

Original Research Article

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Effect of *Azotobacter*, *Pseudomonas* and Bio-Regulators on Yield Attributes and Yield of Garlic (*Allium sativum* L.)

Ganpat Lal Yadav^{1*}, S. P. Singh¹, R. K. Yadav² and Bhagchand Yadav¹

¹Department of Horticulture, S.K.N. College of Agriculture, Jobner
(S.K.N. Agriculture University, Jobner, Jaipur) 303328 Rajasthan, India

²Agricultural Research Station, Ummedganj, AU Kota, India

*Corresponding author

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. Application of bio-fertilizers: *Azotobacter* + also significantly increased the neck thickness, polar diameter, fresh weight of bulb, dry weight of bulb, number of cloves per bulb, bulb yield (kg/plot), bulb yield (q/ha) and net returns as compared to control. Foliar application of thiourea @ 1000 ppm to the garlic crop significantly increased the being statistically at par with application of thiourea @ 500 ppm and salicylic acid @ 100 ppm. Further, it can be concluded that combined application of *Azotobacter* + PGPR (*Pseudomonas*) along with thiourea @ 1000 ppm proved to be most superior treatment combination as it fetched comparable bulb yield (223.58 q/ha) followed by *Azotobacter* + PGPR (*Pseudomonas*) along with thiourea @ 500 ppm and *Azotobacter* + PGPR (*Pseudomonas*) along with salicylic acid @ 100 ppm.

Keywords

Azotobacter, PGPR (*Pseudomonas*), thiourea, salicylic acid and mepiquat chloride

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Introduction

Garlic is the second important bulb crop after onion. Botanically it is known as *Allium sativum*, which belongs to the family Amaryllidaceae. It is a multiple or compound bulb consists of smaller bulblets called 'cloves' and is surrounded by a thin white or pinkish papery sheath. The economic yield is

obtained from these cloves. 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).

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). 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 thiourea in various physiological activities of plant is well known. It has also been reported that thiourea regulate the plant growth by maintaining higher photosynthetic rate upto the reproductive stage and increased the yield by improving carbon partitioning towards sink (Anonymous, 1999). The stimulating action of thiourea in various physiological activities of plant is well known. Thiourea is mainly known for its dormancy breaking and germination stimulating effect (Mayer, 1956; Mayer and Poljak off-Mayber, 1958). The dormancy breaking effect of thiourea was suggested to be related to its growth enhancing effect.

Similarly salicylic acid is one of the important bio-regulator which positively affects growth of 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).

Salicylic acid (SA) or ortho-hydroxy benzoic 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).

Materials and Methods

The experiment was laid out in Split Plot Design and replicated three times. The treatments were randomly allotted to different plots using random number table of Fisher and Yates (1963). The seeds of cv. G 282 procured from NHRDF, Karnal (Haryana). The seeds (cloves) of garlic were first treated with Carbendazim @ 2 g per kg seed to control seed borne diseases. The seeds were sown on 3rd November, 2016 and 8th November, 2017 manually with a seed rate of 500 kg /ha in row at 15 cm apart.

It is also known as Yamuna Safed-3. The variety has done very well in Northern parts and also in Central parts of India. It was developed by mass selection technique from a local collection obtained from Dindigul (TN) in 1990. The leaves are wider than other varieties. Bulbs are creamy white and bigger sized (5-6 cm diameter), size index 27-29 cm², diameter of cloves 1.2-1.5 cm., 15-18 number of cloves per bulb, TSS 38-42%, dry matter 39-43% and medium storer. Average yield is 175-200 q/ha. The variety is suitable for export and was notified in the year 1999 vide notification no.1092 (E) dated 26/10/1999.

Application of bio-fertilizers have done as per treatments. For this, 125g 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 was 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.

The recommended dose of NK for garlic was applied @ 120:100 kg/ ha, respectively. Full dose of potassium and half dose of nitrogen were applied as basal dose just before sowing and rest half dose of nitrogen was applied as top dressing in two split doses. To protect the crop from blight and purple blotch the crop was sprayed twice with Diathane M-45 at the rate of 0.25 per cent while for the garlic thrips, the crop was also sprayed twice with Malathion @ 0.1%.

Results and Discussion

It is apparent from the data presented in Table-1 that different levels of bio-fertilizers significantly influenced the neck thickness of garlic during both the years as well as in pooled analysis. Analysis of pooled data indicated that application of *Azotobacter* + PGPR represented maximum (0.73 cm) in neck thickness, which was significantly superior over all the bio-fertilizers applied. This treatment (B₃) registered an increase of 19.67 per cent higher neck thickness over

control. Spray of thiourea @1000 ppm recorded maximum neck thickness (0.72 cm) whereas, minimum was recorded under control. The magnitude in increase of neck thickness with the application of thiourea @1000 ppm / ha was 20.0 per cent over control. The maximum value was recorded in the treatment *Azotobacter* + PGPR which was significantly superior over rest of the treatments. These findings are in close conformity with the findings of Kore *et al.*, (2006).

An increase in yield attributing characters with foliar application of thiourea might have induced large number of reproductive sinks leading to greater activity of carboxylating enzymes resulting in higher photosynthetic rates with greater translocation and accumulation of metabolites in sink and ultimately higher yield (Nehra *et al.*, 2006). Analysis of pooled data indicated that combined application of *Azotobacter* + PGPR represented maximum (6.73 cm) and significantly superior polar diameter of bulb among bio-fertilizers applied. This treatment had better effect and represented the maximum increase of 19.96 per cent in polar diameter of garlic over control. Further bio-regulators also significantly increased the polar diameter of bulb of garlic during both the years as well as in pooled analysis.

Spray of thiourea @1000 ppm recorded maximum polar diameter of bulb (6.58 cm). The magnitude in increase of polar diameter of bulb with the application of thiourea @1000 ppm / ha was registered 21.17 per cent over control. Pachouri *et al.*, (2005) and Anonymous (2007) reported that application of *Azotobacter* + PGPR resulted in significantly highest bulb yield over rest of the treatments. Foliar spray of thiourea was also recorded by Balai and Keshwa (2011), Shanu *et al.*, (2013) in coriander and Gupta and Yadav (2009) in fenugreek.

Different levels of bio-fertilizers significantly influenced the fresh weight of bulb in garlic during experimentation. Combined application of *Azotobacter* + PGPR represented significantly maximum (49.77 g) fresh weight of bulb among all the treatments. This treatment also represented the maximum increase of 22.64 per cent fresh weight of bulb over control.

Examination of pooled data revealed that spray of thiourea @1000 ppm recorded maximum fresh weight of bulb (49.53 g) whereas minimum (39.05 g) was recorded under control. The increase in fresh weight of bulb with the application of thiourea @1000 ppm / ha was registered 26.83 per cent higher over control. The obtained results are agreement with the result of Jaafari and Hadavi (2012a) and Jaafari and Hadavi (2012b).

Combined application of *Azotobacter* + PGPR represented maximum and significantly higher dry weight of bulb (29.11g) among all the treatment and represented 55.25 per cent increase in dry weight of bulb over control. Foliar spray of thiourea @1000 ppm recorded maximum dry weight of bulb (27.72 g) and found significantly superior over rest of the treatments except P₁ and P₃ which were statistically at par to it.

The magnitude in increase of dry weight of bulb with the application of thiourea @1000 ppm / ha was 50.98 per cent over control. Similar response with foliar spray of thiourea was also recorded by Balai and Keshwa (2010) and Bochalia *et al.*, (2011) in fenugreek. Interactive effect of different levels of bio-fertilizers and bio-regulators had significantly effected on fresh weight of bulb during both the years as well as in pooled data. Data mentioned in table 2 indicated that combined application of *Azotobacter* + PGPR and thiourea @1000 ppm (B₃P₂) showed

significantly higher fresh weight of bulb over rest of treatments except (B₃P₁), (B₃P₃), (B₃P₂), (B₁P₂) and (B₃P₃) which were found statistically at par to it.

It is apparent from the data presented in table 3 that different levels of bio-fertilizers significantly influenced the number of cloves per bulb of garlic during both the years as well as in pooled data. Analysis of pooled data indicated that combined application of *Azotobacter* + PGPR represented significantly maximum number of cloves per bulb (18.89) among all bio-fertilizers. This treatment also represented the maximum increase of 19.70 per cent in number of cloves/bulb over control. Analysis of pooled data indicated that combined application of *Azotobacter* + PGPR produced significantly maximum bulb yield of 3.73 kg/plot among all the treatment and registered 22.69 per cent higher bulb yield per plot of garlic over control.

Spray of thiourea @1000 ppm recorded maximum bulb yield (3.71 kg/plot) and found significantly superior over rest of the treatments except P₁ and P₃ which, were statistically at par to it. The magnitude in increase of bulb yields per plot with the application of thiourea @1000 ppm / ha was 26.62 per cent over control.

Combined application of *Azotobacter* + PGPR represented significantly maximum bulb yield (207.37 q/ha) over all the treatments and this treatment represented the maximum increase of 22.63 per cent in bulb yield q/ha over control. Spray of thiourea @1000 ppm recorded significantly maximum bulb yield (206.36 q/ha) over rest of the treatment except P₁ and P₃ which found statistically at par to each other, where as it was noted minimum (162.71 q/ha) under control. The magnitude in increase of bulb yields with the application of thiourea @1000 ppm/ha was 26.82 per cent over control.

Table.1 Effect of bio-fertilizers and bio-regulators on neck thickness, polar diameter, fresh and dry weight of garlic bulbs

Treatments	Neck thickness (cm)			Polar diameter (cm)			Fresh weight (g)			Dry weight (g)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Bio-fertilizers												
B₀ (Control No inoculation)	0.60	0.62	0.61	5.46	5.75	5.61	39.36	41.80	40.58	18.15	19.35	18.75
B₁ (<i>Azotobacter</i>)	0.66	0.67	0.67	5.99	6.19	6.09	44.93	45.64	45.28	24.33	25.65	24.99
B₂ (PGPR)	0.68	0.69	0.69	6.13	6.26	6.20	46.33	47.32	46.82	25.15	26.35	25.75
B₃ (<i>Azotobacter</i> + PGPR)	0.73	0.73	0.73	6.71	6.75	6.73	49.25	50.29	49.77	28.80	29.42	29.11
SEm_±	0.02	0.01	0.01	0.14	0.11	0.11	0.91	0.85	0.68	0.63	0.65	0.49
CD (P=0.05)	0.05	0.03	0.03	0.45	0.32	0.32	2.88	2.44	2.11	1.97	1.88	1.52
Bio-regulators												
P₀ (Control water spray)	0.58	0.61	0.60	5.35	5.51	5.43	38.36	39.75	39.05	16.91	19.80	18.36
P₁ (Thiourea @ 500 ppm)	0.70	0.72	0.71	6.36	6.65	6.51	47.59	48.60	48.09	26.38	27.16	26.77
P₂ (Thiourea @ 1000 ppm)	0.71	0.73	0.72	6.44	6.72	6.58	48.90	50.15	49.53	27.29	28.14	27.72
P₃ (Salicylic acid @ 100 ppm)	0.69	0.71	0.70	6.29	6.51	6.40	46.86	48.60	47.73	26.20	26.81	26.50
P₄ (Mepiquat chloride @ 100 ppm)	0.65	0.62	0.64	5.92	5.79	5.85	43.12	44.22	43.67	23.75	24.04	23.90
SEm_±	0.01	0.02	0.01	0.10	0.16	0.09	0.72	1.02	0.65	0.52	0.77	0.48
CD (P=0.05)	0.03	0.05	0.03	0.29	0.49	0.26	2.06	3.21	1.83	1.49	2.41	1.36

Table.2 Interactive effect of bio-fertilizers and bio-regulators on fresh weight (g) of bulb

Treatments	B ₀	B ₁	B ₂	B ₃
2016-17				
P ₀	37.55	38.00	38.55	39.33
P ₁	40.10	48.46	49.05	52.75
P ₂	41.55	49.83	51.13	53.10
P ₃	39.05	48.35	48.49	51.55
P ₄	38.55	40.00	44.41	49.50
			SEm±	CD (P=0.05)
For B at same level of P			1.44	4.14
For p at same or diff. level of B			2.49	7.18
2017-18				
P ₀	40.35	38.75	39.52	40.36
P ₁	41.10	49.42	50.07	53.79
P ₂	43.55	50.68	52.16	54.22
P ₃	43.02	49.33	49.45	52.59
P ₄	40.99	40.02	45.38	50.49
			SEm±	CD (P=0.05)
For B at same level of P			1.70	4.90
For p at same or diff. level of B			2.88	8.31
Pooled				
P ₀	38.95	38.38	39.04	39.85
P ₁	40.60	48.94	49.56	53.27
P ₂	42.55	50.26	51.65	53.66
P ₃	41.04	48.84	48.97	52.07
P ₄	39.77	40.01	44.90	50.00
			SEm±	CD (P=0.05)
For B at same level of P			1.30	3.66
For p at same or diff. level of B			2.10	5.94

Table.3 Effect of bio-fertilizers and bio-regulators on number of cloves per bulb and bulb yield

Treatments	Number of cloves per bulb			Bulb yield kg/plot			Bulb yield q/ha		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Bio-fertilizers									
B₀ (Control No inoculation)	15.23	16.32	15.78	2.95	3.14	3.04	164.00	174.18	169.09
B₁ (<i>Azotobacter</i>)	16.98	17.58	17.28	3.37	3.42	3.40	187.20	190.17	188.68
B₂ (PGPR)	17.48	17.98	17.73	3.47	3.55	3.51	193.03	197.15	195.09
B₃ (<i>Azotobacter</i> + PGPR)	18.65	19.12	18.89	3.69	3.77	3.73	205.19	209.54	207.37
SEm_±	0.36	0.39	0.28	0.07	0.06	0.06	2.96	3.60	2.28
CD (P=0.05)	1.14	1.12	0.85	0.22	0.18	0.18	9.31	10.34	7.03
Bio-regulators									
P₀ (Control water spray)	15.44	16.54	15.99	2.88	2.98	2.93	159.82	165.60	162.71
P₁ (Thiourea @ 500 ppm)	17.91	18.47	18.19	3.57	3.64	3.61	198.29	202.48	200.39
P₂ (Thiourea @ 1000 ppm)	18.11	18.88	18.49	3.67	3.76	3.71	203.76	208.97	206.36
P₃ (Salicylic acid @ 100 ppm)	17.64	18.17	17.90	3.51	3.64	3.58	195.25	202.49	198.87
P₄ (Mepiquat chloride @ 100 ppm)	16.34	16.69	16.52	3.23	3.32	3.28	179.65	184.25	181.95
SEm_±	0.36	0.42	0.30	0.05	0.10	0.05	2.98	3.47	2.58
CD (P=0.05)	1.03	1.32	0.84	0.14	0.30	0.14	8.56	10.95	7.30

Table.4 Interactive effect of bio-fertilizers and bio-regulators on bulb yield kg/plot

Treatments	B ₀	B ₁	B ₂	B ₃
2016-17				
P ₀	2.82	2.85	2.89	2.95
P ₁	3.01	3.63	3.68	3.96
P ₂	3.12	3.74	3.83	3.98
P ₃	2.93	3.63	3.64	3.87
P ₄	2.89	3.00	3.33	3.71
			SEm_±	CD (P=0.05)
For B at same level of P			0.10	0.28
For p at same or diff. level of B			0.18	0.51
2017-18				
P ₀	3.03	2.91	2.96	3.03
P ₁	3.08	3.71	3.76	4.03
P ₂	3.27	3.80	3.91	4.07
P ₃	3.23	3.70	3.71	3.94
P ₄	3.07	3.00	3.40	3.79
			SEm_±	CD (P=0.05)
For B at same level of P			0.13	0.36
For p at same or diff. level of B			0.24	0.68
Pooled				
P ₀	2.92	2.88	2.93	2.99
P ₁	3.05	3.67	3.72	4.00
P ₂	3.19	3.77	3.87	4.02
P ₃	3.08	3.66	3.67	3.91
P ₄	2.98	3.00	3.37	3.75
			SEm_±	CD (P=0.05)
For B at same level of P			0.10	0.28
For p at same or diff. level of B			0.17	0.47

Table.5 Interactive effect of bio-fertilizers and bio-regulators on bulb yield (q/ha)

Treatments	B ₀	B ₁	B ₂	B ₃
2016-17				
P ₀	156.46	158.33	160.63	163.88
P ₁	167.08	201.92	204.38	219.79
P ₂	173.13	207.63	213.04	221.25
P ₃	162.71	201.46	202.04	214.79
P ₄	160.63	166.67	185.04	206.25
			SEm_±	CD (P=0.05)
For B at same level of P			5.96	17.16
For p at same or diff. level of B			9.49	27.34
2017-18				
P ₀	168.13	161.46	164.67	168.17
P ₁	171.25	205.92	208.63	224.13
P ₂	181.46	211.17	217.33	225.92
P ₃	179.25	205.54	206.04	219.13
P ₄	170.79	166.75	189.08	210.38
			SEm_±	CD (P=0.05)
For B at same level of P			7.20	20.74
For p at same or diff. level of B			11.38	32.78
Pooled				
P ₀	162.29	159.90	162.65	166.02
P ₁	169.17	203.92	206.50	221.96
P ₂	177.29	209.40	215.19	223.58
P ₃	170.98	203.50	204.04	216.96
P ₄	165.71	166.71	187.06	208.31
			SEm_±	CD (P=0.05)
For B at same level of P			5.17	14.60
For p at same or diff. level of B			7.98	22.54

Interaction effect of bio-fertilizers and bio-regulators on bulb yield (kg/plot)

Application of *Azotobacter* + PGPR and thiourea @1000 ppm/ha recorded significantly higher bulb yield (4.02 kg/plot) over rest of the treatment combinations (Table 4). Similar results were reported by Singh and Pandey, (2006), Chattoo *et al.*, (2007) Bhandari *et al.*, (2012) and Sharma (2014).

The increase in bulb yield owing to this treatment may be due to the fact that N and P play an important role in synthesis of chlorophyll and amino acid (Black, 1967) and *Azotobacter* and PGPR ensured the continuous supply of these nutrients, very limited work has been earned out on the use of bio-fertilizers in garlic. However, Yadav *et al.*, (2004), Senapati *et al.*, (2005), Velmurugan and Chezhiyan (2005), Singh and Pandey (2006), Anonymous (2007), Balemi *et al.*, (2007), Bhandari *et al.*, (2012), Choudhary *et al.*, (2014), Banjare *et al.*, (2015) and Sachin *at al.* (2017) in garlic (Table 5). Maximum bulb yield of garlic was registered with treatment *Azotobacter* + PGPR both were found significantly inferior to rest of the treatments. Insufficient supply of nutrients along with reduced growth parameters and poor yield attributes might be the reason for lower bulb yield in these treatments.

Application of *Azotobacter*+PGPR along with thiourea @1000 ppm/ha. recorded maximum bulb yield of garlic (223.58 q/ha). The combined application of *Azotobacter* + PGPR with thiourea @1000 ppm /ha. showed significantly higher bulb yield over rest of treatment combinations.

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 yield, net returns and B: C ratio (223.58 q ha⁻¹, ₹ 189332 and 2.40), respectively. Although, application of (*Azotobacter* + PGPR (*Pseudomonas*) + thiourea @ 500 ppm) and (*Azotobacter* + PGPR (*Pseudomonas*) + salicylic acid @ 100 ppm) were found statistically at par to it.

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