥٧
٢٠١٣	١٣٥,٤٥	٤٢	٢٩٠,٤	١٢٧	٥٩٤,٨٥	٤٠٦,٦٩	٣٩٨,٢	٨٠٤,٨٩	١٠٩٨		٢٤٩٧,٧٤	٥٨٧٧,٦٥	٧٤٠٥,٨٤	٤٩٠٨,١٠
المتوسط	**٧٠,٩**	**٢٦**	**١٩٠**	**٧٣,١**	**٣٥٢,٥٨**	**٢٦١,٢٧**	**٢٢٤,١**	**٤٨٥,٣**	**٦٥٠,٤**		**١٤٨٨,٣٣**	**١٨٩٧,٩١**	**٢٠٨٤,١٦**	**٥٩٥,٨٤**

- معاملات التحويل: ٠,٦٧ للعمل البشرى ، ١,١ للعمل الآلى ، ١,١٥٩ للعمل الحيواني ، ١,٠٥ للتقاوي ، ١,١ للأسمدة الكيماوية ، ١,٢ للمبيدات، أما باقي البنود فكان معامل تحويلها =١.
- سعر الحدود = (سعر فوب – تكاليف الشحن) × سعر الصرف.
- إجمالي العائد عند سعر الحدود = الإنتاجية الفدانية × سعر الحدود.

( ): القيم بين القوسين تُعبر عن صافي العائد السالب.

المصدر: جُمعت وحُسبت من:

- البيانات الواردة بالجداول أرقام (٣)، (٤).
- الجهاز المركزي للتعبئة العامة والإحصاء، قاعدة بيانات التجارة الخارجية " مصر إنترنت"، بيانات غير منشورة.
- الموقع الإلكتروني، للأمم المتحدة، قاعدة بيانات التجارة الخارجية، بيانات منشورة comtrade.un.org/data.

جدول (٤). الأسعار المحلية لتكاليف إنتاج فدان محصول عباد الشمس بالجنيه / فدان وفقاً لبنود التكاليف خلال الفترة (٢٠٠٠-٢٠١٣)

البيان	مستلزمات الإنتاج							الموارد المحلية			جملة التكاليف بالإيجار	إجمالي العائد	صافي العائد
	التقاوي	السماد البلدي	السماد الكيماوي	مصاريف عمومية	إجمالي مستلزمات الإنتاج	قيمة العمل البشري	قيمة العمل الآلي	جملة عنصر العمل	إيجار الأرض				
٢٠٠٠	٤٠	-	٩٥,٤	٤٨,٣	١٨٣,٧	٢٤٦,٥	١٥٥,٥	٤٠٢	٣٨٧,٦		٩٧٣,٣	٨٥١,٤٠	(١٢١,٩٠)
٢٠٠١	٤٠	-	٨٤,٧	٤٦,٦	١٧١,٣	٢٥٢,٨	١٤٠,٢	٣٩٣	٣٨٩,٣		٩٥٣,٦	٨٥٦,٨٠	(٩٦,٨٠)
٢٠٠٢	٣٦	٣	٩٢	٤٠	١٧١	٢٢٤	٩٣	٣١٧	٣٣٧		٨٢٥	١٠٣٣,٨٥	٢٠٨,٨٥
٢٠٠٣	٣٠	٣٩	١٠٢	٥١	٢٢٢	٢٦٤	١٢٩	٣٩٣	٣٤٦		٩٦١	١٦٦٩,٤٥	٧٠٨,٤٥
٢٠٠٤	٥١	٢٧	١٣٨	٧٣	٢٨٩	٣٠٠	١٦٢	٤٦٢	٣٤٩		١١٠٠	١٧٧٦,٦٥	٦٧٦,٦٥
٢٠٠٥	٥٢	٣١	١٤٥	٦٦	٢٩٤	٣٣٨	١٧٢	٥١٠	٣٤٠		١١٤٤	١٧٨٠,٤٣	٦٣٦,٤٣
٢٠٠٦	٥٩	٥	١٦٠	٦٢	٢٨٦	٣٨٥	١٦٦	٥٥١	٣٦٢		١١٩٩	١٨٤٤,٨٦	٦٤٥,٨٦
٢٠٠٧	٦٨	٢	٢٢١	٦٠	٣٥١	٣٨٨	١٨٢	٥٧٠	٣٠٤		١٢٢٥	١٩٢١,٢٢	٦٩٦,٢٢
٢٠٠٨	٧٠	-	٢٣١	٧٩	٣٨٠	٤٠٨	٢٢٩	٦٣٧	١٠١٩		٢٠٣٦	٢٢٠٤,٨٤	١٦٨,٨٤
٢٠٠٩	٧٤	-	٢٠١	٧٥	٣٥٠	٤٢٦	٢٣٠	٦٥٦	١٠١٧		٢٠٢٣	٢٧١٩,١٣	٦٩٦,١٣
٢٠١٠	٨٢	٣٦	٢١٧	٩٠	٤٢٥	٥١٢	٢٥٢	٧٦٤	١٠٢٥		٢٢١٤	٢٩٠١,٢٥	٦٨٧,٢٥
٢٠١١	٩٩	٤٩	٢٢٢	٩١	٤٦١	٥٣٠	٢٦٤	٧٩٤	١٠٢٢		٢٢٧٧	٢٨٩٢,٥٥	٦١٥,٥٥
٢٠١٢	١١٥	٢٦	٢٤٥	١١٥	٥٠١	٥٧٨	٣١٥	٨٩٣	١١١٠		٢٥٠٤	٣٦٢٦,٧٣	١١٢٢,٧٣
٢٠١٣	١٢٩	٤٢	٢٦٤	١٢٧	٥٦٢	٦٠٧	٣٦٢	٩٦٩	١٠٩٨		٢٦٢٩	٣٨٧٠,٢٩	١٢٤١,٢٩
المتوسط	**٦٧,٥**	**٢٦,٠**	**١٧٢,٧**	**٧٣,١**	**٣٣١,٩٣**	**٣٩٠,٠**	**٢٠٣,٧**	**٥٩٣,٦٤**	**٦٥٠,٤**		**١٥٧٦,٠**	**٢١٣٩,٢٥**	**٥٦٣,٢٥**

( ): القيم بين القوسين تُعبر عن صافي العائد السالب.

المصدر: جُمعت وحُسبت من وزارة الزراعة واستصلاح الأراضي، قطاع الشئون الاقتصادية، الإدارة المركزية للاقتصاد الزراعي، نشرة الاقتصاد الزراعي القاهرة، أعداد مختلفة.

الشمس بالدرجة الأولى خاصة في الأراضي الجديدة، مع التركيز على استخدام البذور المحسنة ذات الإنتاجية العالية، وتشجيع المزارعين على إنتاج عباد الشمس وذلك بدعم مستلزمات الإنتاج الزراعي من خلال إعادة الاهتمام بدور الجمعيات الزراعية والبنك الزراعي.

ثانياً: التكاليف والإيرادات الفدانية بالأسعار المحلية والعالمية لمحصول عباد الشمس في مصر

تبين من خلال البيانات الواردة بجدولي رقم (٤)،(٥) التكاليف والإيرادات الفدانية بالأسعار المحلية والعالمية لمحصول عباد الشمس في مصر خلال الفترة ٢٠٠٠-٢٠١٣ ما يلي:

١- **مستلزمات الإنتاج:** حيث بلغ متوسط قيمة التقاوي بالأسعار المحلية حوالي ٦٧,٥ جنيه /فدان ، في حين بلغ متوسط قيمتها بالأسعار العالمية حوالي ٧٠,٩ جنيه/فدان، وبلغ متوسط القيمة للسماد البلدي بالأسعار المحلية حوالي ٢٦ جنيه/فدان ، في حين بلغ متوسط قيمته بالأسعار العالمية حوالي ٢٦ جنيه/فدان، وبلغ متوسط القيمة للسماد الكيماوي بالأسعار المحلية حوالي ١٧٢,٧ جنيه /فدان ، في حين بلغ متوسط قيمته بالأسعار العالمية حوالي ١٩٠ جنيه/فدان، بلغ متوسط القيمة للمصاريف العمومية بالأسعار المحلية حوالي ٧٣,١جنيه /فدان، في حين بلغ متوسط قيمتها بالأسعار العالمية حوالي ٧٣,١ جنيه/فدان، بلغ متوسط القيمة لجملة مستلزمات الإنتاج بالأسعار المحلية حوالي ٣٣١,٩٣ جنيه /فدان، في حين بلغ متوسط قيمتها بالأسعار العالمية حوالي ٣٥٢,٥٨ جنيه/فدان.

٢- **قيمة الموارد المحلية:** حيث بلغ متوسط القيمة للعمل البشري بالأسعار المحلية حوالي ٣٩٠ جنيه /فدان ، في حين بلغ متوسط قيمته بالأسعار العالمية حوالي ٢٦١,٢٧جنيه/فدان، وبلغ متوسط القيمة للعمل الآلي بالأسعار المحلية ٢٠٣,٧جنيه/فدان، في حين بلغ متوسط قيمته بالأسعار العالمية حوالي ٢٢٤,١جنيه/فدان، وبلغ متوسط القيمة لجملة عنصر العمل بالأسعار المحلية حوالي ٥٩٣,٦٤ جنية /فدان ، في حين بلغ متوسط قيمته بالأسعار العالمية حوالي ٤٨٥,٣جنيه/فدان، بلغ متوسط القيمة لإيجار الأرض الزراعية بالأسعار المحلية حوالي ٦٥٠,٤ جنيه /فدان، في حين بلغ متوسط قيمتها بالأسعار العالمية حوالي ٦٥٠,٤ جنيه/فدان.

٣- **جملة العائد الفداني:** حيث بلغ متوسط القيمة لجملة العائد الفداني بالأسعار المحلية بلغت حوالي ٢١٣٩,٢٥ جنيه/فدان ، في حين بلغ متوسط قيمته بالأسعار العالمية حوالي ٢٠٨٤,١٦جنيه/فدان.

النتائج والمناقشات

أولاً: الموقف الراهن للإنتاج المحلي لمحصول عباد الشمس في مصر

تبين من خلال البيانات الواردة بالجدول رقم (٣) أن مساحة محصول عباد الشمس تراوحت بين حد أدنى بلغ حوالي ١٥,١٦ ألف فدان عام ٢٠١٣، وحد أقصى بلغ حوالي ٤٥,٤٨ ألف فدان عام ٢٠٠٤، بمتوسط سنوي بلغ حوالي ٣٠,٠٧ ألف فدان ، كما تبين أن الإنتاجية تراوحت بين حد أدنى بلغ ٠,٩٥ طن/ فدان عام ٢٠٠٢، وحد أقصى بلغ حوالي ١,٢٦ طن/فدان عام ٢٠١٣، بمتوسط سنوي بلغ حوالي ١,٠٣ طن/فدان ، أما بالنسبة لإجمالي إنتاج محصول عباد الشمس فقد تراوح بين حد أدنى بلغ حوالي ١٨,٣٢ ألف طن عام ٢٠١١ ، وحد أقصى بلغ حوالي ٤٤,٣ ألف طن عام ٢٠٠٤، بمتوسط سنوي بلغ حوالي ٣٠,٢٩ ألف طن خلال الفترة ٢٠٠٠–٢٠١٣.

جدول (٣). المساحة والإنتاجية والإنتاج لمحصول عباد الشمس في مصر خلال الفترة (٢٠٠٠ – ٢٠١٣)

السنة	المساحة (ألف فدان)	الإنتاجية (طن/ فدان)	الإنتاج (ألف طن)
٢٠٠٠	٢٧,٩٨	٠,٩٨	٢٧,٥٢
٢٠٠١	٤٤,١٤	٠,٩٥	٤٢,٠٨
٢٠٠٢	٣٣,٧٩	٠,٩٥	٣٢,٠٩
٢٠٠٣	٣٢,٣٧	٠,٩٨	٣١,٥٩
٢٠٠٤	٤٥,٤٨	٠,٩٧	٤٤,٣
٢٠٠٥	٢٩,٩	٠,٩٧	٢٨,٩٩
٢٠٠٦	٣٥,٦٥	١	٣٥,٧٧
٢٠٠٧	٢٧,١٨	١,٠٢	٢٧,٦٣
٢٠٠٨	١٩,٢٣	١,٠٦	٢٠,٣٩
٢٠٠٩	٣٩,٦٥	١	٣٩,٥٧
٢٠١٠	٣٥,٢٦	١,٠٤	٣٦,٨٢
٢٠١١	١٧,٥٤	١,٠٥	١٨,٣٢
٢٠١٢	١٧,٧١	١,١٣	١٩,٩٩
٢٠١٣	١٥,١٦	١,٢٦	١٩,٠٤
المتوسط	**٣٠,٠٧**	**١,٠٣**	**٣٠,٢٩**
معدل النمو%	**(٥,٦)****	**١,٥****	**(٤,١)***

( ): القيم بين القوسين تُعبر عن معدلات التناقص السنوي(%).

** معنوي عند المستوى الاحتمالي ٠,٠١ * معنوي عند المستوى الاحتمالي ٠,٠٥

المصدر: جُمعت وحُسبت من وزارة الزراعة واستصلاح الأراضي، قطاع الشئون الاقتصادية، الإدارة المركزية للاقتصاد الزراعي، نشرة الاقتصاد الزراعي، القاهرة، أعداد مختلفة.

يتضح من خلال معدلات النمو لكل من المساحة المخصصة لمحصول عباد الشمس وإنتاجية الفدان والإنتاج الكلي، أن معدلات النمو الثلاثة متباينة وهو أمر طبيعي، ويُلاحظ أن معدل نمو المساحة متناقص وبمعدل عالي نسبياً حيث بلغ نحو ٥,٦% سنوياً ومعنوي إحصائياً عند المستوى الاحتمالي ٠,٠١، في حين بلغ معدل نمو الإنتاجية نحو ١,٥% سنوياً ومعنوي إحصائياً عند المستوى الاحتمالي ٠,٠١، ويشير ذلك إلى أن معدل نمو الإنتاجية الفدانية لا يواكب معدل تناقص المساحة فقط بل أنه يأخذ إتجاهاً معاكساً مما يضاعف حجم المشكلة، ألا وهي تناقص كمية الإنتاج الكلي من بذور عباد الشمس وانعكس أثر ذلك على كمية مستخلصات بذور عباد الشمس وعلى رأسها زيت الطعام والزيوت الدوائية، وقد أكدها الأثر السلبي لقيمة معدل النمو السالب للإنتاج الكلي البالغ نحو ٤,١% ومعنوي إحصائياً عند المستوى الاحتمالي ٠,٠٥، وهنا تبرز أهمية زيادة المساحة التي يشغلها عباد

حيث تُمثل:

A : إجمالي العوائد بالأسعار المحلية.
B : تكلفة الموارد التجارية بالأسعار المحلية.
C : تكلفة الموارد المحلية بالأسعار المحلية.
D (= A-B-C): تقيس صافي العوائد الخاصة.
I (= A-E): تقيس تحويلات الناتج.
J (=B-F): تقيس تحويلات المدخلات التجارية.
E : إجمالي العوائد بالأسعار العالمية.
F : تكلفة الموارد التجارية بالأسعار العالمية.
G : تكلفة الموارد المحلية بالأسعار العالمية.
H (=E-F-G) : تقيس صافي العوائد الاجتماعية.
K (= C-G): تقيس تحويلات الموارد المحلية.
L (= D-H; or I-J-K) : تقيس صافي التحولات.

جدول (٢). المعاملات الاقتصادية المشتقة من مصفوفة تحليل السياسة ونتائجها المحتملة وأثر السياسة الزراعية المتبعة

م	المعامل	كيفية التقدير	النتائج المحتملة	الدلالة
١	**معامل الحماية الإسمي للمدخلات (NPCI) Nominal Protection Coefficient of the Inputs**	B/F	**1 <**	السعر المحلي للمدخلات أعلى من السعر العالمي لها، مما يعني تحمل المنتج ضرائب غير مباشرة.
			1 >	السعر المحلي للمدخلات أقل من السعر العالمي لها، مما يعني قيام الدولة بدعم المنتج.
			1 =	عدم وجود اختلال في السياسة السعرية.
٢	**معامل الحماية الإسمي للمخرجات (NPCO) Nominal Protection Coefficient of Outputs**	A/E	**1 <**	السعر المحلي للمحصول أعلى من السعر العالمي، هذا يعنى دعم المنتج و تحمل المستهلك أسعار أعلى مع وجود الحماية.
			1 >	السعر المحلي للمحصول أقل من السعر العالمي، هذا يعنى تحمل المنتج ضرائب غير مباشرة ودعم المستهلك.
			1 =	تصف حالة المساواة بين المنتجين و المستهلكين مما يعنى عدم وجود إنحراف أو إختلال سعري ، ولا يوجد تدخل حكومى.
٣	**معامل الحماية الفعال (القيمة المضافة) (EPC) Effective Protection Coefficient**	(A-B)/(E-F)	**1 <**	المنتجين يتسلمون عوائد أكبر على مواردهم وهذا يعنى وجود دعم لهم **(حماية موجبة).**
			1 >	المنتجين يتسلمون عوائد أقل على مواردهم وهذا يعني أى وجود ضرائب على المنتجين**(حماية سالبة).**
			1 =	هذا يعنى وجود سياسة حيادية.
٤	**معامل تكلفة الموارد المحلية (الميزة النسبية) (DRC) Domestic Resources Cost Coefficient**	G/(E-F)	**1 <**	الدولة لا تتمتع بميزة نسبية في إنتاج المحصول بالمقارنة بإستيراده من الخارج مما يعكس إنخفاض الكفاءة النسبية في إستخدام الموارد المتاحة لإنتاج المحصول أو السلعة.
			1 >	الدولة تتمتع بميزة نسبية في إنتاج المحصول أي وجود كفاءة في إستخدام الموارد الإنتاجية الطبيعية المحلية المتاحة في إنتاج المحصول أو السلعة.
			1 =	يعكس وضع توازنى أو نقطة التعادل ، وهنا يكون التوجه نحو الإنتاج المحلي بهدف زيادة عملية التشغيل وزيادة نسبة الإكتفاء الذاتي.
٥	**معامل الربحية (PC) Profitability Coefficient**	D/H	**1 <**	الربحية المتحققة من إنتاج المحصول في ظل السياسة المتبعة تفوق تكلفة فرصتها البديلة ،أي يشير إلى نجاح السياسة المتبعة.
			1 >	الربحية المتحققة من إنتاج المحصول في ظل السياسة المتبعة أقل من تكلفة فرصتها البديلة ،أي يشير إلى فشل السياسة المتبعة.
			1 =	هذا يعنى وجود سياسة حيادية.

المصدر: راجع في ذلك كل من: أحمد أبو اليزيد الرسول (٢٠٠٤)، جامعة الدول العربية (٢٠٠٠)، على يوسف خليفة، أحمد زبير جعاطة (٢٠٠٠)، علي يوسف خليفة (٢٠٠١).

المنتجين الزراعيين، ويوجد العديد من النماذج الرياضية المختلفة التي يتم الإستفادة منها في إجراء بعض التحليلات للسياسات الاقتصادية الكلية، أو لتحليل أثر بعض السياسات على بعض جوانب الأداء الاقتصادي ومن بين النماذج شائعة الإستخدام في هذا الشأن مصفوفة تحليل السياسة(Policy Analysis Matrix (PAM والتي تُعد واحدة من أهم الأدوات والأساليب المستخدمة في تحليل السياسات الزراعية (خاصة السياسة السعرية) حيث أن السياسة السعرية تُعد من أهم أدوات السياسة الزراعية في مصر وتستخدم لإستنتاج مجموعة من المعايير التي تساعد في التعرف على توجهات السياسة الزراعية في القطاع الزراعي بصفة عامة ، كما أنها طريقة للتحليل المنطقي لتقييم أثر السياسات العامة وكذلك أثر التشوهات السوقية على الأنشطة الاقتصادية، وتبين الكفاءة الاقتصادية في إستخدام الموارد المتاحة في ظل النشاط السلعي من خلال قياس بعض المؤشرات الهامة مثل الربحية الخاصة (المالية أو المحلية) للمحصول والربحية الإجتماعية (الاقتصادية أو العالمية) للمحصول والتحويلات (المنظمة العربية للتنمية الزراعية ،٢٠٠٠) من خلال مقارنة الربحية المالية لمحصول معين بالربحية الاقتصادية لنفس المحصول في ضوء أن المحصول يخضع لظروف التجارة الحرة. حيث يمكن إستخدام هذا النموذج للتوصل إلى أثر تطبيق سياسة اقتصادية أو زراعية على إجمالي عوائد إنتاج المنتجين، وأسعار مواردها الإنتاجية والتي تتمثل في تكلفة مواردها التجارية (التقاوي والأسمدة والمبيدات) وتكلفة مواردها الطبيعية (الأرض والمياه والعمل) وصافي العوائد.

تصميم مصفوفة تحليل السياسة

تُصمم مصفوفة تحليل السياسة لتحليل تشوهات السوق وسياسات التدخل ، وتعتمد مصفوفة تحليل السياسة على متطابقة حسابية بسيطة هي: **الربح = إجمالي العائد – إجمالي التكاليف.** وتُقسم التكاليف إلى مدخلات قابلة للإتجار(الأسمدة، التقاوي، المبيدات) وأخرى غير قابلة للإتجار يطلق عليها الموارد المحلية الطبيعية (الأرض ، المياه ، العمل)، ويُحسب الربح والعائد ونوعي التكاليف بإستخدام كلاً من الأسعار الخاصة (المحلية) والأسعار الإجتماعية (العالمية)، ويسمى الفرق بين أسعار السوق والأسعار الاقتصادية بالتحولات. ويتمثل هيكل مصفوفة تحليل السياسة بالجدول رقم(١)، حيث يستند تقدير النموذج إلى إستخدام التحليل الاقتصادي الرياضي والوصفي من خلال مجموعة من المعادلات الرياضية تشتق حساباتها من خلال مصفوفة تحليل السياسة وصولاً إلى احتساب معاملات الحماية الإسمية للإنتاج والموارد ومعامل تكلفة الموارد المحلية ومعامل الربحية بالجدول رقم(٢).

جدول (١). مكونات هيكل مصفوفة تحليل السياسة Policy Analysis Matrix(PAM)

البيان Items	إجمالي العوائد Revenues	التكاليف Costs		صافي العوائد Profits
		المدخلات التجارية Tradable Inputs	الموارد المحلية Domestic Factors	
الأسعار الخاصة (المحلية) Private prices	A	B	C	D = A – B - C
الأسعار الاجتماعية (العالمية) Social prices	E	F	G	H =E – F - G
أثر السياسة (التحويلات) Effects of policy	I = A - E	J =B - F	K = C - G	L = D – H = I – J - K

Source: Abdul Fatah, F , V. C. and Stephan, The Policy Analysis Matrix of Profitability and Competitiveness of Rice Farming in Malaysia, International conference of Agricultural Economics, Roma, Italy, 8 -14 August 2015.

المعايير أو المكونات والتوازن فيما بينهم (المنظمة العربية للتنمية الزراعية، ٢٠٠٠). كما أن الإدارة المزرعية الكفؤة هى العنصر الحاكم في تحقيق مكونات وعناصر السياسة الاقتصادية من خلال الإستخدام الأمثل للموارد المتاحة وليس هذا فحسب بل تمتد للإستفادة من المخلفات المزرعية (الشرقاوى، ٢٠١٦).

ولذا تأتي أهمية هذا البحث في محاولة التعرف على مدى نجاح أو فشل السياسة الاقتصادية الزراعية لمحصول عباد الشمس بمصر في تحقيق أهدافها والقدرة على الإستمرار ، حيث تعكس مؤشرات ومعالم ومعاملات مصفوفة تحليل السياسة تأثير أسعار محصول عباد الشمس ومدخلاته خلال فترة زمنية محددة. ويعكس ذلك ضمنياً مدى النجاح الذي حققته السياسات والبرامج الاقتصادية الزراعية ومدى إستجابة وتفاعل المنتجين مع تلك السياسات وصولاً لمعالجة السياسة أو الإستمرار في إتباعها.

الإسلوب البحثي

اعتمد البحث في تحقيق أهدافه على إستخدام الأساليب التحليلية الإحصائية والاقتصادية من خلال المتوسطات الحسابية والنسب المئوية ومعدلات النمو، هذا بالإضافة إلى إستخدام إسلوب تحليل مصفوفة السياسة Policy Analysis Matrix (PAM) كأحد أهم الأدوات الاقتصادية لتحليل أثر السياسة الزراعية المتبعة لمحصول عباد الشمس في مصر واشتقاق عدد من المعاملات الاقتصادية وهي التى يمكن من خلالها التعرف على مستويات الحماية التي يتمتع بها منتجي عباد الشمس أو مدى تحملهم أعباء كالضرائب غير المباشرة، وبالتالي الوقوف على التشوهات في أسواق مستلزمات الإنتاج والمنتج النهائي، معامل الحماية الاسمي لمستلزمات الإنتاج (المدخلات) Nominal Protection Coefficient of the Inputs (NPCI) ، معامل الحماية الاسمي للإنتاج (المخرجات) Nominal Protection Coefficient of Outputs (NPCO) ، معامل الحماية الفعال(القيمة المضافة)Effective Protection Coefficient (EPC) ، معامل تكلفة الموارد المحلية (الميزة النسبية) Domestic Resources Cost Coefficient (DRC) ، معامل الربحية Profitability Coefficient (PC).

مصادر البيانات

اعتمد البحث على البيانات المنشورة وغير المنشورة من مصادرها المختلفة مثل نشرات الاقتصاد الزراعى والجهاز المركزى للتعبئة العامة والإحصاء وقد تم الإستعانة بمعاملات التحويل Conversion Factors الصادرة من البنك الدولي لتقدير القيمة الاقتصادية لمستلزمات الإنتاج لمحصول عباد الشمس في مصر **(وزارة الزراعة وإستصلاح الأراضي، ٢٠٠٠)** ، كما تم الإستعانة بشبكة المعلومات الدولية خاصة بنك المعرفة المصري Egyptian Knowledge Bank (EKB)للحصول على الدراسات والبحوث الأجنبية ذات الصلة بأسلوب تحليل مصفوفة السياسة.

الإطار النظري والتحليلي لإسلوب تحليل مصفوفة السياسة (PAM)

تعتبر البيانات والمعلومات هي المادة الخام الأساسية التي ترتكز عليها أعمال التحليل الاقتصادي والإجتماعي بصفة عامة، ومنها بيانات الأسعار التي تلعب دوراً هاماً في اقتصاديات الإنتاج الزراعي فهي تعكس حالة النشاط الاقتصادي الزراعي، وللأسعار مكانة هامة في القطاع الزراعي وذلك لتأثيرها على مستوى دخول

٤٤-٤٨%. ويتميز زيته بالخلو من المواد السامة والكوليسترول بدرجة كبيرة، كما أنه يستخدم فى صناعة السمن الصناعى والصابون وبعض أنواع البويات. كما تستخدم مخلفات النباتات الخضراء لعباد الشمس في تصنيع الأعلاف الحيوانية. وأيضاً تقدم البذور بعد تقشيرها وتحميصها لبعض أنواع الطيور خاصة الأصناف ذات البذور صغيرة الحجم. وتقوم الدول الأوروبية بإدخال منظور جديد للزراعة بإستخدام المحاصيل الزراعية كمصدر لإنتاج الوقود الحيوي وحل مشكلة الطاقة العالمية (Konstadinos Mattas, et al., 2015) مما يزيد مشكلة الأمن الغذائي للدول النامية ومنها مصر خاصة في السلع الإستراتيجية الهامة ومنها المحاصيل الزيتية.

مشكلة البحث

مواجهة المشكلات الاقتصادية وإيجاد الحلول المناسبة لها يتطلب إتخاذ بعض التدابير والأساليب المناسبة لذلك، من أجل تحقيق هدف ما وهي ما تسمى بالسياسة الاقتصادية Economic Policy وهي إحدى مكونات السياسات القومية للمجتمع، وعليه فإن هناك ارتباطاً وثيقاً بينها وبين غيرها من السياسات خاصة السياسة الزراعية العامة Common Agricultural Policy (CAP) ، وهي تلك التي يتم تخطيطها وإعدادها وتطبيقها في قطاع الزراعة ونجاحها يتوقف على مدى التنسيق والتكامل بينها وبين غيرها من السياسات الاقتصادية الأخرى. وتتمثل مشكلة البحث في عزوف المزارعين عن زراعة محصول عباد الشمس، نظراً لما يواجهه من مشاكل في الإنتاج والتسويق بجانب تعرضه للعديد من الأمراض الخطيرة التي تقلل من إنتاجية الفدان، وبالرغم من ارتفاع تكاليف إنتاجه فإن محصول عباد الشمس غير مربح نسبياً إذا ما قورن بالمحاصيل الأخرى، الأمر الذي أدى إلى أن مصر تستورد نحو ٩٥% من إحتياجاتها من البذور الزيتية وعلى رأسها بذور عباد الشمس. لذلك فقد حان الوقت للتفكير بجدية في حل مشاكل إنتاج المحاصيل الزيتية في مصر خاصة محصول عباد الشمس. ومن هنا أصبح من الأهمية بمكان التعرف على السياسات الزراعية المتبعة في إنتاج محصول عباد الشمس خلال الفترة ٢٠٠٠-٢٠١٣.

أهداف البحث

استهدف البحث دراسة وتحليل وتقييم أثر السياسة الاقتصادية الزراعية المتبعة في إنتاج وتسويق محصول عباد الشمس في مصر والتعرف على الوضع الراهن لاتجاهات متغيراته الاقتصادية خلال الفترة ٢٠٠٠-٢٠١٣.

أهمية ومبررات البحث

تجدر الإشارة من الناحية العملية أن مؤشر الرفاهية الاقتصادية Economic Welfare يعتبر دالة لكافة السياسات والأنشطة الاقتصادية والتي يسعى المجتمع إلى تعظيمها وتحقيق أكبر قيمة ممكنة لها، ولذلك يتم إستخدام مجموعة من المعايير للحكم على السياسات الاقتصادية وفاعليتها لتحقيق الأهداف التنموية المنشودة ومن ثم الرفاهية كهدف نهائي ومنها الكفاءة Efficiency، النمو Growth، العدالة Equity (نصار، ٢٠١٥ أ)، الحوافز المشروطة أو الدعم المشروط Conditional Support ، والممارسات الزراعية الجيدة (GAP) Good Agricultural Practices (نصار، ٢٠١٥ ب)، الإستقرار Stability ، المخاطرة Risk ، توزيع الدخل Income Distribution ، الإستجابة السوقية Market Responsiveness ، الحفاظية Conservativeness حيث أن السياسة الاقتصادية المُثلى هي تلك التي تؤدي إلى تعظيم محصلة هذه

تحليل السياسة الاقتصادية الزراعية لمحصول عباد الشمس في مصر

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**معهد بحوث الاقتصاد الزراعي – مركز البحوث الزراعية – القاهرة

الملخص: استهدف البحث دراسة وتحليل السياسة الاقتصادية الزراعية المتبعة في إنتاج وتسويق محصول عباد الشمس في مصر خلال الفترة ٢٠٠٠-٢٠١٣ بإستخدام مصفوفة تحليل السياسة حيث أوضحت نتائجها حصول المزارع على دعم سواء لمستلزمات الإنتاج أو للمنتج النهائي إذ بلغ معامل الحماية الإسمي للمدخلات والناتج نحو ٠,٩٤ ، ١,٠٣ على الترتيب. كما بلغ معامل الحماية الفعال نحو ١,٠٤ مما يؤكد على أن هناك دعماً لأسعار بذرة عباد الشمس لصالح المنتجين، وأن مصر لديها ميزة نسبية في إنتاج بذور عباد الشمس إذ بلغ معامل تكلفة الموارد المحلية (الميزة النسبية) نحو ٠,٦٦ مما يعكس الكفاءة النسبية في إستخدام الموارد المحلية لإنتاجه وتصديره ، وأخيراً بلغ معامل الربحية للفدان نحو ٠,٩٥ حيث أن الربح المتحقق لإنتاج فدان من بذور عباد الشمس في ظل السياسة المتبعة حالياً يمثل نحو ٥% أقل عن الربح المتحقق من إستيراده، أي أنه يشير إلى فشل السياسة المتبعة في إنتاج بذور عباد الشمس.

وأخيراً يوصي البحث: بضرورة إيجاد حافزاً مجزياً لدى المزارعين لزراعة محصول عباد الشمس والاستفادة من الميزة النسبية التي تتمتع بها مصر في إنتاجه وكاحد محاصيل الزيوت النباتية التي يمكن أن تقلل من فجوة الزيوت النباتية.

الكلمات الدالية: مصفوفة تحليل السياسة، معاملات الحماية الإسمية ، معامل تكلفة الموارد المحلية، معامل الربحية، عباد الشمس.

المقدمة:

يُعد تحرير القطاع الزراعي في مصر والسماح للقطاع الخاص بالإتجار في مستلزمات الإنتاج الزراعي والمحاصيل والمنتجات الزراعية بالأسواق الداخلية والخارجية وفقاً لآلية السوق والذى إنعكس بطبيعة الحال على إرتفاع بنود التكاليف الفدانية سواء للمدخلات التجارية والموارد المحلية مما أدى إلى إرتفاع العبء على كلاً من المنتج والمستهلك (الشرقاوي، ٢٠١٢)، وتعتبر المحاصيل الزيتية مصدراً هاماً لتوفير الزيوت النباتية والتي تُعد أحد مكونات الغذاء الأساسية كمصدر للطاقة، كما تتعدد الإستخدامات وتتنوع المنتجات التي تُصنع من الزيوت النباتية وعلى رأسها المسلي الصناعي النباتي كبديل رخيص الثمن للمسلي البلدي. كما تعد المحاصيل الزيتية مصدراً رئيسياً من مصادر الدخل الزراعي. ونظراً لأن مصر تواجه فجوة غذائية فى إنتاج الزيوت المختلفة، فإن الأمر يستلزم التوسع فى زراعة محاصيل البذور الزيتية التي تغطي حالياً من ٤٠-٥٠% فقط من الاستهلاك المحلى من الزيوت النباتية، ويلعب إنتاج بذور عباد الشمس دوراً هاماً في زيادة إنتاج الزيت مما زاد من الإهتمام بالتوسع الزراعي في إنتاج هذا المحصول الزيتي الهام، خاصة وأن مصر تستورد كميات كبيرة منه حالياً . ويتميز محصول عباد الشمس بأن بذوره تحتوي على نسبة زيت تتراوح بين ٣٥-٥٠% كما تتراوح نسبة البروتين فى البذور بين

المحتويات

هيئة التحرير

مجلـة

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