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Response of Garlic Plants (*Allium sativum* L.) to Foliar Application of Some Bio-Stimulants

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THIS STUDY was carried out in a clay loamy soil, at the Experimental Farm, Fac. of Agric., Tanta Univ., Egypt, during 2011/2012 and 2012/2013 seasons. The aim of this study was to evaluate the effect of some bio-stimulants, dry yeast (at three concentrations 2, 3 and 4 $g.L^{-1}$) and chitosan (at three concentrations 2, 4, 6 $ml.L^{-1}$) on growth, yield, quality and storability of "clone sids-40" garlic plants. Bio-stimulants were applied at 30, 45, 60 and 75 days from planting date. The experiment was designed in a completely randomized blocks (CRB) with three replicates. Foliar application of dry yeast (3 and 4 $g.L^{-1}$) and chitosan (4 and 6 $ml.L^{-1}$) effectively increased plant height, leaf number, leaves fresh weight, yield and its components in both seasons. Same treatments were positively affected N, P and K contents of leaves and bulbs, total carbohydrate and volatile oil of the bulbs in both seasons. Weight loss of the bulbs was the least with the application of chitosan (6 and 4 $ml.L^{-1}$) in both seasons. In general, foliar application of dry yeast (3 and 4 $g.L^{-1}$) and chitosan (4 and 6 $ml.L^{-1}$) can be recommended to improve productivity, quality and storability of garlic plants grown in clay loamy soil.

Keywords: Bio-stimulate, Yeast, Chitosan, Productivity, Storability, garlic (*Allium sativum* L.).

Garlic (*Allium sativum* L.) is one of the most important vegetables in Egypt for both local consumption and exportation. It is cultivated for its flavor and medicinal properties, with the latter steadily arising worldwide (Collin, 2004). There are many factors, such as fertilization and bio-stimulating substances affect plant growth, productivity and bulb quality (El-Morsy, 2004). Using natural products, such as dry yeast and chitosan in garlic production has been adopted for safe agricultural system during the last years.

Yeast is considered as a natural source of phytohormones (especially cytokinins), vitamins, enzymes, minerals and amino acids. It has stimulatory effects on plant growth and productivity of beans (Barnett *et al*., 1990, Amer, 2004 and El-Tohamy & El-Greadly, 2007), on eggplants (El-Tohamy *et al*., 2008), on tomatoes (Fathy *et al*., 2000), on peas (El-Desuki and El-Greadly, 2006), on potatoes (Taha & Omar, 2010 and Ahmed *et al*., 2011), on cucumber (Shehata *et al*., 2012), and on garlic (Shalaby and El-Ramady, 2014).

It also has a positive effect on cell division and enlargement, protein and nucleic acid synthesis, and chlorophyll formation of potatoes (Kraig & Haber, 1980, and Castelfranco & Beale, 1983). The cytokinins content of the yeast has an important role on plant stress resistance (Barnett *et al*., 1990).

Chitosan is a natural polymer derived from deacetylation of chitin. Chitin is readily available from shellfish waste from food processing. As a high molecular polymer, nontoxic, bioactive agent, Chitosan has become a useful appreciated compound due to its fungicidal effects and elicitation of defense mechanisms in plant tissues (Terry and Joyce, 2004 and Shehata *et al*., 2012). Chitosan coating is known for its ability to extend the storage life of fruits and vegetables. Chitosan forms a semipermeable film that regulates gas exchange, reduces respiration and transpiration rates, and slows down the ripening processes. (Shehata *et al*., 2012). This effect has been reported for numerous horticultural commodities such as tomatoes, strawberries, apples, mangoes, bananas, and bell peppers (Jiang & Li, 2001 and Bautista-Baños *et al*., 2006). Foliar application of chitosan has been shown to stimulate plant growth (Kim, 2005), to increase vegetative growth, yield and quality of vegetable crops (Walker *et al*., 2004, Abdel-Mawgoud *et al*., 2010, Ghoname *et al*., 2010, El-Tanahy *et al*., 2012 and Fawzy *et al*., 2012), and to improve storability of fruits and vegetables (El Ghaouth *et al*., 1991).

Therefore, the aim of this study was to investigate the effect of some bio stimulants *i.e.* chitosan and yeast extract on growth, yield, bulb quality and storability of garlic under clay soil.

Materials and Methods

This experiment was carried out during two successive seasons, 2011/2012 and 2012/2013, at the Experimental Farm, Fac. of Agric., Tanta Univ., Egypt, on "clone sids-40" garlic plants. The soil type is loamy clay, and the chemical properties of the soil are presented in Table 1. Soil samples were collected at 25 cm below soil surface.

TABLE 1. Chemical physical analysis of soil before sowing according to Ryan *et al*. (1996).

Sand = 14.98 %	N (available) = 45.7 mg/100g	Ca++ = 5.99 (meql)	HCO3- = 4.81 (meql)
Silt = 38.45 %	P (available) = 7.2 mg/100g	Mg++ = 6.13 (meql)	Cl- = 9.27 (meql)
Clay = 46.57 %	K (available) = 178.9 mg/100g	Na+ = 14.61 (meql)	SO4-- = 8.84 (meql)
pH = 7.84		EC = 1.3 (ds/m)	

The experimental unit area was 12 m^2, with four rows, 5 m length and 60 cm width. Garlic cloves were selected uniform in shape and size. The cloves were sown on both two sides of the rows at distance of 7 cm apart. Planting date was on October 3^{rd} and 8^{th} during the first and the second seasons, respectively. The cultural practices were done according to the recommendation of Ministry of Agriculture, Egypt.

Treatments and experimental design

The experiment included 7 treatments from some bio-stimulants as follows: Three different concentrations of yeast extract (2, 3 and 4 $g.L^{-1}$), chitosan extract (2, 4, 6 $ml.L^{-1}$) and control were applied at 30, 45, 60 and 75 days from sowing date in both seasons. Tap water was sprayed to the control of plants. The experiment was designed in a complete randomized blocks (CRB) with three replicates.

Data recorded

Ten plants were selected randomly from each replicate at 145 days after planting to measure plant height (cm), number of leaves per plant, and leaf fresh and dry weight (g) per plant. At harvest (190 days after sowing), all plants for each replicate were harvested and the total yield per feddan was calculated after curing for 7 days . A random sample of 10 bulbs was taken from each replicate to determine bulb fresh weight (g), number of cloves/ bulb, and bulbing ratio. Bulbing ratio was calculated according to Mann (1952) = Neck diameter (cm) / Bulb diameter (cm). Total nitrogen (N), phosphorus (P) and potassium (K) in dry matter of leaves and bulbs, and the total hydrolysable carbohydrates in dry bulbs were determined according to the methods described in A.O.A.C (1995). Volatile oil content was determined in fresh garlic cloves, also, according to the methods described in A.O.A.C (1995).

After curing, random samples of 10 kg of garlic plants were taken from each replicate and stored for 9 months at normal room conditions. Monthly average air temperature and relative humidity during storage time are presented in Table (2). The percentages of total weight loss were calculated every two months up to the end of the storage period (1, 3, 5, 7 and 9 months). Sprouting and decay were recorded only at the end of storage period.

Statistical analysis

Data were analyzed by MSTATC computer software program (Bricker, 1991) using ANOVA with the least significant difference (LSD) at the $P \leq 0.05$.

TABLE 2. Monthly average of maximum and minimum air temperature and relative humidity during storage period.

Month	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (RH%)	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (RH%)
	2012			2013		
April	33.24	15.06	30.05	29.13	12.38	38.94
May	37.01	18.50	30.71	35.53	18.00	32.13
June	41.33	21.83	31.32	37.24	20.05	33.51
July	42.71	23.67	33.52	37.21	20.26	38.98
August	41.75	24.24	34.08	38.10	21.26	37.98
September	38.05	21.66	41.79	35.17	19.79	44.50
October	33.87	20.30	47.08	30.17	15.58	48.14
November	27.48	16.85	55.48	27.11	14.62	51.55
December	21.75	11.26	53.67	20.09	8.48	54.71

Data recorded by Gemmeza Meteorological Station, Gharbia governorate, Egypt.

Results and Discussion

Growth characteristics

Foliar application of dry yeast (2, 3 and 4 $g.L^{-1}$) and chitosan (2, 4 and 6 $ml.L^{-1}$) was significantly improved leaf fresh weight, plant height, and the number of leaves per plant, compared to the control during both seasons (Table 3). The most pronounced effect was noticed with the highest concentrations of both dry yeast and chitosan, but no effect was noticed on plant dry weight at any concentration during both seasons. The positive effect of yeast extract on plant vegetative characteristics was mainly due to the natural content of cytokinins, enzymes, amino acids, vitamins and mineral nutrients (Khedr and Farid, 2002, Mahmoud, 2001) that positively affect cell division and elongation, nucleic acid synthesis, protein and chlorophyll formation (Kraig & Haber, 1980 and Castelfranco & Beale, 1983). Improving growth and productivity of vegetable crops by application of yeast extract were recorded by several studies such as (El-Tohamy & El-Greadly, 2007 and Fawzy *et al*., 2010) on beans, (El-Tohamy *et al*., 2008) on eggplant, (Fathy *et al*., 2000) on tomatoes, (El-Desuki and El-Greadly, 2006) on pea, (Ghoname *et al*., 2010) on sweet pepper, (Ahmed *et al*., 2011 and Ahmed *et al*., 2013) on potato, Shehata *et al*. (2012) on cucumber and Shalaby and El-Ramady (2014) on garlic.

The stimulatory effect of chitosan on vegetative growth and bulb growth is may be due to some amino acids components that required for plant growth. Chitosan application was proved to stimulate plant growth and production in strawberry (Abdel-Mawgoud *et al*., 2010), on sweet pepper (Ghoname *et al*., 2010), on cowpea (El-Tanahy *et al*., 2012), and on cucumber (Shehata *et al*., 2012).

TABLE 3. Effect of some bio-stimulants on the vegetative growth characteristics of garlic plants during 2011/2012 and 2012/2013 seasons.

Treatments	2011/2012				2012/2013			
	Plant height (cm)	No. of leaves/ plant	Leaves fresh wt. (g)	Leaves dry wt. (%)	Plant height (cm)	No. of leaves/ plant	Leave fresh wt. (g)	Leaves Dry wt. (%)
Yeast (2 $g.1^{-1}$)	79.67	9.52	28.71	13.77	75.43	9.21	29.34	14.38
Yeast (3 $g.1^{-1}$)	84.32	10.11	30.51	13.91	79.99	10.11	32.15	14.55
Yeast (4 $g.1^{-1}$)	88.12	10.57	31.38	14.09	82.34	10.75	33.21	14.75
Chitosan (2 $ml.1^{-1}$)	78.03	9.99	28.85	13.54	74.11	8.75	29.42	14.21
Chitosan (4 $ml.1^{-1}$)	83.11	9.91	31.09	13.72	78.47	9.86	31.99	14.05
Chitosan (6 $ml.1^{-1}$)	86.21	10.26	31.77	13.98	80.91	10.38	32.53	14.51
Control	74.98	8.98	28.1	13.47	69.88	8.11	28.52	13.93
L.S.D. ($P \leq 5\%$)	3.01	0.34	1.05	n.s	2.17	0.23	0.99	n.s

Total yield and yield components

All bio-stimulants treatments clearly improved total yield, bulb weight and bulb diameter compared to the control (Table 4). The application of dry yeast (4 $mg.L^{-1}$) and chitosan (6 $ml.L^{-1}$) had the most pronounced effect on total yield, 8.299 and 8.189, ton/ feddan in the first season, and 8.359 and 8.254 ton/ feddan in the second season, respectively. The same treatments increased bulb weight and diameter significantly in both seasons. The lowest yield was obtained from the control in both seasons. Bulbing ratio and number of cloves were not significantly affected with any treatments in both seasons. The positive effect of dry yeast is may be attributed to the increase in plant nutrient contents, amino acids, vitamin B and cytokinins (Glick, 1995 and Fathy & Farid, 1996). Vitamins and amino acids increase the metabolic processes and the levels of endogenous hormones, *i.e.* GA_3 and IAA, which affect the final bulb weight and size (Chaliakhyan, 1957 and Sarhan & Abdullah, 2010). Similar trend of results, as previously, were reported by several scientists for several crops (Shehata *et al*., 2012 on cucumber, Ahmed *et al*., 2013 on potato and Shalaby and El-Ramady, 2014 on garlic).

Chitosan has a stimulatory effect on plant growth, and improve the total yield, as well as plant resistance to diseases in several vegetable and fruit crops (Shehata *et al*., 2012). The increase in bulbs yield could be attributed to the increase in some amino acids and nutrients required for growth. This results are in agreement with previously reported results on strawberry (Abdel-Mawgoud *et al*., 2010), sweet pepper (Ghoname *et al*., 2010), cowpea (El-Tanahy *et a*l., 2012), and cucumber (Shehata *et al*., 2012).

TABLE 4. Effect of some bio-stimulants on yield and quality of garlic bulbs during 2011/2012 and 2012/2013 seasons.

Treatments	2011/2012					2012/2013				
	Total bulb yield (ton /fed.*)	Bulb fresh wt. (g)	Cloves no. / bulb	Bulb diam-eter (cm)	Bulb-ing ratio	Total Bulb Yield (ton /fed.*)	Bulb fresh wt. (g)	Cloves no. / bulb	Bulb diam-eter (cm)	Bulb-ing ratio
Yeast (2 $g.1^{-1}$)	7.917	81.65	22.58	6.04	0.23	8.085	75.23	21.76	5.81	0.22
Yeast (3 $g.1^{-1}$)	8.124	87.12	21.95	6.25	0.23	8.250	84.34	22.56	6.04	0.21
Yeast (4 $g.1^{-1}$)	8.299	90.23	22.99	6.52	0.22	8.359	88.99	23.55	6.15	0.19
Chitosan (2 $ml.1^{-1}$)	7.927	79.89	20.65	5,99	0.23	8.041	74.21	21.61	5.81	0.23
Chitosan (4 $ml.1^{-1}$)	8.055	85.87	20.37	6.20	0.22	8.187	81.34	24.00	6.05	0.21
Chitosan (6 $ml.1^{-1}$)	8.189	87.11	23.19	6.34	0.21	8.254	84.45	23.01	6.11	0.20
Control	7.763	76.11	18.95	5.41	0.25	7.959	70.21	20.11	5.32	0.24
L.S.D. ($P \leq 5\%$)	0.205	2.15	n.s	0.51	n.s	0.175	1.99	n.s	0.45	n.s

*Fed. = 4200 m^2.

Chemical constituents

The total content of N, P, K in leaves and bulbs, as well as total carbohydrates and voliate oil content in bulbs were increased as a resullt of foliar application with all bio-stimulants treatments compared to the control in both seasons. Foliar application of dry yeast at 4 $mg.L^{-1}$ resulted in the highest values of N content in leaves (1.86 and 1.83%), N content in bulbs (1.47 and 1.44%), and total carbohydrate content (83.2 and 81.32) in bulbs in both seasons, respectively (Tables 5 and 6). This effect of dry yeast is may be attributed to its high contents of carbohydrates, amino acids, sugars, fatty acids, proteins, hormones, macro and micro- nutrients (Khedr and Farid, 2002). The highest values of P (0.77 and 0.71% in leaves, 0.54 and 0.55% in bulbs) and K (2.69 and 2.53% in leaves, 1.55 and 1.51% in bulbs) content were obtained by foliar application of chitosan at 6 $ml.L^{-1}$ in 2011/2012 and 2012/2013 seasons, respectively. All results were significant in both seasons, but K contents of leaves and bulbs were insignificant in the first season only (Tables 5 and 6).

The obtained results are in harmony with those of Ghoname *et al*. (2010), Fawzy *et al*. (2010), Ahmed *et al*. (2011) and Shehata *et al*. (2012) who studied the effects of application of yeast on sweet pepper, Snap bean, potato, cucumber and garlic plants, respectively. The results of the effect of chitosan on chemical content are in agreement with results, which obtained with Abdel-Mawgoud *et al*. (2010) and Shehata *et al*. (2012).

TABLE 5. Effect of some bio-stimulants on chemical contents of garlic leaves during 2011/2012 and 2012/2013 seasons.

Treatments	2011/2012			2012/2013		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Yeast (2 g.l^{-1})	1.71	0.55	2.64	1.75	0.49	2.39
Yeast (3 g.l^{-1})	1.84	0.59	2.65	1.82	0.55	2.42
Yeast (4 g.l^{-1})	1.86	0.64	2.70	1.83	0.59	2.41
Chitosan (2 ml.l^{-1})	1.70	0.62	2.66	1.73	0.60	2.47
Chitosan (4 ml.l^{-1})	1.78	0.71	2.66	1.79	0.68	2.51
Chitosan (6 ml.l^{-1})	1.81	0.77	2.69	1.81	0.71	2.53
Control	1.66	0.51	2.59	1.69	0.42	2.38
L.S.D. ($P \leq 5\%$)	0.03	0.06	n.s	0.02	0.05	0.04

TABLE 6. Effect of some bio stimulants on chemical contents of garlic bulbs during 2011/2012 and 2012/2013 seasons.

Treatments	2011/2012 season					2012/2013 season				
	N (%)	P (%)	K (%)	Total carbo-hydrate (mg/ 100g)	Volatile oil (g/100 g f.w.)	N (%)	P (%)	K (%)	Total carbo-hydrate (mg/ 100g)	Volatile oil (g/100g f.w.)
Yeast (2 g.l^{-1})	1.42	0.39	1.49	73.76	0.331	1.35	0.42	1,40	70.55	0.328
Yeast (3 g.l^{-1})	1.46	0.45	1.52	80.59	0.357	1.42	0.44	1.40	77.78	0.352
Yeast (4 g.l^{-1})	1.47	0.46	1.54	83.02	0.379	1.44	0.47	1.43	81.32	0.361
Chitosan (2 ml.l^{-1})	1.38	0.49	1.51	69.99	0.342	1.35	0.46	1.45	68.99	0.349
Chitosan (4 ml.l^{-1})	1.42	0.51	1.51	78.99	0.371	1.39	0.50	1.49	74.32	0.368
Chitosan (6 ml.l^{-1})	1.42	0.54	1.55	79.56	0.386	1.42	0.55	1.51	78.21	0.409
Control	1.34	0.33	1.44	67.21	0.329	1.29	0.37	1.39	63.92	0.317
L.S.D. ($P \leq 5\%$)	0.02	0.04	n.s	0.52	0.009	0.03	0.05	0.01	0.45	0.015

Storability

The storability of garlic bulbs was markedly influenced by foliar application of chitosan and yeast (Fig. 1, 2, 3 and 4). In general, chitosan treatments had better storability effect, compared to the control and other treatments. i.e., reduced total weight loss, decay and sprouting percentages of garlic bulbs during storage periods in both seasons. The percentage of weight loss was steadily increased until 7 months of storage, then, it rapidly increased until the end of storage (Fig. 1 and 2). These results may be due to the stimulatory effect of chitosan on growth parameters, which may be reflected on quality and storability of garlic bulbs during storage (Table 3). The effect of chitosan on garlic bulbs storability may be due to the positive effect of chitosan coatings effect that extend the storage through the reduction of respiration rate and water loss (Shehata *et al*., 2012).

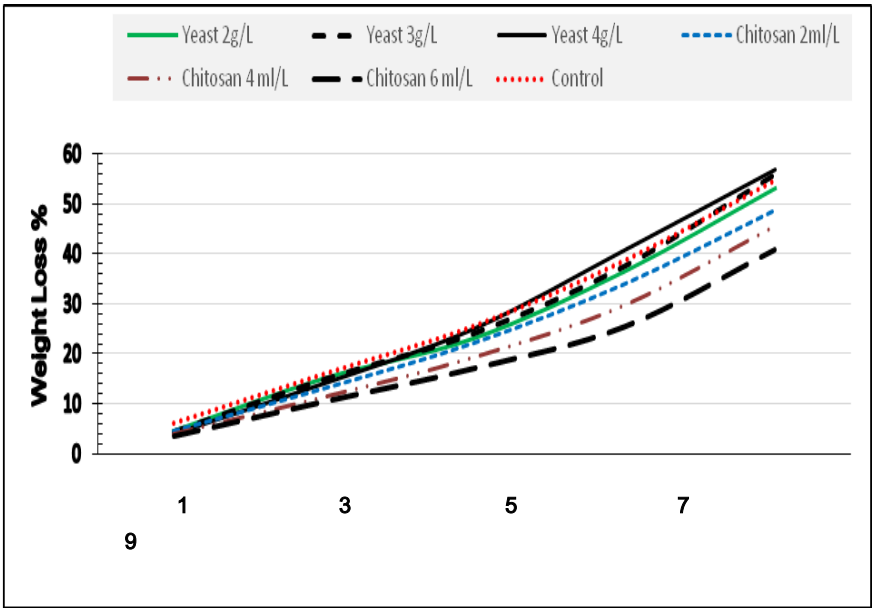

Fig. 1. Effect of some bio-stimulants on weight loss (%) of garlic bulbs during 2011/2012 season.

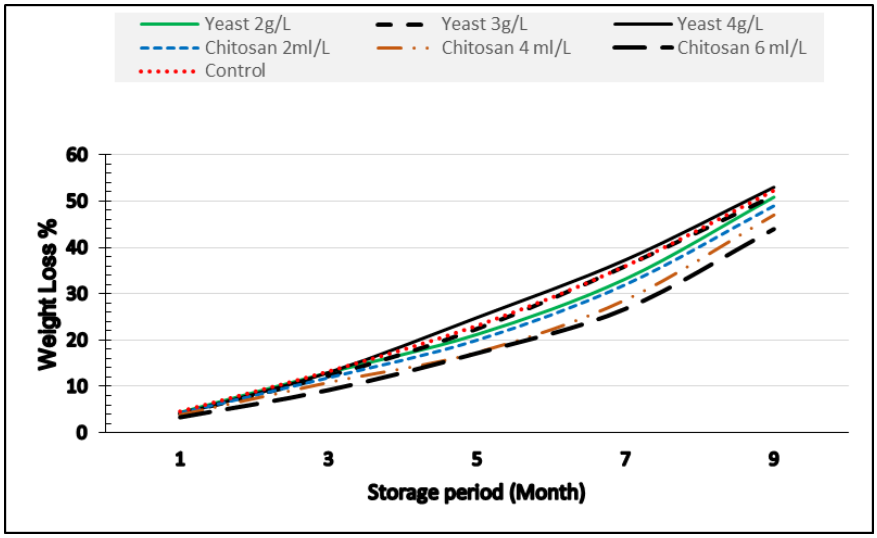

Fig. 2. Effect of some bio-stimulants on weight loss (%) of garlic bulbs during 2012/2013 season.

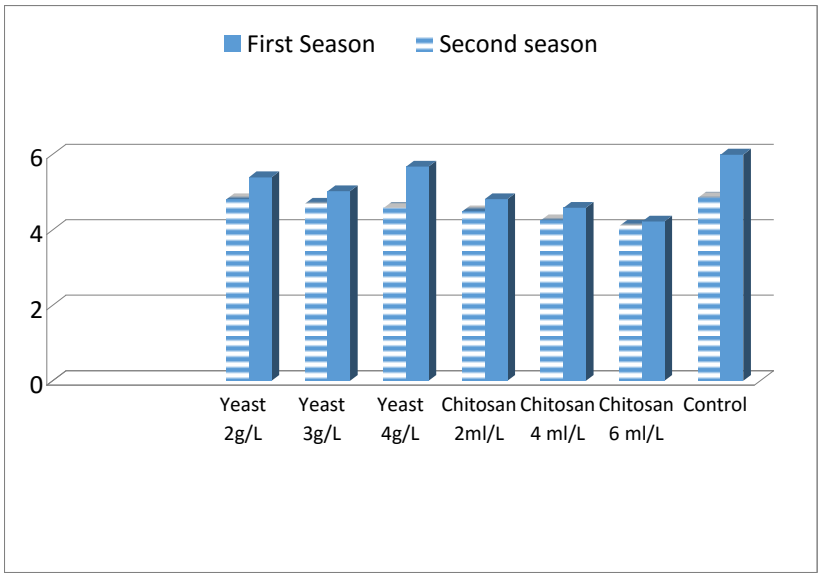

Fig. 3. Effect of some bio-stimulants on Sprouting (%) of garlic bulbs during 2011/2012 and 2012/2013 seasons.

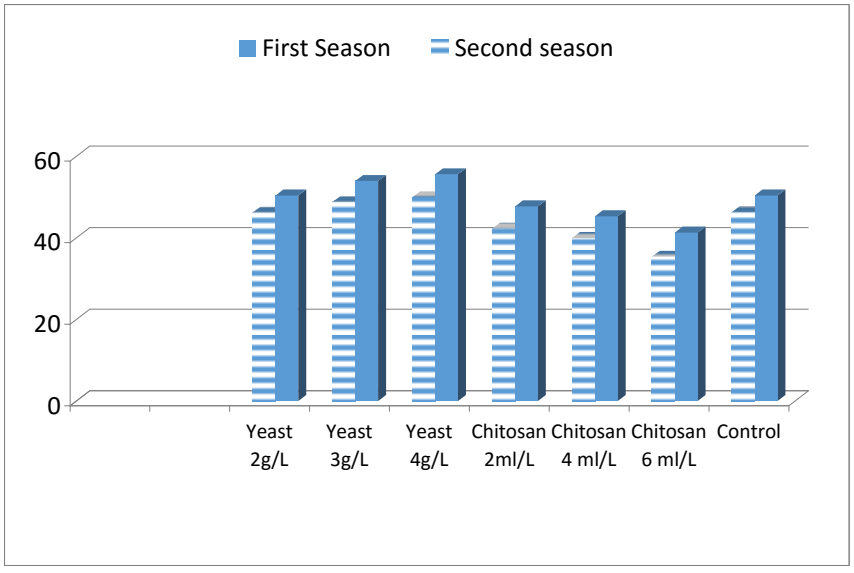

Fig. 4. Effect of some bio-stimulants on decay (%) of garlic bulbs during 2011/2012 and 2012/2013 seasons.

Conclusion

From the previous results it could be concluded that the foliar application of dry yeast (3 and 4 $g.L^{-1}$) and chitosan (4 and 6 $ml.L^{-1}$) can be used to enhance yield, quality andstorability of garlic plants grown in loamy clay soil.

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إستجابة نباتات الثوم للرش ببعض المنشطات الحيوية

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أجريت هذه الدراسة خلال موسمى ٢٠١٢/٢٠١١ و ٢٠١٣/٢٠١٢ بالمزرعة البحثية بكلية الزراعة – جامعة طنطا لدراسة تأثير الرش الورقى ببعض المنشطات الحيوية مثل الخميرة الجافة (بثلاثة تركيزات ٢ و٣ و٤ جرام لكل لتر) والشيتوسان (بثلاثة تركيزات ٢ و٤ و٦ مل لكل لتر) على نمو وانتاجية وجودة والقدرة التخزينية لنباتات الثوم (سلالة سدس ٤٠). اضافة المنشطات الحيوية كانت عند ٣٠ و٤٥ و٦٠ و٧٥ يوم من تاريخ الزراعة. وقد استخدم تصميم القطاعات كاملة العشوائية فى التجربة.

وكانت أهم النتائج المتحصل عليها:

الرش الورقى لنباتات الثوم بالخميرة الجافة بتركيز ٣ او ٤ جرام فى اللتر وكذلك الرش بالشيتوسان بتركيز ٤ او ٦ مل فى اللتر ادى الى زيادة معنوية فى صفات النمو الخضرى (ارتفاع النبات وعدد الاوراق والوزن الطازج للأوراق) وكمية المحصول ومكوناته فى كلا الموسمين. ادت نفس المعاملات السابقة الى الحصول على اعلى القيم لمحتوى الاوراق والابصال من النتروجين والفوسفور والبوتاسيوم وكذلك محتوى الابصال من الكربوهيدرات الكلية والزيوت .

الرش بالشيتوسان بتركيز ٤ او ٦ مل فى اللتر ادى الى تحسن القدرة التخزينية لرؤوس الثوم حيث اعطت اقل نسبة فقد فى وزن الابصال اثناء التخزين وكذلك اقل نسبة تزريع وعفن فى كلا الموسمين.

عموماً يمكن التوصية برش نباتات الثوم بالخميرة بتركيز ٣ او ٤ جرام فى اللتر وكذلك الرش بالشيتوسان بتركيز ٤ او ٦ مل فى اللتر لتحسين نمو وانتاجية وجودة والقدرة التخزينية للثوم تحت ظروف الاراضى الطميية.