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INFLUENCE OF INORGANIC NUTRIENTS AND BIO-FERTILIZERS IN COMBINATION WITH BIO-CONTROL AGENTS AND BOTANICAL EXTRACTS ON QUALITATIVE PARAMETERS AND ECONOMICS OF GARLIC (*ALLIUM SATIVUM* L)

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ABSTRACT

Garlic (*Allium sativum* L.,) is used as a spice and condiment. It is one of the important cultivated *alliu* species after onion having tremendous medicinal properties. In current investigation the attempt wa made to test the inorganic nutrients and bio-fertilizers in combination with bio-control agents an botanical extracts on qualitative properties and economics of garlic under southern Telangana region. Th results revealed that, significant difference in Total Soluble Solids, sulphur content of bulb and essentia oil content of bub was observed with combined application of soil nutrients and bioformulations. Th highest TSS (39.29 ^{0}Brix) was reported in treatment N_3B_1 (50%N +50%P+ 100%K+ *Azotobacter* at kg/ha+ PSB 5 kg/ha+ *Trichoderma viride* at 10ml/l + Neem oil at 0.5%) and highest dry matter conten (34.52%), sulphur content (1.25%) was recorded in N_2B_3 (50%N +100%P+50%K+ *Azotobacter* a 5kg/ha+ KSB at 6 kg/ha+ *Bacillus subtilis* at 10ml/l + Sesame oil at 1%). The highest ascorbic aci content (8.66 mg/100g) was recorded in N_3B_3 (50%N +50%P+ 100%K+ *Azotobacter* at 5 kg/ha+ PSB kg/ha+ *Bacillus subtilis* at 10ml/l + Sesame oil at 1%). Further, the highest total biomass 7.80 t/ha harves index (82.97 %) Grass returns Rs. 782333.3, net returns Rs.579319.35 and highest benefit cost rati (2.86) was obtained under N_2B_1 (50%N +100%P+50%K+ *Azotobacter* at 5kg/ha+ KSB at 6 kg/ha *Trichoderma viride* at 10ml/l + Neem oil at 0.5%) among all the tested treatments.
***Keywords* :** Quality, Bio-fertilizers *Trichoderma viride*, *Bacillus subtilis,* Neem oil.

Introduction

All bulb crops belong to monocotyledon family Alliaceae and the genus *Allium*. Among bulb crops Garlic is cultivated in *Rabi* season from ancient time. Garlic (*Allium sativum* L.) is one of the important bulb crop grown and used as a spice or condiment throughout India. Garlic has been cultivated for thousands of years. It is the most ancient cultivated vegetables giving pungency of the genus *Allium*. Original abode of Garlic is said to be Central Asia and Southern Europe especially, Mediterranean region.

Garlic contains about 0.1% volatile oil. The chief constituents of oil are diallyl disulfide (60%), diallyltrisulfide (20%), allyl propyl disulfide (6%), a small quantity of diethyl disulfide and probably diallyle polysulfide. The Diallyl disulfide possesses the true garlic odour. However, when cloves are crushed allicin was obtained which has some amount of fat, vitamin-C and sulphur (Memane *et al.*, 2008). Garlic has Anti-bacterial, Antiviral, Antifungal and anti-protozol properties and it has anti-oxidant and anti-cancer properties (Harris *et al.*, 2001). The person suffering with risk of vascular calcification with high blood cholesterol can be reduced by supplementation with garlic extract (Durak *et al.*, 2004).

Nitrogen, phosphorus and potassium play key roles in garlic's development and output in terms of yield (Mallangouda *et al.*, 1995). Garlic requires nitrogen supplementation to ensure good vegetative growth (Kakara *et al.*, 2002). In plant metabolism, potassium is essential for processes like photosynthesis, food transport, pore modulation, catalyst activation, and disease and pest resistance. Potassium enhances the crop's color, glossiness, and dry matter buildup in addition to its storage quality (Dorais *et al.*, 2001). In addition to improving the biological fixation of atmospheric nitrogen and producing hormones and anti-metabolites. Biofertilizers are products that contain living cells of various microorganisms that can convert nutritionally important elements. They are also known to play a major role in increasing the availability of N and P (Bhat *et al.*, 2013).

Biological agents are living organisms that may considerably reduce the density of plant pathogens and have gained popularity as an alternative to conventional pesticides for pest and disease control (O'Brien, 2017). By accomplishing this, the over-reliance on pesticides that causes development of resistance against the pesticide, flora and fauna extinction, environmental degradation, and by using the bio-organics the health issues of farmers and consumers may be reduced. The fungus of the *Trichoderma* genus is among the most potent opponents of plant diseases. One of the most often utilized plant pathogen antagonists is *Trichoderma harzianum*, which is a component of several biopreparations. In addition to its ability to act as a mycoparasite, it also generates antibiotics and is a great nutritional competitor because of its rapid growth rate. Additionally, it can activate defensive systems in plants (Benitez *et al.*, 2004). During in vitro experiments, B. *subtilis* is a species that can stop B. *cinerea* from growing. In addition to preventing growth, they were also 80% less likely to germinate conidia on strawberry fruits (Donmez *et al.*, 2011). *Pseudomonas* sp. is a commonly occurring fungi in almost all cultivated areas. It generates phenazines and siderophores, which may function as growth factors, and grows rapidly. Phenazines are a broad class of substances that promote plant growth. Additionally, they are able to generate systemic resistance (Pierson and Pierson 2010).

Materials and Methods

Experimental Site

Field study was conducted two consecutive years at Medicinal and Aromatic Plant Research Station, Rajendranagar, Hyderabad -SKLTGHU, during *Rabi*, 2022-23 and 2023-24 Geographically, the experimental site lies at latitude of 17°.19' N, longitude of 79°.23' E and an altitude of 542.6 m above mean sea level. The average maximum temperatures during crop period i.e., 2022-2023 and 2023-2024 were 33.5 and 36.3 °C and the minimum average temperature were 13.7 °C and 16 °C, respectively. The average maximum relative humidity during crop period i.e., 2022-2023 and 2023-2024 were 92.0% and 92.0% and the average minimum relative humidity were 35.0% and 29.0% respectively. The soil is sandy loam in nature, coarse in texture, good in water holding capacity with low pH (acidic-5.25). In both years, the composite soil samples from the entire experimental plots were collected randomly and analyzed before planting.

Experimental setup

The total experimental area in this study was 410 m^2 which was divided into 3 blocks; each block was then subdivided into ten plots. The plant to plant and row to row distances were followed about 10 and 20 cm respectively. There were 10 treatments and each treatment was repeated thrice by using methodology of Contrast Factorial Randomized Block Design according to the experiment's design, the raised beds of 3.0×2.0 m^2 were prepared and each plot was divided from the others by a 50 cm-wide canal on four sides.

Factor-I : Chemical fertilizers combination with biofertilizers

N_1:100% N +50 % P + 50% K + PSB @ 5Kg/ha +KSB@ 6Kg/ha;

N_2: 50%N +100%P+50%K+ *Azotobacter* @ 5kg/ha+ KSB@ 6Kg/ha;

N_3:50%N +50%P+ 100%K+ *Azotobacter* @ 5kg/ha+ PSB @ 5Kg/ha) and

Factor-II: Bioformulations

B_1: *Trichoderma viride* @ 10ml/l + Neem oil @ 0.5%

B_2: *Pseudomonas fluorescence* @ 10ml/l + Pongamia oil @ 0.5%

B_3: *Bacillus subtilis* @ 10ml/l + Sesame oil @ 1%)

Control (100% RDF+ Imidacloprid 17.8 SL @ 0.3 ml/lit)

The required quantity of bioformulation applied as soil drenching as well as foliar application to the plants.

Method of data collection

Total soluble solids (ºBrix)

Following crop harvest, TSS was measured using a hand refractometer. Using a mortar and pestle, the garlic cloves were smashed and then muslin fabric was used to drain the liquid. The refractometer prism was well cleaned with distilled water and dry tissue paper before to the measurement of TSS. One or two drops of juice was placed on the prism and exposed to light to view the scale therein. The readings were then recorded and the mean value was calculated.

Ascorbic acid content (mg 100-1 g)

Fresh bulbs from representative plants were selected and chopped into little pieces, ideally of uniform size. Using the 2,6 dichlorophenol indophenol visual titration technique A.O.A.C. 1975), the vitamin C content of 100 grams of chopped fresh bulbs from each plot was then estimated in the lab and expressed in milligrams per 100 g of fresh bulbs. The procedures listed as follows

Procedure

1. Standardization of dye: 5 ml of standard ascorbic acid solution and 5 ml of 3 per cent MPA were taken and titrated with the dye solution to pink colour.

2. Dye factor (D.F) = 0.5/titration value

3. Preparation of sample and titration: From 100 g of fresh bulbs, juice was extracted and filtrated and the volume made up to 100 ml with 3 per cent MPA. 5 ml was then taken and titrated with the standard dye till the pink colour was observed.

The ascorbic acid content of the sample was calculated by adopting the following equation

$$= \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract taken for estimation} \times \text{Weight of sample taken for estimation}}$$

Dye factor = 0.5/ Average burette reading for standardization of dye solution

Dry matter content (%)

A 100 g homogenate sample was used to determine the dry matter content. It was subsequently oven-dried for 48 hours at 65ºC. The dry weight was measured using a digital balance and computed using the following formulas:

$$\text{DM}(\%) = \frac{(\text{DW} + \text{CW}) - \text{CW}}{(\text{FW} + \text{CW}) - \text{CW}} \times 100$$

Where, DW = dry weight, FW = fresh weight, CW=container weight

Sulphur content of bulb (%)

The turbidimetric approach was used to quantify sulphur (Bardsley and Lancaster, 1960). Using a gelatine barium chloride solution, a 0.5g plant sample was digested using a 3:1 di-acid combination (nitric acid and perchloric acid) to create turbidity. A spectrophotometer was used to quantify the resulting turbidity and the percentage of sulphur content was calculated using dry weight.

Harvest Index (%)

Harvest index = (Economic yield/Biological yield) x100

Economics

Benefit Cost Ratio

The price of the inputs in rupees prevailing at the time of experimentation were considered for working out the cost of cultivation.Net returns per hectare were calculated by deducting the cost of cultivation from gross return. Benefit cost ratio was worked out as follows: Benefit: Cost ratio was worked out by using the formula,

$$\text{Benefit : Cost ratio} = \frac{\text{Gross income (Rs. ha)}}{\text{Cost of cultivation (Rs. ha)}}$$

Statistical analysis

The data pertaining to all characters studied were subjected to statistical analysis using variance techniques as described by Panse and Sukhatme (1967); Gomez and Gomez (1983) and Rangaswamy (2010). The significant difference of treatments was tested by 'F' test at 5 per cent level of significance. The critical difference (C.D.) was calculated when the difference among the treatments was found to be significant by 'F' test.

Results and Discussion

Effect of soil nutrients

Data regarding total soluble solids (TSS), sulphur content, Ascorbic acid content, Dry matter content and total biomass and harvest index are presented in table 1. Which revealed that significant difference between soil nutrients and bioformulations. Based on two seasons pooled data. The maximum TSS and Ascorbic acid content (36.86 ^{0}Brix and 8.49 mg respectively) was recorded with 50%N +50%P+ 100%K+ *Azotobacter* at 5 kg/ha+ PSB at 5 kg/ha. The highest sulphur content of bulb, dry matter content of bulb, Total biomass and harvest index (1.09% 33.33%, 7.60 t/ha and 80.68% respectively) was observed under treatment of 50%N +100%P+50%K+ *Azotobacter* at 5 kg/ha+ KSB 6 at kg/ha.

Effect of Bio formulations

Application of bio-formulations on TSS, sulphur content, Ascorbic acid content, Dry matter content and total biomass and harvest index differed significantly. The pooled data of both the seasons (Table 1and 2) revealed that highest TSS, Ascorbic acid content, dry matter content of bulb, total biomass and harvest index (36.66 ^{0}Brix, 7.94 mg, 31.63%, 7.25 t/ha and 79.71% respectively) recorded with *Bacillus subtilis* at 10ml/l + Sesame oil at 1%. However, control (100 % RDF-100:50:50 kg NPK/ha+ Imidacloprid) recorded the minimum TSS, Ascorbic acid content, dry matter content and sulphur content of bulb (30.00 ^{0}Brix, 6.69 mg, 24.44% and 0.64% respectively)

Interaction effect of soil nutrients and bioformulations

The two season pooled data (Table.1) revealed that the statistically significant difference regarding Total Soluble Solids and sulphur content of bulb was observed with combined application of soil nutrients and bioformulations. The pooled data related to Total Soluble solids revealed that highest TSS (39.29 ^{0}Brix) was reported in treatment N_3B_1 (50%N +50%P+ 100%K+ *Azotobacter* at 5 kg/ha+ PSB 5 kg/ha+ *Trichoderma viride* at 10ml/l + Neem oil at 0.5%) and highest dry matter content (34.52%), sulphur content (1.25%) was recorded in N_2B_3 (50%N +100%P+50%K+ *Azotobacter* at 5kg/ha+ KSB at 6 kg/ha+ *Bacillus subtilis* at 10ml/l + Sesame oil at 1%). However, the highest ascorbic acid content (8.66 mg/100g) was recorded in N_3B_3 (50%N +50%P+ 100%K+ *Azotobacter* at 5 kg/ha+ PSB 5 kg/ha+ *Bacillus subtilis* at 10ml/l + Sesame oil at 1%). Further, the highest total biomass 7.86 t/ha harvest index (82.97 %) was obtained under N_2B_1 (50%N +100%P+50%K+ *Azotobacter* at 5kg/ha+ KSB at 6 kg/ha+ *Trichoderma viride* at 10ml/l + Neem oil at 0.5%)

The increase in TSS is mostly due to the hydrolysis of starch into sugars. A study by Hussain *et al.* (2017) showed that garlic with higher TSS levels typically contains higher amounts of soluble vitamins and antioxidants. Vitamin C, in particular, is water-soluble and contributes to TSS, while phenolic compounds also increase TSS. Higher TSS levels suggest an increase in the concentration of soluble amino acids, including those involved in the synthesis of sulphur compounds. The formation of sulphur compounds like allicin is a result of enzymatic action that occurs when garlic is crushed or chopped. Higher TSS can reflect a greater concentration of these compounds, which are soluble in the cellular fluid of garlic. Brady *et al.* (2007) discusses how garlic's pungency and sulphur content change with maturation, which can influence both TSS which reduces the pungency of garlic and its health effects. The quality parameters like vitamin C content varied with application of N P and K Rani *et al*. (2020). Increasing the fertigation level of NPK influenced the ascorbic content of garlic due to be potassium, it is a quality nutrient that is highly responsible for carbohydrate metabolism and thus increases the ascorbic acid content. The similar result was also reported by Yadav (2006) in onion. Combination of treatments increased dry matter content this may be due to photosynthates' effectively translocated to bulbs, which increased bulb size. Phosphorus along with *Bacillus subtilis* is also essential for the development of a larger root system, which enables plants to access nutrients and water from deeper soil. Eventually, the plants could be able to produce more assimilates, which would increase the content of dry matter in the bulbs. These results are in conformity with the findings of Mahendran and Kumar (1997), Suresh (1997) and Lal *et al.* (2002), in garlic.

Economics of garlic under crop management practices

The data related to the effect of different levels of soil nutrients and bio formulations and their combined effect on economics of garlic is presented in Table 3**.**

An examination of the data revealed that the maximum gross and net income of Rs. 7,82,333.3 and Rs. 5,79,319.35 as well as highest benefit cost ratio (2.86) was worked out in $N_2B_1$was observed in treatment N_2B_1

In the present study, the maximum benefit cost ratio was obtained in plants treated with 50% N + 100% P + 50% K + *Azotobacter* at 5 kg/ha + KSB at 6 kg/ha + *Trichoderma viride* at 10 ml/l + Neem oil at 0.5%. This is due to higher bulb yield from respective treatment. Bulbs can get higher income per rupee invested by adopting integrated approach.

Conclusion

From the study it can be concluded that the application of different combinations of soil nutrients along with biofertilizer and bio formulations play a significant role in enhancing the soil fertility in terms of macronutrients, secondary nutrients, micronutrients and microbial population. The biofertilizers helped in enriching the soil with the major nutrients like N, P and K which are mainly essential for luxuriant growth of the crop as well as enhancing the quality of produce.

Table 1 : Effect of soil nutrients and bi-formulations on Total soluble solids, ascorbic acid content and sulphur content of garlic

Treatments	Total Soluble Solids			Ascorbic acid content			Sulphur content		
	2022-23	2023-24	pooled	2022-23	2023-24	pooled	2022-23	2023-24	pooled
Nutrients									
N_1	33.8	30.18	**31.99**	8.1	6.91	**7.51**	0.96	0.72	**0.84**
N_2	36.21	35.86	**36.03**	7.17	7.69	**7.43**	1.15	1.03	**1.09**
N_3	36.78	36.94	**36.86**	8.49	8.48	**8.49**	0.91	0.81	**0.86**
S. Em+	0.13	0.10	0.07	0.02	0.03	0.02	0.005	0.006	0.004
CD (5%)	0.35	0.30	0.22	0.07	0.11	0.08	0.01	0.02	0.01
Bio-agents									
B_1	36.63	36.69	**36.66**	8.03	7.85	**7.94**	1.02	0.86	**0.94**
B_2	34.64	32.62	**33.63**	7.74	7.49	**7.61**	0.95	0.8	**0.87**
B_3	35.51	33.66	**34.59**	8	7.74	**7.87**	1.07	0.9	**0.98**
S. Em+	0.13	0.10	0.07	0.02	0.03	0.02	0.005	0.006	0.004
CD (5%)	0.35	0.30	0.22	0.07	0.11	0.08	0.01	0.02	0.01
Interaction (NXB)									
N_1B_1	34.37	31.94	**33.15**	8.31	7.19	**7.75**	1.01	0.75	**0.88**
N_1B_2	32.3	28	**30.15**	7.85	6.63	**7.24**	0.93	0.7	**0.82**
N_1B_3	34.72	30.61	**32.67**	8.14	6.91	**7.53**	0.99	0.73	**0.86**
N_2B_1	36.92	38.15	**37.53**	7.32	7.87	**7.60**	1.1	0.99	**1.05**
N_2B_2	35.06	34.37	**34.72**	7.01	7.53	**7.27**	1.02	0.94	**0.98**
N_2B_3	36.64	35.05	**35.84**	7.17	7.66	**7.42**	1.34	1.15	**1.25**
N_3B_1	38.6	39.99	**39.29**	8.46	8.48	**8.47**	0.94	0.85	**0.90**
N_3B_2	36.56	35.5	**36.03**	8.35	8.31	**8.33**	0.91	0.75	**0.83**
N_3B_3	35.18	35.33	**35.26**	8.68	8.65	**8.67**	0.87	0.83	**0.85**
S. Em+	0.35	0.30	0.22	0.07	0.11	0.08	0.01	0.02	0.01
CD (5%)	1.07	0.90	0.86	NS	NS	NS	0.04	0.05	0.03
Control	31.46	28.54	30	7.09	6.28	6.69	0.68	0.61	0.64
S. Em+	0.35	0.30	0.22	0.07	0.11	0.08	0.01	0.02	0.01
CD (5%)	1.36	1.16	1.03	0.28	0.35	0.23	0.05	0.07	0.03

Table 2 : Effect of soil nutrients and bi-formulations on dry matter content, total biomass and harvest index of garlic

Treatments	Dry matter content			Total Biomass			Harvest Index		
	2022-23	2023-24	pooled	2022-23	2023-24	pooled	2022-23	2023-24	pooled
Nutrients									
N_1	31.12	29.01	**30.06**	7.15	7.02	**7.1**	76.48	79.07	**77.78**
N_2	32.8	33.87	**33.33**	7.55	7.77	**7.6**	80.98	80.39	**80.68**
N_3	28	31.39	**29.7**	6.47	6.35	**6.4**	73.52	76.35	**74.93**
S. Em+	0.19	0.10	0.11	0.01	0.01	0.01	0.18	0.23	0.15
CD (5%)	0.59	0.31	0.35	0.04	0.04	0.03	0.50	0.70	0.63
Bio-agents									
B_1	31.4	31.87	31.63	7.33	7.13	7.25	78.35	81.08	**79.71**
B_2	29.37	30.61	29.99	6.77	6.73	6.75	74.79	75.82	**75.31**
B_3	31.14	31.79	31.47	7.1	7.23	7.13	77.83	78.9	**78.37**
S. Em+	0.19	0.10	0.11	0.01	0.01	0.01	0.18	0.23	0.15
CD (5%)	0.59	0.31	0.35	0.04	0.04	0.03	0.50	0.70	0.63
Interaction (NXB)									
N_1B_1	31.41	29.51	**30.46**	7.25	7.13	**7.19**	76.98	79.67	**78.33**
N_1B_2	30.83	28.97	**29.9**	7.07	6.92	**7.00**	75.69	78.87	**77.28**
N_1B_3	31.11	28.54	**29.825**	7.12	7	**7.06**	76.79	78.66	**77.73**

N_2B_1	32.89	33.64	**33.265**	7.75	7.97	**7.86**	82.59	83.36	**82.98**
N_2B_2	31.52	32.92	**32.22**	7.18	7.4	**7.29**	79.56	77.2	**78.38**
N_2B_3	33.98	35.05	**34.515**	7.72	7.93	**7.83**	80.78	80.61	**80.70**
N_3B_1	29.89	32.45	**31.17**	6.98	6.32	**6.65**	75.48	80.2	**77.84**
N_3B_2	25.77	29.95	**27.86**	6	5.93	**5.97**	69.11	71.4	**70.26**
N_3B_3	28.34	31.78	**30.06**	6.43	6.78	**6.61**	75.96	77.45	**76.71**
S. Em+	0.59	0.31	0.35	0.04	0.04	0.03	0.50	0.70	0.63
CD (5%)	NS	NS	NS	0.13	0.13	0.09	1.60	2.07	1.40
Control	25.81	23.08	24.44	7.68	7.88	7.75	74.77	75.9	75.33
S. Em+	0.59	0.31	0.35	0.04	0.04	0.03	0.50	0.70	0.63
CD (5%)	2.29	1.19	1.37	0.16	0.17	0.12	2.07	2.60	1.81

Table 3 : Economics of garlic influenced by inorganic nutrients and bioformulations

Treatments	Cost of cultivation (Rs/ha.)	Market price of garlic Rs/kg	Gross returns (Rs/ha.)	Net returns (Rs/ha.)	B:C Ratio
T_1-N_1B_1	202366.00	120	676333.33	473967.33	**2.34**
T_2-N_1B_2	208366.00	120	648333.31	439967.31	**2.12**
T_3-N_1B_3	202966.00	120	658333.3	455367.35	**2.25**
T_4-N_2B_1	203014.00	120	782333.3	579319.35	**2.86**
T_5-N_2B_2	209014.00	120	685417.31	476403.31	**2.28**
T_6-N_2B_3	203614.00	120	757666.68	554052.68	**2.73**
T_7-N_3B_1	202510.00	120	620042.66	417532.66	**2.06**
T_8-N_3B_2	208510.00	120	508999.98	300489.98	**1.45**
T_9-N_3B_3	203110.00	120	609000.01	405890.01	**2.00**
T_{10}-control	195870.00	120	703666.65	507796.65	**2.60**

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Authors Contributions

SR carried out the field experiment, collection, noting, recorded data, wrote drafts for research work. JC participated in the design of the study, supervised the whole research and helped in compiling the manuscript. PP contributed to analysis of samples data, technical manuscript writing and.MS contributed to editing the manuscript. BN helped analyze soil and plant samples. VS performed the statistical analysis of data and supervision of pest dynamics in field. All authors read and approved the final manuscript.

Conflict of interest: The authors declare no conflicts of interest.

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