

## Influence of Sources and Rates of Mineral Nitrogenous Fertilization and Bio-Stimulants on Garlic Productivity and Efficiency of Its Extracts against Pathogen Activities

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### ABSTRACT

Garlic is a worldwide important vegetable crop, and increase its growth and productivity a crucial objectives especially via good agricultural practice; i.e., mineral nitrogen fertilization and bio-stimulants. Two field experiments were conducted to study the influence of nitrogen fertilizer sources (ammonium sulphate and ammonium nitrate), rates (90 and 120 kg N/fed.) and bio-stimulants (Halex-2, yeast extract, Halex-2 + yeast extract and without bio-stimulants) as well as their interactions on vegetative growth, productivity, bulb quality and chemical constituents of garlic (*Allium sativum*, L.). Moreover, garlic extracts were tested against two pathogen activities (*Fusarium oxysporium* and *Alternaria alternata*). Garlic cloves were planted during two winter seasons of 2013/2014 and 2014/2015 at a newly reclaimed area at Sadat City, Egypt. Inoculation garlic plants with bio-stimulants showed superior effects on most of the studied characters especially for garlic productivity. Most of the studied vegetative characters, yield, yield components and bulb quality characteristics significantly affected with nitrogen sources, nitrogen rates and bio-stimulants as well as their interactions during the two seasons. Inoculation garlic plants with Halex-2 + yeast extract was found to have positive effective in reducing the mineral nitrogen fertilizer being applied by 25 % as compared with the commonly recommended dose. Data revealed that the fertilization combination treatments; 120 kg nitrogen /fed. at the form of ammonium sulphate + Halex-2 + Yeast extract and 90 nitrogen kg/fed. at the form of ammonium sulphate + Halex-2 + Yeast extract significantly gave the highest values for phenol content. The plant extracts obtained from the same two previous fertilization combinations gave the highest inhibition against *Fusarium oxysporium* and *Alternaria alternata*. The *in vitro* results showed that using concentration 2.5% of the selected extracts gave the highest mycelial growth inhibition for the tested pathogens. These results may contribute to develop environmentally safer alternatives antifungal agent against plant pathogenic fungi.

**Key words:** Garlic (*Allium sativum*, L.), nitrogenous fertilization, bio-fertilizer, Halex-2, yeast extract, bio-stimulants, phenol content, garlic extract, antifungal activities, *Fusarium oxysporium* and *Alternaria alternata*.

### Introduction

Generally, garlic (*Allium sativum*, L.) is one of the oldest horticultural crops worldwide. Garlic is considered one of the most important vegetable crops all over the world. Garlic possess some medicinal characteristics such as power suppliers, insecticidal, anti-bacterial, antifungal, anti-cancer, depressor for both blood sugar and blood lipids, and reduce blood platelet aggregation (Agusti, 1990). Egypt is ranked fourth in the world in terms of the productivity of garlic (244.626 MT) after China, India and Korea (FAO, 2011). Abou El-Magd (2012) pointed out that the productivity of garlic in Egypt in 2008 has reached (301270 ton) according to the Egyptian Statistics of Ministry of Agriculture. Nitrogen fertilization is necessary and important for increasing the productivity and quality of vegetable crops such as garlic and thus is more gainful for farmers (Gulser, 2005). For good agricultural practices of garlic, it needs numerous factors which must be considered include planting time, time, rate of fertilizers, application and optimum plant population (Brewster and Butler, 1989). The published articles declare that adding an appropriate

quantity of nitrogenous fertilization during sprouts stage, leads to promote vigorous vegetative growth and optimum leaf expansion (Stork *et al.*, 2004). Kakara *et al.* (2002) reported that increasing nitrogen units from 50 to 200 kg per/ha exhibited a positive effect on the mean values of plant height, leaf area, leaf count, and fresh and dry plant mass. Therefore, use of nitrogenous fertilization is necessary for establishing successful vegetative growth of garlic. The obtained results of Mudziwa (2010) indicated that *A. sativum* plants that received higher amounts of ammonium sulphate fertilizer (100, 150 and 200 kg/ha) had, significantly, higher yield values as compared to garlic plants that received the lowest level of fertilization (50 kg/ha). Nitrogenous fertilization sources (ammonium sulphate and calcium nitrate) affected plant growth and yield of garlic. The positive and significant response of garlic to applied nitrogen fertilizer has been reported by many researchers (Kilgori *et al.*, 2007; Brahma and Yousuf, 2008; El-Zohri and Abdou, 2009 and Zaman *et al.*, 2011).

Halex-2 is a bio-fertilizer which is considers as a non-symbiotic N<sub>2</sub> fixing bacteria that has greater

amounts of bacteria which responsible for fixing of nitrogen. Application of Halex-2 to the growing plants enhanced the following values; increasing soil fertility, reducing the usage of nitrogenous fertilization and expanding the availability of various nutrients to plant absorption (Abdel-Razzak and El-Sharkawy, 2013).

The role of yeast extract as bio-stimulant for growing plants was reported through its stimulatory effects on cell division and enlargement, as well as, the chlorophyll formation, synthesis of proteins and nucleic acids (Wanas, 2002 and 2006), in addition to its content of cryoprotective agent (sugars, protein, amino acids and several vitamins) as reported by (Mahmoud, 2001).

The pathogenic fungi of the plant cause many plant diseases that lead to large losses of crops production. Synthetic fungicides; generally, are applied by the farmers as a way to conserve and prevent plants, infection plant diseases. The indiscriminate and excessive usage of a wide range of fungicides has led to increased environmental pollution and the emergence of groups of pathogens that have acquired immunity to resist chemical pesticides. Therefore, the demand for organic agricultural products is increasing day by day. Based on these requirements, many researchers have been interested with the integrated control of fungal diseases, including the usage of antagonistic microorganisms and safer chemicals such as food preservatives and derived products from plants (Copping and Menn, 2000). Sulfur compounds, plant extracts, aromatics and volatile compounds resulting from secondary plant metabolism, widely applied in the fields of folk medicine, food flavor and preservation; in addition to their use in the perfume industry. For centuries, sulfur compounds have been known as antifungal (Lazarević *et al.*, 2011). The extracts of garlic, either from leaves or bulbs, were used as antibacterial, fungal and viral infections; as well as, using as an immune system booster (Kaye *et al.*, 1995). Numerous studies indicated that each of garlic and onion plants have biological and medicinal activities. These activities are mainly because they include high percentages of organo-sulphur compounds content. These natural bioactive chemicals have no toxic effects. Therefore, a number of recent researches have started to take advantage towards plant extracts to be commercially produced (Kim *et al.*, 2003 and Bajpai *et al.*, 2009). In the present study, the effects of methanolic extracts of garlic (*Allium sativum*) plant extracts on controlling the growth of two of the most important phytopathogenic fungi (*Fusarium oxysporium* and *Alternaria alternata*) in Egypt were studied.

Hence, the aims of the present study are to minimize the usage of nitrogen in form of mineral fertilizers; in addition to, maximize garlic productivity through the use of appropriate combination between two mineral nitrogen sources, nitrogen rates and bio-stimulants. Moreover, the study was intended to provide natural alternatives to the chemical fungicides to obtain healthier and safer plant products via diagnosis the fungicidal impact of garlic extract in order to perform natural alternative healthier, safer and cheaper bio-fungicide products.

## MATERIALS AND METHODS

Two field experiments were carried out during two winter seasons of 2013/2014 and 2014/2015 at a newly reclaimed area, at the Environmental Studies and Researches Institute Farm, Sadat University, Minufiya Governorate, Egypt. Chinese garlic (*Allium sativum*, L.) cultivar was planted under drip irrigation system on 1<sup>st</sup> of September 2013 and 7<sup>th</sup> of September 2014. Cloves were planted in rows 10 m long, 60 cm wide at spacing of 10 cm within row and on both sides of the rows. The treatments of the given experiments were as follows:

- 1- Nitrogen source treatments:- two mineral nitrogen fertilizers; i.e., ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), 33.5% N. and ammonium sulphate ( $\text{NH}_4)_2\text{SO}_4$ , 20.6% N.
- 2- Applied nitrogen rate treatments:- two nitrogen rates; i.e., 90 kg N/fed. and 120 kg N/fed. (recommended dose by the Egyptian Ministry of Agriculture.
- 3- Bio-stimulants treatments: - four treatments; i.e., Halex-2, yeast extract, Halex-2 + yeast extract in addition to without bio-stimulants treatment.

Each experimental unit (sub-sub-plot) was consisted of two rows with plot area of 12 m<sup>2</sup>. Each replicate had 32 rows represent the applied fertilization treatments.

### Agricultural practices

The following fertilizers were added to the soil at preparation; 75 Kg P<sub>2</sub>O<sub>5</sub>/fed. in the form of mono calcium phosphate (15.5 % P<sub>2</sub>O<sub>5</sub>), sulphur was applied at the rate of 200 Kg/fed. plus 5 tons/fed of compost. Potassium fertilizer was added at the rate of 96 Kg K<sub>2</sub>O / fed. in the form of potassium sulphate (48% K<sub>2</sub>O) throughout the drip irrigation system. All other agricultural practices for garlic production were followed as recommended in the area.

At the 2<sup>nd</sup> of April, 2014 and 10<sup>th</sup> of April, 2015 when the leaves had started withering, plants of each sub-sub-plot were harvested separately. The physical and chemical properties of the soil were measured using laboratory tests suggested by the U.S. Salinity Laboratory Staff (1954 and are presented in Table (1).

**Table 1: Physical properties and chemical analyses of the experimental soil**

Mechanical analysis			Texture	pH	EC. dS/m	CaCO <sub>3</sub> %	O.M. %
Season	Sand%	Silt% Clay%					
2013/2014	90	5 5	sandy	7.25	5.98	5.4	0.79
2014/2015	90	5 5	sandy	7.27	6.02	5.6	0.81

Chemical analysis										
Season	Cations (meq/L)				Anions (meq/L)					
	N <sup>+</sup>	P <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	CL <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
2013/2014	Traces	0.38	53.75	23.71	17.0	2.19	Zero	8.0	68.0	20.74
2014/2015	Traces	0.42	53.75	23.79	17.2	2.13	Zero	8.0	68.0	20.78

Garlic cultivar: Chinese cultivar was tested in this study. This cultivar is characterized by its big cloves, easy peel; the cloves have shiny white skin with purple vertical lines.

#### Source of the bio-stimulants

Halex-2 is a biofertilizer made up of a mixture of growth promoting of non-symbiotic N-fixing bacteria of genera *Azospirillum*, *Azotobacter* and *Klebsiella*. Halex-2 was kindly provided by the Bio-fertilization Unit, Department of Plant Pathology, The Faculty of Agriculture, Alexandria University, Egypt. Cloves of the tested cultivar "Chinese" were inoculated by soaking in cell suspension of the Halex-2 containing 5% Arabic gum, at the rate of 400 g/fed. for half an hour according to the recommendation of the above mentioned Department. The inoculation with Halex-2 was repeated three weeks later as side dressing beside seedlings (Ghoneim, 2005).

Yeast extract:- The brewer's yeast (*Saccharomyces cerevisiae*) was dissolved in water. Sugars were added to the yeast at a ratio of 1:1. The extract was kept for 24 hours in a warm place for reproduction, as explained by Morsi *et al.* (2008).

#### Data recorded

**Vegetative characters:-** Ten randomly plants from each replicate were used to determine each of plant height (cm), number of leaves / plant and leaves fresh weight (gm). The obtained data were averaged and recorded.

**Yield and yield components:-** garlic yield was calculated for plot area then attributed to the feddan (fed.), where fed. = 4200 m<sup>2</sup>. A random sample (10 bulbs) was taken from each treatment to determine both bulb weight (gm) and number of cloves/bulb.

**Bulb quality characteristics:-** Ten randomly bulbs were taken from each replicate and used to determine neck thickness (cm), bulb diameter (cm), bulbing ratio (%), cloves firmness (Kg/cm<sup>2</sup>) and total phenol content (mg/g). Bulbing ratio was calculated according to Mann (1952) = Neck diameter (cm) / Bulb diameter (cm). Cloves firmness (Kg/cm<sup>2</sup>) was measured using fruit pressure tester model FT 327 (3-27 Lbs.), Italy. Total phenolic were determined colorimetrically using Folin Ciocalteureagent at 755 nm as explained

by Chaovanalikit and Wrolstad (2004). The obtained data were averaged and recorded.

**Cloves Chemical analysis:-** The following chemical elements were analyzed in garlic bulbs: Nitrogen (N), Phosphorus (P) and Potassium (K). Harvested bulbs were dried at 65 °C for 72 h and then ground with a mill. Determination was done as outlined by APHA (2005).

**GC-MS analysis:-** The GC-MS analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Central Lab, National Research Center, Egypt. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

**Collection of tested plant pathogenic fungi:-** *Fusarium oxysporium* 9704AUMC, *Alternaria alternate* 10301AUMC, were obtained from Mycology Center Assiut University, and were maintained on PDA medium, which served as the test fungi for antifungal activity assay.

**Medium:-** The PDA medium contained potato (200 g), dextrose (20 g), agar (20 g), and distilled water (1000 ml). The pH of the medium was adjusted to 5.6 with 1N HCl or NaOH. A 20-ml portion of the medium was added to sterilized Petri dish and prepared as the test agar medium.

**Preparation of plant extracts:-** Planting materials of garlic bulbs, produced from the growing plants during the second season of this study, were dried in the shade at room temperature, ground using electrical mill into fine powder and extracted by soaking in methanol at the rate of 1:1 (w/v) for 48 hours. The extracts were filtered through cheese cloth under a strong hand pressure and the solvent was dried under vacuum at 60-65°C using a rotary evaporator. The extracted residue was dissolved in dimethyl sulfoxide (1mg /ml). The crude extracts were preserved under refrigeration until use (Dawood *et al.*, 2003).

**Screening of garlic plant extracts for antifungal activities:-** Sixteen plant extracts of garlic bulb (derived from the applied fertilization treatments) were examined for their inhibitory activities against tested phytopathogenic fungi *Fusarium oxysporium* and *Alternaria alternate* by using agar diffusion

technique (Deans and Ritchie, 1987). Dry potato dextrose agar plates were inoculated by spreading spore suspensions of constant fungal inoculum. Wells were made in the inoculated potato dextrose agar plates for plant extracts inoculation (200 $\mu$ ). All plates were incubated at 26 $\pm$ 2°C for 72 hours. The diameter of inhibition zones was determined for all tested fungi.

**Preparing media for evaluation the selected extracts on fungal linear growth:-** The media were prepared as 100 ml aliquots in 250 ml Eyrlemeyer flasks. After autoclaving, the media were amended with different concentrations of plant extract (0.5% ,1.5% and 2.5%).The medium was poured in petri dishes and left for solidification. Mycelial discs were cut out from the growing edges of the tested fungal colonies and transferred separately to these plates. Control plates were without plant extract. Triplicate dishes were used for each treatment. All dishes were incubated at 26 $\pm$ 2C° during the whole experimental period. Daily measurement of linear growth was carried out (El-Morsy, 1993).The inhibition rates were calculated according to formula by Deans and Svoboda (1990):

$$I = C - T/C \times 100$$

Where; I = percent mycelial inhibition, C = mean colony diameter of control sets, and T = mean colony diameter of treatment sets.

#### Experimental design and statistical analysis

The experimental layout was presented as a split-split-plot in a randomized complete blocks design (R.C.B.D), with three replicates. Two sources of nitrogen fertilizers were assigned in the main plots, two rates of nitrogen units were assigned to the sub-plots and four bio-stimulants which were, randomly, distributed in the sub-sub-plots. Collected data of sixteen samples of garlic extracts were layout as a

randomized complete design (R.C.D). Collected data of the experiments were, statistically, analyzed using the analysis of variance method. Comparisons among the means of different treatments were done, using least significant differences (L.S.D) test procedure at  $p = 0.05$  level of probability, as illustrated by Snedecor and Cochran (1980). Computation was done using Co-Stat software program (2004).

## RESULTS AND DISCUSSION

### Main effects of the studied treatments on the garlic vegetative measurements

The studied vegetative characters were significantly affected with nitrogen fertilization source only during the second season of this experiment (Table, 2). The recorded data divulged that fertilization garlic plants with ammonium nitrate fertilizer; led to enhance the vegetative traits compared to the fertilization with ammonium sulphate fertilizer. This result could be attributed to its easiest solubility in water compared to ammonium sulphate fertilizer. The data showed that ammonium nitrate fertilizer exhibited the best results. The results showed that adding 120 kg N / fed. significantly gave rise to the mean highest values for the vegetative measurements. Generally, inoculation of bio-stimulants to the growing garlic plants led to better results for the vegetative traits compared to non-inoculated one. The highest mean values were obtained when the growing garlic plants inoculated with both "Halex-2" + yeast extract during both seasons of this experiment (Table, 2). Leaves fresh weight character, was significantly, affected with bio-stimulants treatments during the first season only.

**Table 2: Averages of vegetative growth characters of Chinese garlic as affected by nitrogenous sources, its rate and bio-stimulants during the seasons of 2013/2014 and 2014/2015.**

Treatments	Season 2013/2014			Season 2014/2015		
	Plant height (cm)	No. of leaves / plant	Leaves fresh weight (g)	Plant height (cm)	No. of leaves / plant	Leaves fresh weight (g)
<b>Nitrogen source</b>						
Ammonium nitrate	50.03 a	9.19a	21.45a	54.54a	8.46a	19.22a
Ammonium sulphate	48.64 a	8.96a	20.63a	47.55b	7.03b	14.64b
<b>Nitrogen rate</b>						
120 Kg nitrogen /fed.	53.71 a	9.61a	24.66a	57.46a	8.41a	18.65a
90 Kg nitrogen /fed.	44.96 b	8.55b	17.42b	44.63b	7.09b	15.21b
<b>Bio-stimulants</b>						
Without	44.93 c	8.40c	17.67c	46.19d	7.08d	16.07a
Halex-2 (H)	51.00 ab	9.19b	21.35b	52.85b	8.02b	17.14a
Yeast extract (Y)	49.39 b	9.03b	21.43b	50.07c	7.52c	16.68a
Halex-2 + Yeast extract	52.01 a	9.69a	23.72a	55.08a	8.37a	17.84a

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p= 0.05$  level of probability.

The positive effects of tested bio-stimulants on the vegetative characters were mainly due to the role of Halex-2 in increasing the availability of N to plant absorption and containing yeast cytokinins, enzymes, vitamins, minerals and amino acids which have positive role on cell division and elongation, nucleic acid synthesis, protein and chlorophyll formation (Khedr and Farid, 2002, Mahmoud, 2001).

El-zohiri and Abdou (2009) reported that increasing the nitrogen fertilizer level from 60 up to 120 kg-N/fed., significantly, increased all the studied vegetative growth characters of garlic "Seds 40 cv.; i.e., plant height, number of leaves, fresh and dry matter of leaves. Meanwhile, Zaman *et al.* (2011) reported that the increase in plant height trait due to nitrogen application indicates a positive role of nitrogen on plant growth and the soil was deficient in nitrogen. Herein, Naruka (2000) explained that availability of nitrogen is of prime importance for growing plants as it is a major and indispensable source of protein and nucleic acid molecules, since nitrogen is an essential part of chlorophyll molecules, which are responsible for photosynthesis. The same author added that using N fertilizer helps to increase production and is a

somewhat quick method for reaching maximum yields. These results are in accordance with that obtained by Naruka (2000); Naruka and Dhaka (2001); Yadav (2003); Banafar and Gupta (2005); Naruka *et al.* (2005). The findings of Table (2) are in accordance with those obtained by Abdel-Razzak and El-Sharkawy (2013) who reported that the bio-fertilizer "Halex-2" has a positive effect on increasing the availability of N to plant absorption which in turn increases the vegetative growth of garlic plants. Similar findings were recorded by El-Desuki *et al.* (2006); Yaso *et al.* (2007) on onion. The results of Shafeek *et al.* (2015) on onion; cleared that bio-stimulants showed positively influence on the vegetative growth and total production and its quality.

#### Effects of the first-order interactions of studied treatments on garlic vegetative measurements

The results of Table (3) appeared that all the first-order interactions possessed significant differences for the studied vegetative traits during both seasons of the study. In this respect, adding 120 Kg nitrogen per feddan in form of ammonium nitrate; gave the highest and significant results for the studied vegetative characters.

**Table 3: Averages of vegetative growth characters of Chinese garlic as affected by the first-order interactions between nitrogenous sources, its rate and bio-stimulants during the seasons of 2013/2014 and 2014/2015**

Treatments	Season 2013/2014			Season 2014/2015			
	Plant height (cm)	No. of leaves / plant	Leaves fresh weight(g)	Plant height (cm)	No. of leaves / plant	Leaves fresh weight (g)	
<b>Nitrogen source X Nitrogen rate interaction</b>							
Ammonium nitrate	120 Kg N/fed.	54.27 a	9.83a	25.11a	60.39a	9.42a	20.37a
	90 Kg N/fed.	45.78 b	9.57a	17.79b	48.69c	7.51b	18.07ab
Ammonium sulphate	120 Kg N /fed.	53.14 a	8.55b	24.21a	54.54b	7.40b	16.93b
	90 Kg N/fed.	44.13 b	8.54b	17.06b	40.57d	6.67c	12.35c
<b>Nitrogen source X Bio-stimulants interaction</b>							
Ammonium nitrate	Without	44.02e	8.57cd	18.42ef	50.56c	7.31d	18.34b
	Halex-2(H)	51.94ab	9.30b	21.92bc	55.28b	8.97b	19.40a
	Yeast ex.(Y)	50.75abc	8.91bc	19.42de	54.16b	8.16c	18.75a
	H + Y	53.40a	10.01a	26.06a	58.17a	9.41a	20.40a
Ammonium sulphate	Without	45.85de	8.22d	16.93f	41.82e	6.85e	13.80c
	Halex-2(H)	50.05bc	9.08bc	20.78cd	50.42c	7.08de	14.89c
	Yeast ex.(Y)	48.03cd	9.16bc	23.44b	45.98d	6.88e	14.60c
	H + Y	50.61abc	9.38ab	21.39c	51.99c	7.33d	15.29bc
<b>Nitrogen rate X Bio-stimulants interaction</b>							
120 Kg nitrogen /fed.	Without	52.35a	8.78d	21.05c	56.56b	7.34c	17.66ab
	Halex-2(H)	54.29a	9.80ab	25.83ab	57.17b	8.85a	19.04a
	Yeast ex.(Y)	53.25a	9.51bc	24.86b	56.99b	8.19b	18.85a
	H + Y	54.94a	10.34a	26.89a	59.17a	9.27a	19.07a
90 Kg nitrogen /fed.	Without	37.52d	8.02e	14.28e	35.82f	6.83d	14.47b
	Halex-2(H)	47.71bc	8.58de	16.86d	48.56d	7.19cd	15.25b
	Yeast ex.(Y)	45.53c	8.56de	18.00d	43.15e	6.85d	14.50b
	H + Y	49.08b	9.04cd	20.56c	50.99c	7.48c	16.62ab

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

The data of the interaction between nitrogen source and bio-stimulants showed that inoculation growing garlic plants with Halex-2 + yeast extract, significantly, encouraged the vegetative growth of garlic plants during the growing seasons of the research (Table, 3). The interaction between nitrogen rate and bio-stimulants show that the vegetative measurements recorded the highest values when garlic plants were fertilized with 120 Kg nitrogen / Fed. combined with Halex-2 or with bio-stimulants (Halex-2 + yeast extract). Generally, the data of the Table (3) clearly show benefit effects of the bio-stimulants on the vegetative growth of garlic plants. These positive effects were achieved by significant increases in the studied vegetative traits; plant height, No. of leaves per plant, and leaves fresh weight. This can be attributed to the role of bio-stimulants in enhancing both plant nutrients uptake and plant metabolism processes, hence gain in accumulation of proteins, carbohydrates and vitamins. This explanation is in accordance with Shalaby and El-Ramady (2014) and Ahmed (2015) on garlic crop.

#### Effects of the second-order interactions of studied treatments on garlic vegetative measurements

The data of the interaction between nitrogen source x nitrogen rate x bio-stimulants are presented in Table (4). There were highly significant differences among the tested fertilization treatments regarding all the vegetative

characters during both growing seasons. The fertilization treatment No. 4 (composed of 120 kg N / fed. at the form of ammonium nitrate + Halex-2 + yeast extract) gave the highest mean values for all studied vegetative characters during both seasons; as shown in Table (4). Plant height character, there were no significant differences among the fertilization treatments No. 4 and No.1,2,3,9,10, 11 and 12 during the first season. Meanwhile, data showed that there were no significant differences between the previous treatments (No. 4) and No.2 (120 kg nitrogen /fed. at the form of ammonium nitrate + Halex-2) during the second season. The highest values for number of leaves per plant and leaves fresh weight traits were conjoined with the treatment No. 4 with insignificant differences values with the fertilization treatments No. 1,2,10,11 and 12 for number of leaves per plant during the first season. The results of the second season showed that there were no significant differences between the treatment No. 4 and the treatments No. 2 for the number of leaves per plant trait. The data of the second season showed that there were insignificant differences among the eleven fertilization treatments (No. 1, 2,3,4,5,6,7,8, 10, 11 and 12) for leaves fresh weight character. Generally, it could be concluded that the potent treatment of 120 kg N per fed. at the form of ammonium nitrate + Halex-2 + yeast extract; possessed, highest mean values for all the tested vegetative traits which means that this treatment of fertilization enhanced growth attributes of garlic plants.

**Table 4: Averages of vegetative growth characters of Chinese garlic as affected by the second-order interactions between nitrogenous sources, its rate and bio-stimulants during the seasons of 2013/2014 and 2014/2015**

Treatments	Season 2013/2014			Season 2014/2015				
	Plant (cm) height	No. of leaves / plant	Leaves fresh weight(g)	Plant (cm) height	No. of leaves / plant	Leaves fresh weight (g)		
<b>Nitrogen source X Nitrogen rate X Bio-stimulants interaction</b>								
Ammonium nitrate	120 Kg nitrogen /fed.	1- Without	52.25abcd	9abc	21.39def	59.00b	7.45def	19.33ab
		2- Halex-2(H)	54.35ab	9.57a	26.33b	60.78ab	10.4a	21.00a
		3- Yeast ex.(Y)	53.84abc	7.73def	22.17de	59.45b	9.1b	20.10ab
		4- H + Y	56.66a	9.78a	30.56a	62.33a	10.73a	21.06a
		5- Without	35.79h	7.03fgh	15.44hi	42.11e	7.18ef	17.34abcd
90 Kg nitrogen /fed.		6- Halex-2(H)	49.54cde	8.49bcd	17.50gh	49.78d	7.53cde	17.79abc
		7- Yeast ex.(Y)	47.66ef	7.18fg	16.67h	48.88d	7.22def	17.40abcd
		8- H + Y	50.14bcde	6.52gh	21.55def	54.00c	8.08c	19.74ab
Ammonium sulphate	120 Kg nitrogen /fed.	9- Without	52.45abc	7.72def	20.72ef	54.11c	7.22def	15.99bcde
		10- Halex-2(H)	54.23ab	9.25ab	25.33bc	53.5c	7.30def	17.07abcd
		11- Yeast ex.(Y)	52.66abc	8.91abc	27.56b	54.53c	7.28def	17.60abc
		12- H + Y	53.21abc	9.5a	23.22cd	56.00c	7.80cd	17.07abcd
90 Kg nitrogen /fed.		13- Without	39.24gh	6.21h	13.11i	29.52g	6.47g	11.60e
		14- Halex-2(H)	45.88ef	7.44efg	16.22h	47.33d	6.85fg	12.70de
		15- Yeast ex.(Y)	43.40fg	8.22cde	19.33fg	37.42f	6.47g	11.60e
		16- H + Y	48.01de	7.95def	19.56fg	47.98d	6.87fg	13.50cde

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

The results of Table (4) show that the fertilizer formulations containing Halex-2 and/ or yeast extract gave, In general, better results for the vegetative characters than those treatments that did not include either of them at the same rate and source of nitrogen fertilization. Bio-stimulants able to enhance vegetative growth, mineral nutrient uptake and improve the yielding of many plants (Fayad, 2005; Fathy *et al.*, 2008; Hassan *et al.*, 2008; Mohamed *et al.*, 2010). Many researches proved the benefit role of yeast extract on stimulating the vegetative growth of plants; i.e., Wanas (2002) on faba bean; Wanas (2006) on tomato and Mady (2009) on faba bean. The authors illustrated that yeast extract had favorable influence on the plant metabolism and biological activity through stimulating photosynthetic pigments and enzyme activity which in turn promote the plant vigorous. In addition, bio-stimulants improved antioxidant activity, vitamin C and raises leaves pigment of vegetables compared to untreated plants. Thus, the application of bio-stimulants could be considered as a good production strategy for obtaining high production of nutritionally valuable vegetables with lower negative effect on the environment (Sazetak, 2011).

#### **Main effects of the studied treatments on garlic productivity and bulb quality characteristics**

The results of Table (5) showed that mineral nitrogen fertilizer source had significant effects on garlic yield; yield components and bulb quality characteristics over both growing seasons except for the number of cloves per bulb and bulbing ratio during the first season. The data, clearly, appeared that ammonium nitrate fertilizer; gave higher mean values for most studied characters compared to ammonium sulphate fertilizer except for cloves firmness and phenol content. These results are in accordance with the obtained results of the vegetative characters; where ammonium nitrate led to enhance the vegetative growth of the growing plants more than ammonium sulphate (Table, 2). Bulbing ratio significantly affected with nitrogen rate during the second season only. These findings confirmed that applying adequate source and quantity of nitrogen fertilizer are correlated with intensifying vegetative growth and leading to greater productivity.

Hassan (2015) explained that the increasing happened of neck thickness, bulb diameter and bulbing ratio by increasing nitrogen level might be due to its role in photosynthesis, protein synthesis, cell division and enlargement which are the basal steps of plant growth. In addition, nitrogen plays an important role in the enzyme activity which reflects more products needed in plant growth. Increasing nitrogen rate from 90 kg./fed. up to 120 kg./fed.; led to significant positive effects on garlic productivity and its component characters (Table, 5). The results of El-Zohiri and Abdou (2009) concluded that average clove weight, fresh weight of bulb; as well as, total produced yield of garlic were positively affected with increasing

nitrogen level from 60 up to 120 kg N/fed. during both growing seasons. Those authors explained that the increments in total yield production as a result of application of the highest level of nitrogen might be due to the increase in average bulb parameters and number of cloves/bulb and the effect of nitrogen on vegetative growth aspects which in turn affected the total production. Similar results and explanations are also reported by Kilgori *et al.* (2007) on garlic; El-Desuki (2004) and Nasreen *et al.* (2007) on Onion. Zaman *et al.* (2011) found that production of maximum bulb yield was detected by the application of nitrogen at 150 kg/ha. due to production of taller plants with higher number of leaves which in turn leading to increase formation of vegetative structure for nutrient absorption, photosynthesis and subsequence increased production of assimilates to fill the sink which result in increased bulb size and weight.

Further, the results of Table (5) show that inoculation garlic plants with bio-stimulants had significantly positive effects on garlic yield and its component characters; as well as, bulb characteristics except for bulbing ratio during the first season compared with un-inoculated treatment. The results of Ahmed (2015) showed that Bulbing ratio and number of cloves traits were not, significantly, affected with bio-stimulants in both seasons. In this regard, inoculation garlic plants with bio-stimulants (Halex-2 + garlic extract) reflected the highest mean values for most studied characters. Such findings of bio-stimulants on studied characters could be connected with their positive effects on vegetative measurements which in turn effect on produced bulbs. This positive effect on garlic productivity could be attributed to an increase in mineral absorption by plant and thus an increase in plant contents of vitamins, amino acids and carbohydrates. These findings are in accordance with Shalaby and El-Ramady (2014) and Ahmed (2015) on garlic crop

#### **Effects of the first-order interactions of studied treatments on garlic productivity and bulb quality characteristics**

The results of Table (6) clearly exhibited that most studied characters, significantly, affected the first-order interactions except for bulbing ratio. Addition of 120 kg N/fed. in the form of ammonium nitrate significantly gave the highest values for garlic yield, yield components, neck thickness and bulb diameter. These results could be conjoined with the previous obtained results where the same treatment enhanced the studied vegetative characters. These results positively affected on garlic crop and therefore on bulb characteristics. Cloves firmness had significantly increased when garlic plants fertilized with 90 kg N/fed. in the form of ammonium nitrate. The results generally indicated that increasing the nitrogen rate from 90 up to 120 kg N/fed. negatively affected cloves firmness characteristic.

**Table 5: Averages of yield, yield components and bulb quality characters of Chinese garlic as affected by nitrogenous sources, its rate and bio-stimulants during the seasons of 2013/2014 and 2014/2015.**

Treatments	Season 2013/2014										Season 2014/2015									
	No. of cloves/ bulb	Average bulb weight (g)	Yield/ fed. (ton)	Neck thickness (cm)	Bulb diameter (cm)	Bulbing ratio (%)	Cloves firmness Kg/cm <sup>2</sup>	No. of cloves/ bulb	Average bulb weight (g)	Yield/ fed. (ton)	Neck thickness (cm)	Bulb diameter (cm)	Bulbing ratio (%)	Cloves firmness Kg/cm <sup>2</sup>	Phenol content (mg/g)					
<b>Nitrogen source</b>																				
Ammonium nitrate	12.60a	47.91a	6.73a	1.40a	5.09a	27.51a	4.34a	13.74a	48.53a	6.79a	1.23a	4.46a	27.56a	4.32a	1.899b					
Ammonium sulphate	11.31a	41.16b	5.63b	1.32b	4.61b	28.90a	4.06b	10.21b	34.53b	4.75b	1.08b	4.20b	25.55b	4.02b	1.974a					
<b>Nitrogen rate</b>																				
120 Kg nitrogen /fed.	13.04a	49.57a	6.94a	1.43a	5.16a	27.78a	4.08b	13.42a	45.82a	6.38a	1.25a	4.49a	27.76a	4.05b	1.919b					
90 Kg nitrogen /fed.	10.88b	39.51b	5.42b	1.29b	5.55b	28.62a	4.32a	10.53b	37.25b	5.17b	1.06b	4.17b	25.35b	4.30a	1.954a					
<b>Bio-stimulants</b>																				
Without	10.83c	39.96c	5.63c	1.29b	4.57c	28.27a	3.89c	10.64c	38.07b	5.17b	1.01d	4.1c	25.68b	3.87c	1.914c					
Halex-2 (H)	12.17ab	45.50b	6.23b	1.38a	4.96ab	27.84a	4.11b	11.78b	43.53a	6.09a	1.19b	4.36b	27.15a	4.04b	1.940b					
Yeast extract (Y)	11.79bc	44.11b	6.09b	1.37a	4.73bc	29.16a	4.37a	11.50b	39.65b	5.55b	1.13c	4.31b	26.06b	4.37a	1.925c					
Halex-2 + Yeast extract	13.04a	48.58a	6.76a	1.41a	5.14a	27.53a	4.43a	13.97a	44.88a	6.28a	1.25a	4.56a	27.32a	4.42a	1.968a					

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

This could be explained by the fact that excess of nitrogen depress calcium uptake which influence on ripening attributes and firmness. Fertilization garlic plants with nitrogenous fertilizers containing sulfur component (ammonium sulphate fertilizer) at the rate of 120 or 90 kg N/fed. had significantly positive effects on increasing phenol content compared with the fertilization with ammonium nitrate fertilizer. This result is in harmony with the previously obtained result that in Table (5) and the explanation of Farooqui *et al.* (2009) in relation to the role of sulfur element on the active substances in garlic.

The results of the interaction between nitrogen source x bio-stimulants showed that inoculation growing garlic plants with bio-stimulants generally increased the mean values of garlic yield, its component characters and bulb quality characteristics except for bulbing ratio (Table, 6). The presented data showed that combining ammonium nitrate fertilizer with bio-stimulants (Halex-2 + yeast extract) possessed the highest mean values for garlic yield and yield component characters. Same trend of results were also obtained with bulb quality characteristics; where, neck thickness and bulb diameter gave the highest values with combining ammonium nitrate fertilizer with bio-stimulants (Halex-2 + yeast extract) without significant differences with combining ammonium nitrate with each of Halex-2 or yeast extract during the first season and combining ammonium nitrate with halex-2 for neck thickness during the second season (Table, 6). Combining ammonium sulphate fertilizer with yeast extract, significantly, gave the highest values for cloves firmness during the two years of this study (Table, 6). This result confirms the role of yeast extract in its effect on increasing plant roots and then increasing the absorption of elements, especially, the element of calcium, which has a significant impact on the garlic cloves firmness. The combination between ammonium sulphate fertilizer and bio-stimulants (Halex-2 + yeast extract), significantly, gave the highest value for phenol content. The interaction between nitrogen rate X bio-stimulants showed highly significant effects on No. of cloves/bulb, average bulb weight (g) and garlic yield/fed. (Table, 6). The data cleared that combining 120 kg N/fed. with bio-stimulants (Halex-2 + yeast extract), significantly, possessed the highest mean values for garlic yield and its component characters during both seasons. The obtained results from Table (6) regarding garlic yield and its component characters are highly consistent with the previous results of Table (3). It could conclude that the positive stimulation effects happened for the studied vegetative characters (plant height, No. of leaves/plant and leaves fresh weight) had a significant impact on the garlic productivity.

The combination between ammonium sulphate fertilizer and bio-stimulants (Halex-2 + yeast extract), significantly, gave the highest mean value for phenol

content. Data of Table (6) appeared that combining chemical nitrogenous fertilizers at the rate of 120 kg N/fed. with bio-stimulants (Halex-2 + yeast extract) or with Halex-2 only, significantly, possessed the highest mean values for neck thickness and bulb diameter characteristics during the first season. Same trend of results were, also, obtained where combining chemical nitrogenous fertilizers at the rate of 120 kg N/fed. with bio-stimulants (Halex-2 + yeast extract), significantly, gave the highest mean values during the second season. As for cloves firmness and phenol content, the presented data show that the combination between chemical nitrogenous fertilizers at the rate of 90 kg N/fed. with bio-stimulants (Halex-2 + yeast extract), significantly, gave the highest mean values during the two growing seasons.

#### **Effects of the second-order interactions of studied treatments on garlic productivity and bulb quality characteristics**

Interactions of nitrogen sources x nitrogen rates x bio-stimulants statistically affected No. of cloves/bulb, average bulb weight, yield per fed. and bulb quality characteristics during the two seasons except for bulbing ratio parameter (Table, 7). The results showed that combined ammonium nitrate X120 kg N/fed. X bio-stimulants (Halex-2 + yeast extract) resulted highest values for No. of cloves per bulb during the two seasons. The data of average bulb weight and garlic yield showed that combined ammonium nitrate X120 kg N/fed. X bio-stimulants (Halex-2 + yeast extract) gave highest values without significant differences with combined ammonium nitrate X120 kg N/fed. X Halex-2. Such finding is true during the two experimental seasons. This finding can be linked to previously obtained results for the measured vegetative characters (Table, 4); where, the same two fertilization combinations gave the highest values for the studied vegetative traits; i.e., plant height, number of leaves per plant and leaves fresh weight, which reflected in an increasing garlic yield. This result is in agreement with the findings of Baghalian *et al.* (2006) and Brewster (2008). The data of Table (7) showed that the fertilization combinations containing bio-stimulants (Halex-2 + Yeast extract) produced, in general, best results for garlic yield which outperformed mostly the combinations that did not contain either or both of them at the same rate and source of nitrogen fertilization.

The data of the second-order interaction (nitrogen source x nitrogen rate x bio-stimulants) showed that fertilization combination No. 4, significantly, gave the highest mean values for neck thickness and bulb diameter characteristics during the two growing seasons without significant differences with the combinations No. 2 and 3 during the first season. The results of cloves firmness appeared that fertilization treatments No. 15 and 16, significantly, gave the highest mean values during the two seasons of this study.

**Table 6: Averages of yield, yield components and bulb quality characters of Chinese garlic as affected by the first-order interactions between nitrogenous sources, its rate and bio-stimulants during the seasons of 2013/2014 and 2014/2015.**

Treatments	Season 2013/2014										Season 2014/2015															
	No. of cloves/ bulb		Average yield/ fed.		Neck thickness (cm)		Bulb diameter (cm)		Bulbing ratio (%)		Cloves firmness Kg/cm <sup>2</sup>		No. of cloves/ bulb		Average yield/ fed.		Neck thickness (cm)		Bulb diameter (cm)		Bulbing ratio (%)		Cloves firmness Kg/cm <sup>2</sup>		Phenol content (mg/g)	
	bulb weight (g)	(ton)	(cm)	(cm)	(%)	(cm)	(cm)	(%)	(%)	(Kg/cm <sup>2</sup> )	(Kg/cm <sup>2</sup> )	(Kg/cm <sup>2</sup> )	bulb weight (g)	(ton)	(cm)	(cm)	(%)	(cm)	(cm)	(%)	(Kg/cm <sup>2</sup> )	(Kg/cm <sup>2</sup> )	(Kg/cm <sup>2</sup> )	(Kg/cm <sup>2</sup> )	(mg/g)	
<b>Nitrogen source X Nitrogen rate interaction</b>																										
Ammonium nitrate	120 Kg N/fed	13.79a	54.42a	7.70a	1.47a	5.37a	27.46a	4.24b	14.83a	54.27a	7.59a	1.39a	4.72a	29.37a	4.20b	1.866c										
	90 Kg N/fed	11.42c	41.40c	5.76c	1.32c	4.81b	27.55a	4.44a	12.94b	42.81b	6.00b	1.08b	4.20bc	25.74a	4.44a	1.933b										
Ammonium sulphate	120 Kg N/fed	12.29b	44.72b	6.17b	1.38b	4.95b	28.10a	3.92c	12.00b	37.37c	5.17c	1.12b	4.27b	26.15a	3.90c	1.972a										
	90 Kg N/fed	10.33d	37.61d	5.08d	1.26d	4.28c	29.70a	4.20b	8.42c	31.69c	4.34d	1.03c	4.14c	24.96a	4.15b	1.976a										
<b>Nitrogen source X Bio-stimulants interaction</b>																										
Ammonium nitrate	Without	11.33cde	41.51d	5.96c	1.32d	4.83bc	27.28a	4.39b	12.72bc	43.28c	6.06b	1.13d	4.17de	26.99a	4.28c	1.897d										
	Halox-2(H)	12.92ab	50.11ab	6.98ab	1.41ab	5.16ab	27.48a	4.42b	13.34b	52.19a	7.30a	1.26ab	4.48b	28.13a	4.40bc	1.912cd										
Ammonium sulphate	Without	10.33e	38.42d	5.30d	1.25e	4.30d	29.27a	3.39e	8.56e	32.87c	4.28d	0.98f	4.03e	24.37a	3.45f	1.931c										
	Halox-2(H)	11.42cd	40.89d	5.48d	1.34cd	4.77bc	28.20a	3.80d	10.22d	34.87c	4.88cd	1.11e	4.23cd	26.18a	3.69e	1.958b										
<b>Nitrogen rate X Bio-stimulants interaction</b>																										
120 Kg nitrogen /fed	Without	12.58bc	42.85c	6.20c	1.34e	4.88cd	27.60a	3.94e	12.45b	41.87cd	5.74cd	1.13c	4.25c	26.48a	3.90fg	1.893c										
	Halox-2(H)	12.75b	50.64b	7.02b	1.46ab	5.31ab	27.50a	4.17c	13.00b	48.37ab	6.76ab	1.27b	4.46b	28.46a	4.12cd	1.932b										
90 Kg nitrogen /fed	Without	13.92a	56.13a	7.73a	1.49a	5.49a	27.21a	4.05d	15.33a	49.15a	6.88a	1.38a	4.83a	28.39a	4.02de	1.946b										
	Halox-2(H)	11.58cd	40.36cd	5.44cd	1.30c	4.62cd	28.18a	4.05d	10.56c	38.69c	5.42de	1.10c	4.20c	25.85a	3.97ef	1.948b										
90 Kg nitrogen /fed	Without	9.08e	37.08d	5.06f	1.23d	4.26e	28.95a	3.84f	8.83d	34.28a	4.61f	0.98d	3.95d	24.88a	3.83g	1.935b										
	Halox-2(H)	11.58cd	40.36cd	5.44cd	1.30c	4.62cd	28.18a	4.05d	10.56c	38.69c	5.42de	1.10c	4.20c	25.85a	3.97ef	1.948b										
90 Kg nitrogen /fed	Without	12.17bc	41.03c	5.79d	1.33c	4.79cd	27.88a	4.80a	12.61b	40.60d	5.69cd	1.13c	4.29c	26.27a	4.82a	1.990a										
	Halox-2(H)	11.58cd	40.36cd	5.44cd	1.30c	4.62cd	28.18a	4.05d	10.56c	38.69c	5.42de	1.10c	4.20c	25.85a	3.97ef	1.948b										

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability

**Table 7: Averages of yield, yield components and bulb quality characters of Chinese garlic as affected by the second-order interactions between nitrogenous sources, its rate and bio-stimulants during the seasons of 2013/2014 and 2014/2015.**

Treatments	Season 2013/2014										Season 2014/2015															
	No. of cloves/ bulb		Yield/ fed.		Neck thickness (cm)		Bulb diameter (cm)		Bulbing ratio (%)		Cloves firmness (kg/cm <sup>2</sup> )		No. of cloves/ bulb		Average yield/ fed.		Neck thickness (cm)		Bulb diameter (cm)		Bulbing ratio (%)		Cloves firmness (kg/cm <sup>2</sup> )		Phenol content (mg/g)	
	bulb weight (g)	(ton)	(cm)	(cm)	(%)	(kg/cm <sup>2</sup> )	bulb weight (g)	(ton)	(cm)	(cm)	(%)	(kg/cm <sup>2</sup> )	bulb weight (g)	(ton)	(cm)	(cm)	(%)	(kg/cm <sup>2</sup> )	(mg/g)							
<b>Nitrogen source X Nitrogen rate X Bio-stimulants interaction</b>																										
<b>Ammonium nitrate</b>																										
<b>120 Kg nitrogen /fed.</b>																										
1- Without	13.33b	43.52d	6.61d	1.36def	5.08bcd	26.89a	4.28de	13.22bcd	48.07c	6.73c	1.22c	4.33de	28.15a	4.20d	1.868gh											
2- Halex-2(H)	13.33b	58.17ab	8.08ab	1.50ab	5.51ab	27.62a	4.44c	14.00b	59.00a	8.25a	1.39b	4.64bc	29.97a	4.43c	1.890fg											
3-Yeast ex(Y)	13.33b	54.50bc	7.65b	1.47ab	5.20abcd	28.29a	3.93f	13.78bc	51.67bc	7.23bc	1.41b	4.72b	29.82a	3.90e	1.850h											
4- H + Y	13.17a	61.50a	8.44a	1.56a	5.70a	27.31a	4.30de	18.33a	58.33ab	8.17ab	1.53a	5.19a	29.54a	4.27cd	1.855h											
5- None	9.33fg	39.50def	5.30ef	1.28fg	4.61efg	27.66a	4.50bc	12.22de	38.49def	5.39def	1.03ef	4.00fg	25.82a	4.37c	1.927de											
6- Halex-2(H)	12.50bc	42.06de	5.89d	1.32defg	4.81cdef	27.35a	4.40cd	12.67cde	45.38cd	6.35cd	1.14cd	4.32de	26.30a	4.37c	1.935cde											
7-Yeast ex(Y)	11.33cde	42.00de	5.88d	1.34cdefg	4.79cdefg	28.08a	4.27e	12.44de	38.84def	5.44def	1.03ef	4.17ef	24.80a	4.34cd	1.933cde											
8- H + Y	12.50bc	42.06de	5.97d	1.35cdefg	5.04bcde	26.85a	4.60b	13.22bcd	48.53c	6.80c	1.12de	4.30de	26.05a	4.70b	1.937cde											
9- Without	11.83bcd	42.17d	5.78de	1.32defg	4.69cdefg	28.31a	3.60h	11.67e	35.67efg	4.99efg	1.03ef	4.17ef	24.80a	3.60f	1.919ef											
10-Halex-2(H)	12.17bcd	43.11d	5.96d	1.41bcd	5.11bcde	27.66a	3.90f	12.00de	37.74ef	5.28ef	1.15cd	4.27de	26.95a	3.80e	1.974b											
11-Yeast ex(Y)	12.50bc	42.83d	5.94d	1.37cde	4.70cdefg	29.33a	4.37cde	12.00de	36.10efg	5.05efg	1.07def	4.17ef	25.61a	4.43c	1.960bc											
12- H + Y	12.67bc	50.75c	7.01c	1.49bc	5.28abc	27.11a	3.80fg	12.33de	39.97de	5.59de	1.22c	4.47cd	27.24a	3.77e	2.038a											
<b>Ammonium sulphate</b>																										
<b>90 Kg nitrogen /fed.</b>																										
13- Without	8.83g	34.67f	4.81f	1.18h	3.90h	30.24a	3.18f	5.44g	30.07g	4.21g	0.93g	3.90g	23.94a	3.30g	1.943cde											
14-Halex-2(H)	10.67def	38.67def	5.00f	1.27g	4.42fgh	28.74a	3.70gh	8.45f	32.00g	4.48fg	1.07def	4.20ef	25.41a	3.57f	1.962bc											
15-Yeast ex(Y)	10.00efg	37.12ef	4.90f	1.30efg	4.24gh	30.95a	4.90a	7.78f	32.00g	4.48fg	1.00g	4.17ef	24.00a	4.80ab	1.956bcd											
16- H + Y	11.83bcd	40.00de	5.60de	1.31efg	4.55efg	28.86a	5.00a	12.00de	32.67fg	4.57fg	1.13cd	4.28de	26.48a	4.94a	2.043a											

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

In this respect, Abdel-Razzak and El-Sharkawy (2013) clarified that inoculation of garlic growing plants with Halex-2; caused increases in tonnage bulbs yield during both growing seasons, and clove weight in the first year compared to non-inoculated plants. They explained that the high bulbs yield and clove weight of garlic could be taken place due to the enhancing effects of non-symbiotic N<sub>2</sub> fixation bacteria on morphology / or physiology of the root system. Noel *et al.* (1996) mentioned that the non-symbiotic N<sub>2</sub> fixing bacteria; *Azotobacter* and *Azospirillum*, created sufficient amounts of Indole acetic acid (IAA), gibberellins (GAs) and cytokinins, as well as, synthesized some vitamins. These products increase the surface area / unit root length and improved the root hair branching with an ultimate lead to increase the uptake of nutrients and adsorption of water from the soil that eventually yield larger and in many cases, more productive plants (Dobbelaere *et al.*, 2001). The role of yeast in influencing the growth of plants, especially vegetables has stated in several reports. El-Ghamriny *et al.* (1999) reported that the dry bread yeast (*Saccharomyces cerevisiae*) is a kind of the used bio-fertilizers in soil fertilization or in foliar application on the shoots of vegetable crops. The authors added that dry yeast is a natural bio-substance and it turned out that it has motivational benefits, nutritional and protective roles when it is applied on to vegetable crops under stressful condition because of it contains a lot of hormones, sugars, amino acids and nucleic acids, in addition to numerous of both vitamins and minerals. The results of Fawzy *et al.* (2012) on onion indicated that, yeast had positive enhancing effects via providing supplemental doses of N, P and K%; as well as, some trace elements components on vegetative growth, yield and its quality; as well as, all chemical compositions compared with the untreated plants.

Data of Table (7) showed that phenol content in garlic bulb was found to be significantly influenced with the tested combination treatments. Data revealed that the

**Table 8: Averages of chemical constituents of Chinese garlic bulbs as affected by nitrogenous sources, its rate and bio-stimulants during the season of 2014/2015**

Treatments	Season 2014/2015		
	Nitrogen (ppm)	Phosphorus (ppm)	Potassium (ppm)
	<b>Nitrogen source</b>		
Ammonium nitrate	4.22a	2.00a	3.38a
Ammonium sulphate	2.96b	1.93a	3.40a
	<b>Nitrogen rate</b>		
120 Kg nitrogen /fed.	4.94a	2.10a	3.43a
90 Kg nitrogen /fed.	2.24b	1.83b	3.35a
	<b>Bio-stimulants</b>		
None	1.60d	1.80b	3.26b
Halex-2 (H)	4.31b	1.75b	3.30b
Yeast extract (Y)	2.48c	2.51a	3.27b
Halex-2 + Yeast extract	5.97a	1.80b	3.75a

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

treatments No. 12 (120 kg nitrogen /fed. at the form of ammonium sulphate + Halex-2 + Yeast extract) and No. 16 (90 nitrogen kg/fed. at the form of ammonium sulphate + Halex-2 + Yeast extract), significantly, gave the highest mean values for phenol content (Table, 7). The findings showed that the combination treatments with ammonium sulfate fertilizer; generally, gave highest phenol content values in garlic bulbs. This finding might be attributed to sulphur element present in ammonium sulphate fertilizer, which could have a positive effect on the active substances especially phenolic in garlic. The results also showed that the addition of bio-stimulants (Halex-2 + yeast extract) to the ammonium sulphate mineral fertilizer could have a positive role in increasing the proportion of phenol in garlic cloves.

As for cloves firmness, the results of Table (7) showed that the two fertilization combinations; ammonium sulphate X 90 kg nitrogen X bio-stimulants (Halex-2 + yeast extract) and ammonium sulphate X 90 kg nitrogen X yeast extract, significantly, gave the highest mean values. These high values given for garlic firmness means that these two fertilization combinations could be desirable for increasing the storability of garlic crop.

#### **Main effects of the studied treatments on chemical constituents of garlic bulbs**

The data presented in Table (8) showed that nitrogen content had, significantly, affected by nitrogen source. The highest mean value for nitrogen was obtained when ammonium nitrate fertilizer was applied. Both of phosphorus and potassium contents did not affect by nitrogen source treatments (Table, 8). Nitrogen rate appeared to have a role in influencing garlic bulb contents of nitrogen and phosphorus. Increasing nitrogen rate from 90 kg/fed. up to 120 kg/fed.; seemed to have a significant positive effect on increasing the garlic bulb contents of both nitrogen and phosphorus.

Meanwhile, potassium content did not affect with increasing nitrogen fertilization rate upon raising from 90 kg/fed up to 120 kg/Fed. Likewise, the gained results showed that bio-stimulants, also, had significant effects on N, P, and K contents respectively. The highest mean values of nitrogen and potassium contents were obtained upon inoculation garlic plants with the combination of both Halex-2 + yeast extract. On the other hand, phosphorus possessed the highest mean value due to yeast extract treatment. These positive effects of bio-stimulants on mineral constituents might be attributed to the role of Halex-2 in increasing nitrogen uptake by plant and the effect of yeast extract because its high contents of carbohydrates, amino acids, sugars, fatty acids, proteins, hormones, macro and micro- nutrients (Khedr and Farid, 2002).

#### Effects of the first-order interactions on chemical constituents of garlic bulbs

The highest level of total nitrogen content of garlic cloves was obtained due to the combination between ammonium nitrate fertilizer and of 120 kg N/Fed. The highest values of phosphorus content were obtained owing to the combinations of ammonium nitrate X 120 kg N/Fed., and ammonium sulphate X 120 kg N/Fed.

without significant differences between them. The interactions between ammonium nitrate X 90 kg N/Fed., and ammonium sulphate X 120 kg N/Fed.; possessed the highest levels of potassium, as shown in Table (9). As for the effect of the interaction between nitrogen source and bio-stimulants, it could be shown that combination of ammonium nitrate fertilizer with bio-stimulants (Halex-2 + yeast extract), significantly, gave the highest value for nitrogen content (Table, 9). On the other extreme, the combinations of ammonium nitrate X yeast extract, and ammonium sulphate X yeast extract; produced the highest level of phosphorus content with significant differences among the other tested interactions. The results of potassium content showed that the highest potassium content in garlic cloves were obtained by both interactions of ammonium nitrate X bio-stimulants (Halex-2 and yeast extract), and ammonium sulphate X bio-stimulants (Halex-2 and yeast extract) without significant differences between them. The interactions of 120 kg N. X bio-stimulants (H+Y), 120 kg N. X yeast extract and 90 kg N. X bio-stimulants (H+Y); gave rise to the highest values for nitrogen, phosphorus and potassium, respectively with significant differences among the other tested interactions.

**Table 9: Averages of chemical constituents of Chinese garlic bulbs as affected by the first-order interactions between nitrogenous sources, its rate and bio-stimulants during the season of 2014/2015**

Treatments	Season 2014/2015			
	Nitrogen (ppm)	Phosphorus (ppm)	Potassium (ppm)	
<b>Nitrogen source X Nitrogen rate interaction</b>				
Ammonium nitrate	120 Kg N/fed.	5.39a	2.13a	3.23b
	90 Kg N/fed.	3.04c	1.86b	3.53a
Ammonium sulphate	120 Kg N/fed.	4.48b	2.06a	3.63a
	90 Kg N/fed.	1.44d	1.81b	3.17b
<b>Nitrogen source X Bio-stimulants interaction</b>				
Ammonium nitrate	Without	1.65f	1.91b	3.11c
	Halex-2(H)	5.85b	1.76bc	3.25bc
	Yeast ex.(Y)	2.28e	2.55a	3.38bc
	H + Y	7.09a	1.77bc	3.80a
	Ammonium sulphate	Without	1.54g	1.70c
Ammonium sulphate	Halex-2(H)	2.76d	1.73bc	3.33bc
	Yeast ex.(Y)	2.68d	2.47a	3.15bc
	H + Y	4.86c	1.84bc	3.70a
<b>Nitrogen rate X Bio-stimulants interaction</b>				
120 Kg nitrogen /fed.	Without	2.04f	1.87cd	3.55b
	Halex-2(H)	5.93b	1.90cd	3.55b
	Yeast ex.(Y)	3.48d	2.63a	3.24cde
	H + Y	8.29a	1.99c	3.39bc
	90 Kg nitrogen /fed.	Without	1.15h	1.73de
Halex-2(H)		2.68e	1.59e	3.03de
Yeast ex.(Y)		1.48g	2.39b	3.30bcd
H + Y		3.66c	1.62e	4.11a

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

### Effects of the second-order interactions on chemical constituents of garlic bulbs

The results of nitrogen content demonstrated that the combination of ammonium nitrate X120 kg nitrogen/Fed. X bio-stimulants (H+Y); resulted in the highest mean value for nitrogen content followed with the combination ammonium sulphate X 120 kg nitrogen/Fed. X bio-stimulants (H+Y). Phosphorus content was found to be significantly influenced with the fertilization combinations; where, the combination of ammonium nitrate X 120 kg N / fed. X yeast extract possessed the highest value without significant differences among the two combinations of ammonium nitrate X. 90 kg N / fed. X yeast extract and ammonium sulphate X 120 kg N / fed. X yeast extract. Meanwhile, potassium contents appeared; somewhat, significantly affected by fertilization treatments since, fertilization combinations of ammonium nitrate X 90 kg N / fed. X bio-stimulants (H+Y) and ammonium sulphate X 120 kg N / fed. X Halex-2; brought about the highest mean values without significant differences between them (Table, 10).

El-Zohiri and Abdou (2009) stated that nitrogen and phosphorus levels were, significantly, increased with increasing nitrogen fertilization from 60 up to 120 kg/fed. The authors explained that raising nitrogen fertilization led to enhance vegetative growth and in turn increased the uptake of nitrogen element. Similar trend of results was also obtained by El-Desuki (2004) on onion.

### Effects of fertilization combination treatments on antifungal activities of garlic extracts against tested phytopathogenic

Antifungal activities of garlic (16 fertilization treatments) extracts are presented in Table (11). The results showed that the most active extract is the one extracted from the planting materials of the growing plants fertilized with ammonium sulphate at the rate of 90 kg N/fed. + Halex-2 + yeast extract against *Fusarium oxysporium*. The garlic extracts which extracted from planting materials fertilized with ammonium sulphate (120 kg N/fed.) + Halex-2 + yeast extract and the one extracted from planting materials fertilized by ammonium sulphate (90 kg N/fed.) + Halex-2 + yeast extract possessed the most effective extracts against *Alternaria alternate*. The results, clearly, showed that the treatment No. 16 significantly gave highest mean values as for Antifungal activities of the tested garlic extracts against the two pathogens of *F. oxysporium* and *A. alternate* without significant differences with the treatment No. 12 for antifungal activities of *A. alternate*. These results could be explained by the fact that these two extracts (No. 12 and 16) which showed the highest efficiency in controlling mycelial growth, having the highest mean values for phenol content; as mentioned before, as well as, its containing sulphur element which exhibit promising fungi static activities and they warrant more consideration as prospective antimicrobials.

**Table 10: Averages of chemical constituents of Chinese garlic bulbs as affected by the second-order interactions between nitrogenous sources, its rate and bio-stimulants during the season of 2014/2015**

Treatments	Season 2014/2015				
	Nitrogen (ppm)	Phosphorus (ppm)	Potassium (ppm)		
	Nitrogen source X Nitrogen rate X Bio-stimulants interaction				
Ammonium nitrate	120 Kg nitrogen /fed.	1- Without	2.15h	2.07def	3.25efg
		2- Halex-2(H)	7.80c	1.96efg	3.11fg
		3- Yeast ex(Y)	3.07g	2.68a	3.32efg
		4- H + Y	8.55a	1.83fgh	3.26efg
	90 Kg nitrogen /fed.	5- Without	1.15l	1.75ghi	2.97gh
		6- Halex-2(H)	3.90f	1.56hi	3.39ef
		7- Yeast ex(Y)	1.50k	2.43abc	3.45def
		8- H + Y	5.63d	1.70ghi	4.33a
Ammonium sulphate	120 Kg nitrogen /fed.	9- Without	1.94i	1.68ghi	3.84bcd
		10- Halex-2(H)	4.06e	1.84fgh	3.90ab
		11- Yeast ex(Y)	3.90f	2.58ab	3.16efg
		12- H + Y	8.02b	2.15cde	3.52cde
	90 Kg nitrogen /fed.	13- Without	1.14l	1.72ghi	2.97gh
		14- Halex-2(H)	1.47k	1.62hi	2.67h
		15- Yeast ex(Y)	1.50k	2.35bcd	3.14efg
		16- H + Y	1.70j	1.54i	3.89bc

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

**Table 11: Mean values of inhibition zone of tested plant pathogens as affected by fertilization treatments.**

No.	Fertilization treatments	Inhibition zone (mm) of tested plant pathogens	
		<i>Fusarium oxysporium</i>	<i>Alternaria alternate</i>
1	Ammonium nitrate (120 kg N/fed.)	17.67c	22.33cd
2	Ammonium nitrate (120 kg N/fed.) + Halex-2	14.33e	24.00bc
3	Ammonium nitrate (120 kg N/fed.) + yeast extract	16.33d	20.00def
4	Ammonium nitrate (120 kg N/fed.)+ Halex-2 + Yeast extract	19.00b	19.67ef
5	Ammonium nitrate (90 kg N/fed.)	15.67d	20.67def
6	Ammonium nitrate (90 kg N/fed.) + Halex-2	14.33e	25.67b
7	Ammonium nitrate (90 kg N/fed.) + Yeast extract	13.00f	16.33gh
8	Ammonium nitrate (90 kg N/fed.) + Halex-2 + Yeast extract	16.33d	21.67cde
9	Ammonium sulphate (120 kg N/fed.)	16.3d	18.33fg
10	Ammonium sulphate (120 kg N/fed.) + Halex-2	18.33bc	18.67fg
11	Ammonium sulphate (120 kg N/fed.) + Yeast extract	13.33ef	18.33fg
12	Ammonium sulphate (120 kg N/fed.) + Halex-2 + Yeast extract	13.33ef	28.33a
13	Ammonium sulphate (90 kg N/fed.)	16.33d	18.33fg
14	Ammonium sulphate (90 kg N/fed.) + Halex-2	16.33d	15.67h
15	Ammonium sulphate (90 kg N/fed.) + Yeast extract	16.33d	21.67cde
16	Ammonium sulphate (90 kg N/fed.) + Halex-2 + Yeast extract	20.33a	28.00a

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

It is known that both phenolic substances and Sulphur have an important role in this regard. Garlic extract have the ability to prevent and manage viral, fungal and even helminthic infections. Newly obtained garlic has been found to impart a significant role in managing food poisoning through killing the causative agents such as *E. coli* (Shuford *et al.*, 2005; Abdulzahra and Mohammed, 2014).

#### Effects of the garlic extract concentrations on the percentage of mycelial inhibitions

The results of Table (12) showed that mycelial growth of tested fungi significantly affected with the differences in concentrations of garlic extracts. Concentration of 2.5 % of garlic extract significantly gave the best results for the percentage of mycelial inhibitions against *F. oxysporium* and *A.alternate*; as well (Table, 12 and Plate, 1). Bagiu *et al.* (2012) showed that garlic plants, inhibited growth of cells of *Candida*

spp. Allicin and *S*-methyl cysteine sulfoxide isolated and identified were capable of inducing antifungal activity.

#### Effects of the fertilization treatments on volatile compounds in garlic bulbs

Table (13) shows differences among three garlic extracts in their content of volatile compounds. The results showed that there were significant differences among the three garlic extracts for the assayed volatile compounds content due to fertilization treatments. The results showed that the garlic extracts resulted from fertilization garlic plants with ammonium sulphate (120 kg N/fed.) + Halex-2 + yeast extract or which fertilized with ammonium sulphate (90 kg N/fed.) + Halex-2 + yeast extract, generally, contained higher concentration from volatile compounds, especially, the compounds having Sulphur element.

**Table 12: Mean values of mycelial growth and the percentage of inhibitions at different concentrations of most active garlic plant extracts on mycelial growth of tested fungi.**

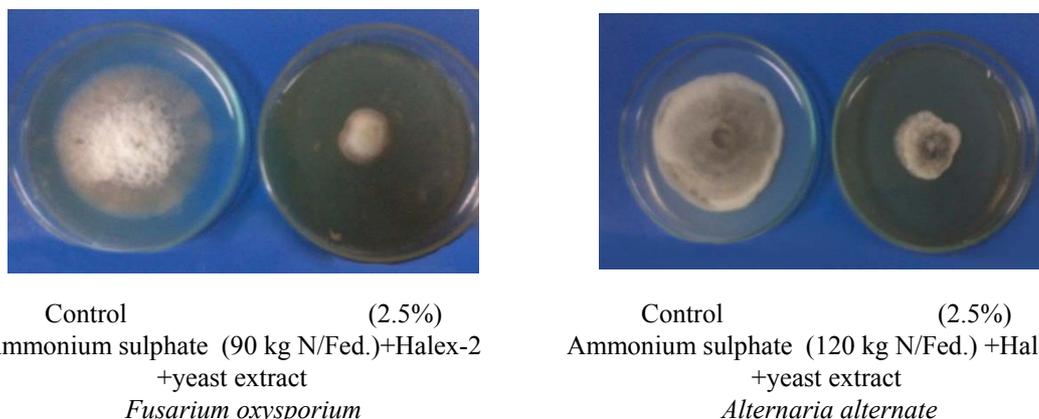
Concentrations of garlic extract	Diameter (mm) of mycelial growth of tested fungi after 6 days			
	Ammonium sulphate (90 kg N/fed.) + Halex-2 + Yeast extract against <i>Fusarium oxysporium</i>		Ammonium sulphate (120 kg N/fed.)+ Halex-2 +Yeast extract against <i>Alternaria alternate</i>	
	Mean of mycelial growth (mm)	% of inhibition	Mean of mycelial growth (mm)	% of inhibition
2.5 %	16.00 d	77.26	22.00 d	68.22
1.5 %	34.33 c	52.46	35.00 c	49.49
0.5 %	41.33 b	43.42	43.00 b	37.95
Control	73.00 a		69.33 a	

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.

Table 13. Mean values of the assayed volatile compounds in garlic bulbs as affected with three fertilization treatments,

Fertilization treatments	Volatile compounds (%)											
	Diallyl sulfide	Disulfide, methyl-2-propenyl	2-Oxazolin-ethione	1,2-Diisopropenyl cyclobutane	Diallyl disulphide	Diallyl tetrasulphide	Trisulfide, methyl-2-propenyl	3-Vinyl-1,2-dithiacyclohex-5-ene	Disulfide, 3-methyl-butyl-1-methyl-propyl	2-vinyl-dithiin	Diallyl trisulfide	2,3-Dicarboxy thiophene
Ammonium nitrate (120 kg N/fed.) (Control)	1.87c	4.91c	2.51c	1.65c	61.13c	Traces c	8.81c	0.94a	0.44c	0.88a	5.12c	1.07c
Ammonium sulphate (120 kg N/fed.)+Halex-2+yeast extract	1.97b	5.35b	4.08a	2.24a	63.31b	0.60b	15.47a	0.83c	0.51b	0.71b	9.39a	1.50b
Ammonium sulphate (90 kg N/fed.)+ Halex-2+yeast extract	3.47a	8.22a	3.45b	1.82b	64.18a	0.72a	12.36b	0.90b	0.60a	0.69c	9.23b	1.78a

Means having an alphabetical letter in common, within a comparable group of means, do not significantly differ, using least significant differences test procedure (L.S.D) at  $p=0.05$  level of probability.



**Plate 1: Effect of different concentrations of most active garlic plant extracts on mycelial growth of tested fungi**

The obtained results of Table (13) in addition to the previously results of phenolic contents (Table, 7) might explain the superiority of the two previous fertilization treatments in inhibition of the two studied pathogens.

In particular, diallyl disulfide is the main reason for inhibiting the growth of molds and bacteria by garlic oil (Amonkar and Banerji, 1971). This might be due to damage caused by garlic to the outer surface of the fungal cells together with alterations in the fat content of the cell. It is also probable that garlic may reduce the adhesion of fungal cells to epithelial membrane. Numerous studies indicated that garlic plants have biological and medicinal activities. These activities are mainly because they include high percentages of organo-sulphur compounds content. The sulphur-containing constituting in *Allium* crops are the S-alk(en)yl-L-cysteine sulphoxide (Corzo-Martinez *et al.*, 2007). One of these important organo-sulphur compounds that accumulate in garlic plant extracts has been identified as allicin (Baustista *et al.*, 2005). Garlic extracts are the breakdown products of allicin, including diallyltrisulphide (DATS) and ajoene, which have greater antifungal effects than allicin (Corzo-Marinez *et al.*, 2007).

### CONCLUSIONS

Under the conditions of this study, the results showed that addition of ammonium nitrate fertilizer through the drip irrigation system; gave better results for the studied traits compared to addition of ammonium sulphate fertilizer. Inoculation growing garlic plants with bio-stimulants was found to have positive effective in reducing the mineral nitrogen fertilizer being applied by 25 % as compared with the commonly recommended dose (120 kg nitrogen per feddan). Also, the obtained data cleared that garlic productivity could be maximized throughout

inoculation garlic plants, which fertilized with 120 kg nitrogen per fed, with bio-stimulants (Halex-2 + yeast extract). This maximization in garlic productivity reached 27.69 % and 21.40 % during the first and second seasons, respectively compared to the addition of 120 kg nitrogen of ammonium nitrate fertilizer. Garlic extracts may be an attractive alternative for the use of a natural product to control the fungi that attack economical crops, especially, *Fusarium oxysporum* and *Alternaria alternate*, avoiding chemical fungicides application.

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