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Effect of bio-regulators and bio-fertilizers on growth of garlic (*Allium sativum* L.)

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Abstract
A field experiment was conducted at Horticulture Farm, S.K.N. College of Agriculture, Jobner (Jaipur) during *Rabi* season 2016-17 and 2017-2018. The experiment consisting four bio-fertilizers (control, *Azotobacter*, PGPR (*Pseudomonas*) and *Azotobacter* + PGPR (*Pseudomonas*) and five bio-regulators (control, Thiourea @ 500 ppm, Thiourea @ 1000 ppm, salicylic acid @ 100 ppm and mepiquat chloride @ 100 ppm). The total 20 treatment combinations were tested in split-plot design with three replications. It was observed that the application of bio-fertilizers *Azotobacter* + PGPR (*Pseudomonas*) significantly increased the plant height at 45 DAP and at harvest, number of leaves per plant, chlorophyll content in leaves, fresh weight of leaves at 30, 45 and 60 DAP, leaf area at 45 DAP, dry weight of leaves at 30, 45 and 60 DAP, crop growth rate at 30-45 and 45-60 DAP and dry matter accumulation as compared to control. The results of the study clearly indicated that foliar application of thiourea @ 1000 ppm to the garlic crop significantly increased the plant height, number of leaves per plant, chlorophyll content in leaves, fresh weight of leaves, leaf area, dry weight of leaves, crop growth rate and dry matter accumulation, being statistically at par with application of thiourea @ 500 ppm and salicylic acid @ 100 ppm.

Keywords: Bio-regulators, bio-fertilizers, *Allium sativum* L

Introduction
Garlic is the second important bulb crop after onion. Botanically it is known as *Allium sativum* which belongs to the family Amaryllidaceae. The economic yield is obtained from these cloves. It is especially rich in protein, carbohydrates and ascorbic acid. Garlic is used in flavoring foods, preparing chutneys, pickles, curry powder, tomato ketchup *etc.* It also contains phosphorus, potash, calcium, magnesium and a colourless as well as odourless water soluble amino acid called 'Allin' after crushing of bulb cloves an enzyme allinase acts upon allin and breaks down to produce Allicin. The principal ingredient of which is odoriferous diallyl-disulphide, which is the major flavouring component of garlic. Garlic extracts and oil have potential uses as an effective insecticide and fungicide in the present scenario of organic farming (Kumara *et al*., 2014) [11]. Allicin present in aqueous extract of garlic reduces blood cholesterol level (Shankaracharya, 1974) [21]. Garlic oil or its juice is recommended to inhale in cases of pulmonary tuberculosis, rheumatism, sterility, impotency, cough and redness of eyes (Pruthi, 1979) [18]. India ranks second in area and third in production of garlic in the world. In India, the major garlic producing states are Madhya Pradesh, Rajasthan, Uttar Pradesh and Gujarat however, In Rajasthan, it is grown extensively in Chittorgarh, Baran, Jodhpur, Jhalawar, Kota, Bundi, Jaipur and Sikar districts. The low productivity of the crop in India and Rajasthan may be due to its unscientific cultivation and lesser care of growers to its nutritional management (Anonymous, 2017) [2]. As bio-fertilizers are the recent sources for fixation of atmospheric nitrogen in to the soil and making it readily available for the growth of plants. Among the bio-fertilizers, *Azotobacter* though having limited use in vegetables, yet has established its bio-activity in cereals, oilseeds and other crops for mobilizing the useful macro nutrients from unusable to usable state and increase the crop production by enhancing soil fertility. In addition, the bio-fertilizers not only supplement the nutrition but also improve the efficiency of applied

nutrients (Somani *et al.*, 1990) [25]. Further, *Pseudomonas fluorescens*is common non-pathogenic saprophyte that colonizes in soil, water and on plant surfaces. It produces a soluble greenish fluorescent pigment. It suppress plant diseases by protecting the seeds and roots from fungal infections by production the number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide. This microbe has the unique ability to enter the plant vascular system and reach to the various parts of the plant system and act as a systemic bio-control agent against various fungal and bacterial diseases. It is applied as Seed treatment @ 4-5 g per kg of seeds as per standard wet treatment (Yawalkar *et al*., 1996) [30]. *Pseudomonas spp*. is ubiquitous bacteria in agricultural soil and have many traits that make them well suited as PGPR. The most effective strain of *pseudomonas* has been *fluorescent pseudomonas spp*. and considerable research is underway globally to exploit the potential of one group of bacteria that belonging to *fluorescent pseudomonas* (FLPs). FLPs helps in the maintenance of soil health and are metabolically and functionally most diverse (Lata *et al.*, 2002) [12].

Besides nutrients, thiourea also plays an important role in mechanizing yield potentials of the crop in arid and semi-arid regions as, it may prove beneficial by inducing stress tolerance. Furthermore, thiourea plays a vital role in the physiology of plants both as a sulfhydryl compound and to some extent as an amino compound like urea. The stimulating action of it in various physiological activities of plant is well known. Thiourea regulate the plant growth by maintaining higher photosynthetic rate up to the reproductive stage and increased the yield by improving carbon partitioning towards sink (Anonymous, 1999) [1]. Thiourea is mainly known for its dormancy breaking and germination stimulating effect (Mayer, 1956; Mayer and Poljak off-Mayber, 1958) [13-14]. The dormancy breaking effect of thiourea was suggested to be related to its growth enhancing effect.

Similarly salicylic acid is positively affects growth of the plants. It is classified as phenolic growth regulator, a non- enzymatic antioxidant, a signaling or messenger molecule in plants to induce responses of plants to environmental stressors. SA plays an important role in the regulation and development of ion uptake, transport and membrane permeability (Simaei *et al*., 2012) [23]. Salicylic acid is a common plant-produced phenolic compound. Which contributes in the regulation of physiological, biochemical and molecular processes and therefore, it affects the plant growth, development and productivity (Hayat *et al.*, 2010) [7]. However, higher concentrations of salicylic acid had an inhibitory effect. Salicylic acid has been reported to induce flowering in a number of plants. Likewise, Mepiquat enhances reproductive organs by allowing plants to direct more energy towards reproductive structures (Wang *et al*., 1995) [28]. Redistribution of assimilates between vegetative and reproductive growth may be one mean by which yield can be increased foliar spray with mepiquat chloride (pix) compound, has interaction on garlic productivity and storability throughout the growth period. The present investigation, is therefore, an attempt to bridge up this knowledge gap to obtain the higher productivity levels of the crop. Bio-fertilizers and bio-regulators were applied as treatments in present experiment to study the growth of garlic.

Materials and Methods

The present study was carried out at Department of Horticulture, S.K.N. College of Agriculture, Jobner during *Rabi*, 2016-17 and 2017-18. The experiment was comprised of twenty treatment combinations with four bio-fertilizers B_0 (Control- no inoculation), B_1 (*Azotobacter*) B_2 (PGPR-*Pseudomonas*) and B_3 (*Azotobacter* + *PGPR*) and inoculation with Bio-regulators P_0 (Control-water spray, P_1 (Thiourea 500 ppm), P_2 (Thiourea 1000 ppm), P_3 (Salicylic acid 100 ppm) and P_4 (Mepiquat chloride 100 ppm). The experiment was laid out in Split Plot Design and replicated 3 times. The treatments were randomly allotted to different plots using random number table of Fisher and Yates (1963) [6]. Bio-regulators: thiourea, salicylic acid and Mepiquat chloride were applied as foliar spray in the plots as per treatments. Thiourea was sprayed @ 500 ppm and 1000 ppm at 30 and 45 DAS. Similarly, salicylic acid @ 100 ppm and Mepiquat chloride was also sprayed @ 100 ppm at 30 and 45 DAS in respective plots. Application of bio-fertilizers was done as per treatment. For this 125 g of Jaggery was mixed in one litre of boiled water. Appropriate quantity of *Azotobacter* 50 g of culture was poured in Jaggery solution separately and stirred well. The seeds were allowed to air dry in shade. The cloves were sown on the same day after inoculation. The process of inoculation was preceded by clove treatment with fungicide then clove inoculation with *Azotobacter* and *Pseudomonas fluorescens* before the sowing by putting seeds in 20 per cent sucrose solution and then inoculated with respective culture @ 10 g/kg of seeds by putting the uniform coating of chalk powder on seeds and were allowed to air dry in shade. The seeds were sown on the same day after inoculation. The seeds of control plot were treated with sucrose solution only.

Results and Discussion

Effect of Bio-fertilizers: Different levels of bio-fertilizers significantly influenced the plant height of garlic at 45 days after planting and at harvest on pooled basis. Combined application of *Azotobacter* + PGPR represented the significantly maximum (33.94cm) and (37.70cm) plant height at 45 DAP and at harvest respectively over control. The number of leaves (8.98) per plant found significantly maximum under B_3 treatment and chlorophyll content of leaves (1.19 mg/g) also recorded maximum among all the bio-fertilizers applied. However, Kore *et al*. (2006) [10] revealed that *Azotobacter* + PGPR /ha significantly attained higher values of plant height and number of leaves per plant in garlic. The bio-fertilizers had better effect and registered the maximum increase the plant height 22.48 per cent at 45 DAP and 23.60 per cent at harvest over control. Number of leaves (46.01%) per plant and chlorophyll content in leaves (36.78%) registered more under treatment B_3 over control. Combined application of same treatment represented significantly maximum (22.37, 24.50 and 26.88 g) fresh weight of leaves at 30, 45 and 60 DAP over rest of the treatment and had better effect registered 28.41, 26.15 and 25.08 per cent increase in fresh weight of leaves over control at 30, 45 and 60 DAP, respectively. Maximum leaf area (107.70 cm^2) at 45 DAP was observed under same treatment over rest of the treatments and percentage wise maximum increase of 46.05 per cent higher over control. Combined application of *Azotobacter* + PGPR represented the significantly maximum crop growth rate (5.36 and 5.39) at 30-45 and 45-60 DAP and dry matter accumulation (44.80 g) over rest of the treatment. This treatment registered the increase of 40.68 and 31.78 per cent more crop growth rate and 26.91 per cent dry matter accumulation over control. Balemi *et al*. (2007) [3], in other crops, Prabhu *et al*. (2002) [17] obtained tallest plant and maximum leaves in okra with the application of *Azotobacter* + PGPR /ha. Similarly, Singh and Pandey (2006) [26] concluded that combined use of FYM, chemical fertilizers and bio-fertilizers would be a sound integrated nutrient management practice for production of onion.

Effect of Bio-regulators

Spray of thiourea @1000 ppm recorded maximum plant height of 33.39 cm and 37.05 cm at 45 DAP and at harvest and total chlorophyll content (1.14 mg/g) in leaves under treatment P_2, whereas minimum plant height of 26.98 cm, 28.26 cm and total chlorophyll content (0.83 mg/g) respectively were recorded under control. The maximum number of leaves per plant (8.59) whereas minimum number of leaves per plant (6.13) were recorded under control. The magnitude in increase of plant height at 45 DAP and at harvest with the application of thiourea @1000 ppm / ha was 23.75 and 31.10 per cent over control, respectively. This treatment also registered 40.13 per cent more number of leaves per plant and total chlorophyll content 37.34 per cent over control. The maximum fresh weight of leaves at 30, 45 and 60 DAP (22.02, 24.07 and 26.25 g) and dry weight of leaves (5.54 g, 5.71 g and 5.81 g) under same treatment whereas, minimum fresh weight of leaves (17.13, 18.99 and 21.25 g) and dry weight of leaves (4.06, 4.22 and 4.25) at 30, 45 and 60 DAP was recorded under control. Maximum leaf area at 45 DAP (103.03) recorded maximum under same treatment whereas minimum (73.53) was recorded under control and 40.11 per cent increment higher over control. Application of bio-regulators had significant influence on the crop growth rate at 30-45 and 45-60 DAP of garlic during experimentation. Spray of thiourea @1000 ppm recorded maximum crop growth rate (5.26 and 5.29) at 30-45 and 45-60 DAP under treatment P_2, whereas minimum crop growth rate (3.84 and 3.81) at 30-45 and 45-60 DAP were recorded under control. The magnitude in increase of crop growth rate at 30-45 and 45-60 DAP with the application of same bio-regulator as 36.97 and 38.84 per cent were noted over control, respectively. The spray of thiourea @1000 ppm recorded maximum dry matter accumulation (43.88 g) found significantly superior over rest of the treatments. The magnitude in increase of dry matter accumulation with the application of thiourea @1000 ppm / ha was 28.07 per cent over control. Similar, results were obtained by Wani and Konde (1998) [29], Hossain *et al*. (1998) [8], Mengistu and Singh (1999) [15], Turk and Tawaha (2001) [27], Jayathilake *et al*. (2002) [9], Banafar *et al*. (2004) [4], Reddy and Reddy (2005) [19], Talware *et al*. (2012) [26], Mohd *et al*. (2011) [16], Sharma *et al*. (2013) [22] and Sachin *et al*. (2017) [20] in garlic. Reduced growth stature of garlic (Plant height, number of leaves/plants, length and width of leaf, fresh and dry weight/plant and leaf area index) was noticed with the *Azotobacter* + PGPR which might be due to supply of Insufficient quantity of nutrients, denying satisfactory level of growth due to retarded cell division and multiplication.

Table 1: Effect of bio-fertilizers and bio-regulators on plant height

Treatments	Plant height (cm)					
	45 DAP			At harvest		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Bio-fertilizers						
B_0 (Control No inoculation)	27.02	28.40	27.71	30.15	30.85	30.50
B_1 (*Azotobacter*)	29.55	31.25	30.40	33.60	33.88	33.74
B_2 (PGPR)	30.78	32.15	31.47	34.25	34.80	34.53
B_3 (*Azotobacter* + PGPR)	33.02	34.85	33.94	37.65	37.75	37.70
SEm±	0.61	0.63	0.46	0.68	0.71	0.50
CD (P=0.05)	1.91	1.81	1.41	2.15	2.04	1.54
Bio-regulators						
P_0 (Control water spray)	26.31	27.65	26.98	28.34	28.19	28.26
P_1 (Thiourea @ 500 ppm)	31.57	33.35	32.46	35.76	36.63	36.19
P_2 (Thiourea @ 1000 ppm)	32.44	34.35	33.39	36.62	37.48	37.05
P_3 (Salicylic acid @ 100 ppm)	31.27	32.88	32.08	35.61	36.45	36.03
P_4 (Mepiquat chloride @ 100 ppm)	28.87	30.09	29.48	33.23	32.85	33.04
SEm±	0.58	0.69	0.48	0.63	0.73	0.53
CD (P=0.05)	1.65	2.17	1.36	1.81	2.32	1.50

Table 2: Effect of bio-fertilizers and bio-regulators on fresh weight of leaves per plant at different stage (Days)

Treatments	Fresh weight of leaves per plant (g)								
	30 DAP			45 DAP			60 DAP		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Bio-fertilizers									
B_0 (Control No inoculation)	16.24	18.60	17.42	18.12	20.71	19.42	20.19	22.79	21.49
B_1 (*Azotobacter*)	19.68	20.80	20.24	21.22	22.91	22.07	23.44	25.13	24.29
B_2 (PGPR)	20.22	21.40	20.81	22.14	23.49	22.82	24.19	25.74	24.97
B_3 (*Azotobacter* + PGPR)	21.98	22.76	22.37	24.12	24.88	24.50	26.84	26.91	26.88
SEm±	0.41	0.43	0.30	0.50	0.42	0.33	0.58	0.46	0.39
CD (P=0.05)	1.28	1.23	0.91	1.59	1.20	1.03	1.84	1.31	1.20
Bio-regulators									
P_0 (Control water spray)	16.80	17.46	17.13	18.48	19.50	18.99	20.73	21.78	21.25
P_1 (Thiourea @ 500 ppm)	20.55	22.34	21.45	22.47	24.35	23.41	24.73	26.45	25.59
P_2 (Thiourea @ 1000 ppm)	21.28	22.77	22.02	23.15	25.00	24.07	25.40	27.10	26.25
P_3 (Salicylic acid @ 100 ppm)	20.46	22.07	21.27	22.41	24.29	23.35	24.63	26.37	25.50
P_4 (Mepiquat chloride @ 100 ppm)	18.55	19.81	19.18	20.50	21.85	21.17	22.84	24.01	23.42
SEm±	0.40	0.43	0.33	0.47	0.44	0.35	0.44	0.52	0.37
CD (P=0.05)	1.16	1.35	0.92	1.35	1.38	1.00	1.27	1.63	1.04

Table 3: Effect of bio-fertilizers and bio-regulators on leaf area per plant

Treatments	Leaf area (cm^2) at 45 DAP		
	2016-17	2017-18	Pooled
Bio-fertilizers			
B_0 (Control No inoculation)	73.44	74.04	73.74
B_1 (*Azotobacter*)	92.88	93.12	93.00
B_2 (PGPR)	96.12	97.44	96.78
B_3 (*Azotobacter* + PGPR)	107.28	108.12	107.70
SEm±	1.88	1.88	1.33
CD (P=0.05)	5.93	5.41	4.10
Bio-regulators			
P_0 (Control water spray)	78.13	68.93	73.53
P_1 (Thiourea @ 500 ppm)	99.12	102.21	100.67
P_2 (Thiourea @ 1000 ppm)	100.29	105.78	103.03
P_3 (Salicylic acid @ 100 ppm)	97.14	100.43	98.79
P_4 (Mepiquat chloride @ 100 ppm)	87.46	88.54	88.00
SEm±	1.88	1.88	1.48
CD (P=0.05)	5.41	5.93	4.18

Table 4: Effect of bio-fertilizers and bio-regulators on dry weight of garlic leaves per plant at different stages

Treatments	Dry weight (g)								
	30 DAP			45 DAP			60 DAP		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Bio-fertilizers									
B_0 (Control No inoculation)	4.06	4.20	4.13	4.24	4.27	4.26	4.29	4.31	4.30
B_1 (*Azotobacter*)	4.98	5.16	5.07	5.21	5.29	5.25	5.33	5.36	5.35
B_2 (PGPR)	5.11	5.37	5.24	5.32	5.45	5.39	5.53	5.60	5.57
B_3 (*Azotobacter* + PGPR)	5.55	5.76	5.66	5.75	5.88	5.82	5.97	5.99	5.98
SEm±	0.12	0.11	0.09	0.12	0.12	0.09	0.13	0.12	0.10
CD (P=0.05)	0.37	0.33	0.26	0.39	0.34	0.28	0.41	0.35	0.30
Bio-regulators									
P_0 (Control water spray)	3.97	4.16	4.06	4.16	4.27	4.22	4.29	4.22	4.25
P_1 (Thiourea @ 500 ppm)	5.33	5.46	5.39	5.51	5.55	5.53	5.64	5.78	5.71
P_2 (Thiourea @ 1000 ppm)	5.42	5.65	5.54	5.63	5.78	5.71	5.79	5.82	5.81
P_3 (Salicylic acid @ 100 ppm)	5.19	5.42	5.30	5.44	5.51	5.47	5.55	5.68	5.62
P_4 (Mepiquat chloride @ 100 ppm)	4.71	4.93	4.82	4.91	4.99	4.95	5.13	5.07	5.10
SEm±	0.11	0.12	0.09	0.11	0.13	0.09	0.11	0.15	0.10
CD (P=0.05)	0.31	0.39	0.25	0.30	0.42	0.25	0.32	0.46	0.27

Table 5: Effect of bio-fertilizers and bio-regulators on crop growth rate (CGR)

Treatments	Crop growth rate (g/m^2/day)					
	30-45 DAP			45-60 DAP		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Bio-fertilizers						
B_0 (Control No inoculation)	3.74	3.88	3.81	4.09	4.08	4.09
B_1 (*Azotobacter*)	4.84	4.97	4.91	4.86	4.86	4.86
B_2 (PGPR)	4.93	5.07	5.00	4.97	5.03	5.00
B_3 (*Azotobacter* + PGPR)	5.34	5.38	5.36	5.41	5.36	5.39
SEm±	0.11	0.09	0.08	0.12	0.09	0.09
CD (P=0.05)	0.36	0.26	0.26	0.38	0.27	0.27
Bio-regulators						
P_0 (Control water spray)	3.69	3.98	3.84	3.83	3.80	3.81
P_1 (Thiourea @ 500 ppm)	5.08	5.25	5.16	5.19	5.23	5.21
P_2 (Thiourea @ 1000 ppm)	5.18	5.33	5.26	5.27	5.32	5.29
P_3 (Salicylic acid @ 100 ppm)	5.02	5.16	5.09	5.12	5.12	5.12
P_4 (Mepiquat chloride @ 100 ppm)	4.58	4.40	4.49	4.76	4.69	4.72
SEm±	0.08	0.12	0.07	0.08	0.13	0.08
CD (P=0.05)	0.24	0.39	0.21	0.24	0.40	0.21

Conclusion

It may be concluded on the basis of results of two-year experiments that the combined application of *azotobacter* + pgpr (*Pseudomonas*) along with thiourea @ 1000 was found significantly better in terms of growth and yield. Although, application of (*Azotobacter* + pgpr (*Pseudomonas*) + thiourea @ 500 ppm) and (*Azotobacter* + pgpr (*Pseudomonas*) + salicylic acid @ 100 ppm were found statistically at par to it.

Hence, application of *azotobacter* + pgpr (*pseudomonas*) and thiourea @ 500 ppm (b_3p_1) to garlic crop in semi-arid region is recommended.

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