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Impact of bioinoculants and chemical fertilizers on soil properties of garlic (*Allium sativum*) cv. solan selection

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ABSTRACT

The field experiment was conducted at the Vegetable Research Farm of the Department of Vegetable Science, Dr. YSP UHF, Nauni, Solan during the Rabi seasons of 2023-2024. The garlic cultivar Solan Selection was used to study the impact of chemical fertilizers combined with bioinoculants on the soil properties of garlic (Allium sativum) in a randomized complete block design with seven treatments and three replications. The treatments included combinations of B. licheniformis and B. pumilis at 10 ml per plant with 100% and 75% recommended doses of nitrogen (N) and phosphorous (P). Individual treatments of B. licheniformis and B. pumilis @ 10 ml per plant and the recommended dose of fertilizer (RDF) were also included. Various soil parameters, such as soil pH, electrical conductivity (EC), organic carbon, available nitrogen, available phosphorus, available potassium and viable count were observed. The results indicated that the 100% recommended dose of N & P + B. licheniformis @ 10 ml/plant resulted in a minimum soil pH, maximum electrical conductivity, organic carbon content, nitrogen, phosphorus, potassium, and viable count of fungi, bacteria, and actinomycetes under mid-hill conditions of Himachal Pradesh. Challenges faced during conducting research is variability in soil microbial communities and environmental conditions, which can influence the consistency and reproducibility of results.

Keywords: *Bioinoculants, Garlic, Soil properties, Bacillus licheniformis, Bacillus pumilis, Chemical fertilizers, Solan Selection, RDF, Nitrogen, Phosphorus, Potassium.*

Highlights

- Joint application of bioinoculants with fertilizers enhanced soil quality in garlic
- Best responses were recorded with *B. licheniformis* + 100% N & P in respect of soil and microbes.
- The values of pH of the soil reduced whereas the EC, OC, NPK and microbial count significantly enhanced.
- Bioinoculant treatments improved the number of viable microorganisms in the rhizosphere.
- Research advocates sustainable garlic cultivation in Himachal mid-hills

Introduction

Garlic (*Allium sativum* L.) belongs to the family Amaryllidaceae and has a chromosome number of 2n=16. Fresh garlic is packed with numerous vitamins, minerals, and trace elements. It is also a rich source of carbohydrates, proteins, manganese, vitamin B6, vitamin C, copper, selenium, vitamin B1, phosphorus, and calcium. The fresh peeled cloves of garlic have 6.30% protein, 1.0% mineral matter, 29.0% carbohydrates, 60%–65% moisture, 0.10% fat, 0.80% fiber, 0.03% calcium, 0.31% phosphorus, 0.001% iron, 13 mg/100g vitamin C, and 0.40 mg/100g nicotinic acid. Garlic has a higher sulfur content than any member of the Allium genus. It contains about 0.5% volatile oil, which is comprised of sulfur-containing compounds, vitamin B, and flavonoids [1]. The major garlic growing states in India are Madhya Pradesh, followed by Gujarat, Uttar Pradesh, Rajasthan, Assam, and Punjab. Garlic is popular worldwide as a valuable spice for cooking a variety of dishes. Additionally, it is used in the preparation of pickles, chutneys, curry powder, vegetables, and tomato ketchup. Biofertilizers are naturally occurring products containing living microorganisms that are derived from the roots or cultivated soil. They have no negative effect on plant, soil health, and the environment. In addition to their role in fixing atmospheric nitrogen and solubilizing phosphorus, biofertilizers also help in stimulating plant growth hormones. Examples of biofertilizers include *Azotobacter*, PSB and *Azospirillum*, which fix atmospheric nitrogen and solubilize phosphorus to enhance soil fertility and its biological activities. Biofertilizers contain living cells of different types of microorganisms that can convert important nutrients, increase the availability of nitrogen and phosphorus, improve biological fixation of atmospheric nitrogen, and produce hormones and anti-metabolites [2]. The application of chemical fertilizers has been instrumental in replenishing nutrients depleted by crops and increasing crop yields. However, the continuous use of chemical fertilizers alone over the years has led to a loss of soil organic carbon and a decline in soil health. Soil organic matter is a vital component that determines soil quality [3]. It serves as a reservoir of plant nutrients and provides a substrate for soil

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microorganisms [4]. Nitrogen is crucial for plant growth, as it is a major component of protein and nucleic acid molecules and is part of chlorophyll molecules. Phosphorus is an essential component of nucleic acids, phospholipids, and several enzymes, and is necessary for energy transfer within the plant system and various metabolic activities. Phosphorus also has beneficial effects on early root development, plant growth, yield, and quality. Potassium plays a vital role in plant metabolism, including photosynthesis, translocation of photosynthates, regulation of plant pores, activation of plant catalysts, and resistance against pests and diseases. Additionally, potassium improves the color, glossiness, and dry matter accumulation of crops, as well as their keeping quality [5]. The *Bacillus* genus consists of Gram-positive, spore-forming, rod-shaped bacteria that are obligate aerobes. Thanks to their ability to form endospores, these bacteria can survive in various extreme environmental conditions, such as variations in temperature, pH, and salinity, making them widespread in nature [6]. *Bacillus* species enhance plant growth through both direct and indirect mechanisms. Indirectly, they help mitigate the effects of phytopathogens, leading to better plant health [7]. Directly, Bacillus spp. Produce siderophores and phytohormones like indole-3-acetic acid, cytokinin, and gibberellic acid, and they facilitate the solubilization of phosphorus and other essential nutrients. These actions actively promote plant growth and increase root volume [8-10]. *Bacillus pumilis*, a Gram-positive, spore-forming bacterium, is commonly found in environments like marine water, sea sediments, and soil [11-12]. It is highly resistant to environmental stresses such as low nutrient availability, drought, UV radiation, oxidizing enzymes, and chemical disinfectants [13]. *Bacillus pumilis* is classified as a plant growth-promoting bacterium due to these characteristics [14-15]. *Bacillus licheniformis* is a facultative anaerobic, Gram-positive, rod-shaped bacterium that forms endospores. It is closely related to *Bacillus subtilis*. *Bacillus licheniformis* has been found to possess strong plant growth-promoting activity [16] and is known to produce auxins, which are vital for plant growth.

2. Material and Method

2.1 Description of the Study Area

The present study was carried out at Vegetable Research Farm, Department of Vegetable Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during *Rabi* season of 2023-24. The experimental site is located at Nauni, about 14 km from Solan, at an altitude of 1276 meters above mean sea level lying at a longitude of 77˚ 11' 30'' E and a latitude of 30˚ 52' 30'' N and falls in the sub-humid, sub-temperate and mid hill zone of Himachal Pradesh.

2.2 Description of the Plant Material

The cultivar "Solan Selection," a local clonal variety from Himachal Pradesh, was chosen for the study.

2.3 Treatments and experimental design

The experiment consisted of seven treatments which included treatment T_1 (100% recommended dose of N & P + *B. licheniformis* @ 10 ml/plant), T_2 (75% recommended dose of N & P + *B. licheniformis* @ 10 ml/plant), T_3 (100% recommended dose of N & P + *B. pumilis @ 10* ml/plant), T_4 (75% recommended dose of N & P + *B. pumilis* @ 10 ml/plant), T_5 (*B. licheniformis* @ 10 ml/plant), T_6 (*B. pumilis* @ 10 ml/plant) and T_7 (RDF). The experiment was set up in a randomized complete block design with three replications, resulting in a total of 21 experimental plots. The spacing was 15 cm between rows and 10 cm between plants, with raised plots measuring 1.5 m × 1.5 m.

2.4 Experimental Procedures

During planting, cloves were separated from the bulbs and then treated with bioinoculants (*B. licheniformis* & *B. pumilis*). After treatment, the cloves were planted 3-4 cm deep in raised beds by hand on September 25. The experimental fields were ploughed three times and well harrowed to prepare the soil. Clods were broken manually, and the experimental plots were set up on a fine seedbed. Fertilizer was applied according to the specific rate assigned for each experimental plot. Weed control was carried out by hoeing and shallow earthing up. Harvesting took place in May when 70% of the leaves had fallen, and it was done by hand. The harvested bulbs were then cured and sun-dried for 10 days, with the leaves folded over the bulbs to protect them from sunburn. After a week of drying, the necks and roots were trimmed. The yields obtained from the plot were then weighed and recorded after curing.

2.5 Observations Recorded

The soil samples were collected after harvesting the crop, by taking three soil samples randomly from each treatment from 0-15 cm depth and were air dried, crushed and passed through 2 mm sieve. Further analysis for soil pH, EC, OC, N, P, K and viable count was carried out.

3. Results

3.1 pH

In Table 3.1, it is unvieled that the lowest soil pH (6.47) was observed in treatment T_1 (100% recommended dose of N & P + *B. licheniformis* @10 ml/plant).

3.2 Electrical conductivity (EC)

Table 3.1 revealed that the highest electrical conductivity (0.69 dSm^{-1}) was observed in treatment T_1, which consisted of 100% recommended dose of N & P + *B. licheniformis* @ 10 ml /plant.

3.3 Organic carbon (%)

The results in Table 3.1 showed that the highest organic carbon content (1.49%) was found in treatment T_1 (which involved using 100% of the recommended dose of N & P + *B. licheniformis* @ 10 ml per plant).

3.4 Available nitrogen

According to the results in Table 3.2, treatment T_1 (100% recommended dose of N & P + *B. licheniformis* @ 10 ml/plant) showed the highest available nitrogen content at 328.01 kg/ha.

3.5 Available phosphorus

The results presented in Table 3.2 demonstrated that treatment T_1, which involves applying 100% of the recommended dose of N & P + *B. licheniformis* @ 10 ml / plant, resulted in the highest available phosphorus content of 68.23 kg/ha.

3.6 Available potassium

It is clear from the data presented in Table 3.2 that all treatments showed a significant increase in available potassium as compared to the control. The treatment T_1 (100% recommended dose of N & P + *B. licheniformis* @ 10 ml/plant) exhibited the highest available potassium content of 469.66 kg/ha.

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3.7 Viable count
The data in Table 3.3 showed that treatment T_1 (100% recommended dose of N & P + *B. licheniformis* @ 10 ml/plant) had the highest population densities of fungi (6.82), bacteria (9.41), and actinomycetes (5.27). The increase in microbial population after harvesting the crop may be attributed to higher levels of organic manure, which could have supplied secondary elements and micronutrients.

4. Discussion
4.1 pH
The study indicated a general decrease in pH levels when bioinoculants are added to chemical fertilizers. This decline in soil pH could be due to the release of organic acids during the decomposition process [17].

4.2 Electrical conductivity (EC)
Analysis of the data revealed that the different treatments, including fertilizer levels, farmyard manure (FYM), and bioinoculants, did not have a significant effect on electrical conductivity (EC) during the study period [18].

4.3 Organic carbon (%)
The application of organic manures and biofertilizers may have created a favorable environment for the formation of humic acid, which in turn stimulated the activity of soil microorganisms, leading to an increase in soil organic carbon content. Similar findings were also reported by [17].

4.4 Available nitrogen
The application of 100% recommended dose of N & P + *B. licheniformis* @ 10 ml/plant led to the highest available nitrogen content. This was likely due to the direct absorption of nitrogen by the soil, which enhanced microbial activity and resulted in the release of organic complexing substances. Similar findings were reported by [18].

4.5 Available phosphorus
Amongst the various treatments tested, T_1 consistently showed the highest available phosphorus content. This outcome can be attributed to the activity of phosphate-solubilizing bacteria, which facilitated the release of organic acids responsible for converting previously unavailable phosphorus into an available form [19].

4.6 Available potassium
The increased availability of nutrients from inorganic sources may have contributed to the rise in available potassium in the soil. Similar results were also reported by [19].

4.7 Viable count
[20] suggested that this increase may be due to the organic carbon provided by VAM, vermicompost, and FYM. Moreover, the release of root exudates, sloughed cells, and decaying root tissues in the rhizosphere likely contributed to the microbial population, which was significantly affected by integrated nutrient applications. These factors resulted in higher nutrient levels in the rhizosphere, influencing the microbial population. Bacteria and fungi play vital roles in the decomposition of dead organic matter in the soil, including plant residues, decaying roots, and microorganisms.

Table 3.1: Effect of chemical fertilizers in combination with bioinoculants on pH, Electrical conductivity, Organic carbon in soil of garlic field

Treatment	Soil parameters		
	pH	Electrical conductivity (dSm^{-1})	Organic carbon (%)
T_1	6.47	0.69	1.49
T_2	6.48	0.66	1.45
T_3	6.56	0.67	1.48
T_4	6.63	0.68	1.45
T_5	6.70	0.53	1.38
T_6	6.74	0.56	1.28
T_7	6.79	0.50	1.24
SE (m) ±	**0.06**	**0.01**	**0.01**
CD $_{(0.05)}$	**0.19**	**0.04**	**0.05**

Table 3.2: Effect of chemical fertilizers in combination with bioinoculants on Nitrogen, Phosphorus, Potassium in soil of garlic field

Treatment	Soil parameters		
	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)
T_1	328.01	68.23	469.66
T_2	316.84	65.35	425.00
T_3	323.95	66.75	389.00
T_4	311.92	64.34	401.00
T_5	292.30	58.84	399.00
T_6	278.53	58.49	361.00
T_7	268.56	51.14	351.00
SE (m) ±	**4.96**	**1.23**	**3.83**
CD $_{(0.05)}$	**15.45**	**3.84**	**11.93**

Table 3.3 Effect of chemical fertilizers in combination with bioinoculants on viable count in soil of garlic field

Treatment	Viable Count		
	Fungi (10^{-3})	Bacteria (10^{-6})	Actinomycetes (10^{-5})
T_1	66.33	255.66	185.00
	(6.82)	(9.41)	(5.27)
T_2	60.00	244.00	169.00
	(6.78)	(9.38)	(5.22)
T_3	62.78	250.33	178.67
	(6.79)	(9.40)	(5.25)
T_4	57.33	228.33	150.33
	(6.75)	(9.35)	(5.18)
T_5	56.67	220.33	154.00
	(6.75)	(9.34)	(5.18)
T_6	54.00	226.33	148.67
	(6.73)	(9.35)	(5.17)
T_7	52.00	164.00	143.67
	(6.71)	(9.21)	(5.15)
CD $_{(0.05)}$	0.031	0.036	0.031

Figures in parentheses are log transformed values

5.0 Conclusion
The present studies demonstrated that the treatment involving a combination of 100% of the recommended dose of N & P + *B. licheniformis* @ 10 ml per plant, showed superior results across a range of soil parameters. Specifically, this treatment was found to positively influence soil pH, electrical conductivity, organic carbon content, and the levels of key nutrients such as nitrogen, phosphorus, and potassium (NPK). Furthermore, the viable count of beneficial microorganisms in the soil was also notably higher under this treatment. These findings suggest that the integration of *B. licheniformis*, a well-known plant growth-promoting bacterium, with optimal levels of N and P fertilizers can significantly enhance soil health and fertility.

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The improvement in soil parameters not only supports better plant growth but also indicates a healthier soil ecosystem. Overall, the treatment involving the full recommended doses of N & P + *B. licheniformis* @ 10 ml per plant, emerged as the most effective strategy for optimizing soil conditions and promoting sustainable agricultural practices.

Author contribution statement

Kritika has carried out most of the experiments (field experiment and laboratory analysis), performed statistical analysis, and wrote the manuscript. Meenu Gupta (MG) planned and optimised all the experiments (field plot experiment and laboratory analysis) and co-wrote the manuscript.

Declaration of competing interests

The authors declared that they have no competing interests concerning the work reported in this research article.

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Future Scope

The future scope includes optimizing bioinoculant and fertilizer combinations to enhance soil health, nutrient cycling, and sustainable garlic production. Further research can explore long term impact on microbial diversity and soil fertility under varying agro-climatic conditions, especially for cultivar Solan Selection.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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