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Effect of Biostimulants and Nutrient Management on Soil Nutrients Level in Onion (*Allium cepa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

During rabi season of 2021, a field experiment was conducted by embracing different organic manures, inorganic fertilizers and biostimulants (*Pseudomonas fluorescens* and *Bacillus subtilis*) over growth, yield, quality and persisting soil fertility of onion in the mineralized soils of Himachal Pradesh. The study was organized in thirteen treatments. The analysis revealed that lowest soil pH (6.94), electrical conductivity (0.186 dSm^{-1}), highest soil organic carbon (0.85%), available nitrogen (257.79 kg/ha) and available phosphorus (26.14 kg/ha) were recorded with treatment T_7 [75% RDF + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha)]. The highest available potassium was recorded in treatment T_{12} [100% RDF (125:75:60 kg/ha)] and highest available sulphur was recorded in treatment in T_{11} [100% RDF + 40 kg S/ha].

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Keywords: Biostimulant; onion; soil nutrient level; sulphur.

1. INTRODUCTION

"Onion is one of the most important commercial vegetable crops cultivated extensively in India and belongs to family Alliaceae" [54]. "The crop is native of Asia" [28]. Onion is an indispensable item in every kitchen as condiment, therefore onion is popularly known as 'queen of kitchen' [48]. "Pungency in onion is due to the presence of a volatile oil 'allyl propyl disulphide'– an organic compound rich in sulphur [51]. Onion has a paramount effect in preventing heart diseases, diabetes [46, 60] and also contains several anti cancerous agents which have shown to prevent cancer [12]. "The beneficial compound called 'quercetin' present in onion is a powerful antioxidant" [16]. "Onion is used by processing industries to greater extent for preparing dehydrated products like powder and flakes" [43]. "India occupies second position after China in production, cultivating onion over an area of 1431 thousand ha with annual production of 26148 thousand metric tonnes and in Himachal Pradesh, onion is cultivated over an area of 3411.08 ha with annual production of 74827 metric tones [6].

"In recent years, it has been realized that judicious application of nutrients has expanded many folds with the adoption improved technology for obtaining higher yield and better quality of onion" [52]. "In modern agriculture, fertilizers constitute major portion of cost of production of onion. Inorganic fertilizers application will cause deleterious effect on soil health leading to soil acidity or alkalinity".

Ramesh [42]. "Moreover, chemical fertilizers are very expensive and sometimes unavailable to small scale farmers and therefore sole application of inorganic fertilizers deteriorates soil fertility level day by day, that affect the production, economics of production and human health"[3, 25]"This anxiety has now led farmers to devise ways and means to switch over from conventional to organic farming systems which used no synthetic fertilizer and pesticide in crop production"[19]. Use of chemical fertilizers supplemented with organic manures and biofertilizers will be environmentally benign [24], indirectly it improves the physical properties of soil such as aggregation, aeration, permeability and water holding capacity [18, 63].

"Improved management of nitrogen, phosphorus, potassium, sulphur and other inputs in the soil could improve the yield and quality of onion. Availability of nitrogen is salient for growing plants as it remains the major constituent of protein and nucleic acid molecules. Application of nitrogen with different doses increases the growth and yield of onion" [36]. "Phosphorous is desired for the transfer of energy within the plant system and is involved in several metabolic activities" [44]. "It has its favourable outcomes on early root development, plant growth, yield and quality" [62]. Potassium plays prime role in plant metabolism [4] and it improves color, glossiness and dry matter accumulation besides improving keeping quality of onion [20]. Sulphur is an essential macronutrient and at an optimum concentration accelerates the plant growth [55, 7]. It also influences the taste and pungency in the crop [56].Therefore, integrated nutrient management is available strategy for advocating judicious and efficient use of chemical fertilizers with matching addition of organic manures for sustainable onion cultivation.

"Organic manures like FYM and Vermicompost activate many species of living organism which release phytohormones and may stimulate the plant growth and absorption of nutrients. The increase in microbial population in the presence of organic manures may also be attributed to greater availability of organic carbon and mineralized nutrients for their proliferation and further cellular development" [31]. "Apart from improving physical and biological properties of soil organic manures help in improving the use and efficiency of chemical fertilizers" [22]. "Combined use of FYM and inorganic fertilizers is of special significance under intensive cropping system as these are complementary and supplementary to each other in sustaining crop yields and soil productivity" [1]. "FYM improves the soil physical, chemical and biological properties along-with the provision of macro and micro nutrients" [8]. "Use of organic manures in combination with chemical fertilizers in an appropriate proportion improves the overall soil health for sustainable onion production" [24].

"Importance of nutrients supply in integrated manner in sustaining productivity is emphasized to restore and sustain soil health and productivity in the long run which otherwise is likely to deteriorate due to continuous and intensive cultivation without adequate nutrients management. Therefore, biofertilizers are widely accepted as low cost supplements to chemical fertilizers with no deleterious effect on either soil health or environment" [13]. "Bio-fertilizer has recently gained with momentum for affecting the sustainable increase in crop yield under various agro-climatic conditions. Biofertilizers are live carrier based microbial preparations used in agriculture as low input resources to enhance the availability of plant nutrients or promote the growth by way of synthesizing growth factors and also improve building hormones along with anti-metabolite properties" [49]. "Role of bio-fertilizer on the crop growth and yield was documented by [59, 40]. A small dose of biofertilizer is sufficient to produce desirable results because each gram of carrier of biofertilizers contains at least 10 million viable cells of a specific strain" [5]. "These may be helpful for increasing the crop production by enhancing the soil fertility, therefore use of biofertilizers not only supplement the nutrient but also improve the efficiency of applied nutrients" [9].

Various biostimulants enhance emergence of seed, plant growth and improve crop production by residing in the rhizosphere of plants and enhancing growth by direct and indirect mechanisms such as nitrogen fixation, solubilization of nutrients (phosphorous, potassium), siderophores production and water & minerals uptake [14, 37]. These biostimulants affect plant growth by producing growth substances such as IAA, GA and Cytokinins [58, 21], fix nitrogen from the atmosphere and provide the plants with this element [15] and are antagonistic towards phytopathogenic micro-organisms [57]. Alternatively, these biostimulants supplement the role of chemical fertilizers, pesticides and other inputs by decreasing the inhibitory effects of various pathogens on plant growth and development [30].

Integrated nutrient management is a viable strategy for advocating judicious and efficient use of organic manures and plant growth promoting rhizobacteria in conjunction with chemical fertilizers has been found to be promising not only in sustaining high productivity but also for good growth, improving soil fertility on long term basis and reducing fertilizer input cost. Taking into consideration, current investigation was designed and implemented to appraise the effect of biostimulants (*Bacillus subtilis* and *Pseudomonas fluorescens*) and organic manures on growth, yield, quality and persisting soil fertility of onion in the mineralized soils of Himachal Pradesh.

2. MATERIALS AND METHODS

2.1 Experimental Site

During the *Rabi* season of 2020-21 and 2021-22, the field experiment on onion was carried out at the College of Horticulture & Forestry's research farm Hamirpur (Himachal Pradesh), located at a latitude of 31° 41'47.6" N and a longitude of 76° 28'06.3" E, and an elevation of 650 meters above sea level. The climate of the region is classified as subtropical, with average low to high temperatures of 20.1°Cto 35.9°C and an average annual rainfall of 1225 mm. Maximum rainfall is recorded during monsoon, from June to September. The soil structure at the research location is classified as sandy clay loam. Prior to initiating the experiment, soil samples were collected from different spots of the experimental site from a depth of 0-15 cm and composite sample was prepared. After analysis, soil pH was neutral (7.04) having normal electrical conductivity of 0.211 dSm^{-1} and organic carbon content was medium (0.70 %). The nutrient status of soil was low in available Nitrogen (197.56 kg/ha), however medium in available Phosphorous (15.73 kg/ha), available Potassium (156.78 kg/ha) and Sulphur (30.60 kg/ha).

2.2 Experimental Design and Crop Management

The experiment was laid out in Randomized Complete Block Design with three replications and thirteen treatments viz., [Control], [*Bacillus subtilis* + FYM (250 q/ha)], [*Pseudomonas fluorescens* + FYM (250 q/ha)], [75% Recommended dose of NPK+ *Bacillus subtilis* + FYM (250 q/ha)], [75 % Recommended dose of NPK + *Pseudomonas fluorescens* + FYM (250 q/ha)], [50% Recommended dose of NPK + *Bacillus subtilis* + FYM (250 q/ha)], [50% Recommended dose of NPK + *Pseudomonas fluorescens* + FYM (250 q/ha)], [75% Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha)], [75% Recommended dose of NPK + 40 kg S/ha + *Pseudomonas fluorescens* + FYM (250 q/ha)], [50% Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha)], [50% Recommended dose of NPK + 40 kg S/ha + *Pseudomonas fluorescens* + FYM (250 q/ha)], [100% Recommended Dose of NPK + 40 kg S/ha] and [100% Recommended Dose of NPK (125:75:60 kg/ha)] in a plot size of 1 m x 0.75 mat spacing of 15 x 10 cm accommodating 50 plants per plot. Agrifound Dark Red variety developed by National Horticulture Research and Development Foundation, Nasik, Maharashtra of onion was employed for the study. The experimental field was thoroughly ploughed with the help of tractor followed by planking. Deep ploughing was done to bring the soil to a fine tilth and all the clods of the soil were thoroughly broken. All the stubble and weeds were removed. Plots were prepared according to the layout plan. The organic manure such as farmyard manure and recommended dose of fertilizers were applied at the time of field preparation as per the treatments in the respective plots. First irrigation was done immediately after sowing and daily irrigation is done till the plants are fully established thereafter, irrigation is done twice a week and then once in 6-7 days to retain optimum soil moisture, depending upon weather conditions. Weeding was done manually on a regular basis to keep the plot free from weeds and to keep the soil loose and airy. Two shallow weeding were done at 20 and 40 DAP to keep the field free from weeds. Harvesting was done at 60-70% neck fall, while the leaves were still green.

- **Application of inorganic fertilizers, organic manures and bio-additives**

Calculated amount of inorganic fertilizers nitrogen, phosphorous, potassium and sulphur (125:75:60:40 kg/ha) were applied in the form of urea (203.8 kg/ha), SSP (356.25 kg/ha), MOP (75 kg/ha) and Sulphur (40 kg/ha) in respective treatments before transplanting of seedlings. One third dose of N along with the full doses of P, K and S were applied as basal dose. Remaining dose of N was given in two splits; after 30 and 60 DAP. Organic manures such as FYM (250 q/ha) were applied during field preparation in the respective treatments. Seedling roots were dipped in bio-additives viz., *Bacillus subtilis* and *Pseudomonas fluorescens* prior to sowing for 30 minutes as per the treatments and immediately transplanted in the field.

2.3 Soil Sampling and Analysis

After completion of two-year experiment, soil samples were collected from 15 cm depth of the treatment plots. These samples were air-dried, crushed and sieved through a 2 mm mesh and stored in cloth bags for subsequent chemical analysis of parameters such as soil pH, electrical conductivity (EC), organic carbon content, and available nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) content. The pH and EC of soil samples were measured using a digital pH meter and an electrical conductivity meter, respectively. Organic carbon content of the samples was determined using the Chromic and Titration method proposed by [61]. Alkaline Potassium Permanganate Method was used to determine available N [53]), P was measured by Olsen method [35], Available K was measured by Normal Neutral Ammonium Acetate Method [32] and Sulphur was determined by 0.15% $CaCl_2$ Extractant and Turbidimetric Method [17]. The mean values of data were subjected to analysis of variance [23] for Randomized Complete Block Design.

2.4 Statistical Analysis

The merging of data was subject to statistical analysis of variance as delineated by [23]. The main aim of the analysis was to regulate the impact of treatments on different soil parameters.

3 RESULTS AND DISCUSSION

3.1 Soil pH

Application of organic manure, inorganic fertilizer and plant growth promoting rhizobacteria improved the soil pH as compared to initial status. Perusal of data presented in Table 1, revealed that minimum pH (6.94) was recorded by the treatment T_7 (75 % Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha). The soil pH decreased moderately due to inoculation with plant growth promoting rhizobacteria like *Bacillus* and *Pseudomonas* significantly enhanced the population of soil microorganisms in the rhizosphere which increased their dehydrogenase activity and then dehydrogenase released hydrogen ions in the rhizosphere resulting in the formation of carbonic acid that decreases the pH value as reported by [29]. Decrease in pH values might be ascribed to H+ ion released during sulphur oxidation. When elemental sulphur is applied to soil, a biological reaction takes place carried out by sulphur oxidizing bacteria, producing sulfuric acid that reduces soil pH as stated by [34].

3.2 Electrical Conductivity (dSm^{-1})

Electrical conductivity is important trait as it indicates the availability of nutrients and provides information related to the concentration of soluble salts present in the soil. The data revealed that maximum electrical conductivity (0.202 dSm^{-1}) was observed in treatment T_0 (Control) while, the minimum electrical conductivity (0.186 dSm^{-1}) was recorded in treatment T_7 (75% Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM) after harvesting the crop (Table 1). This may be due to plant uptake of soluble salts through root systems by plants or leaching of cations and at the same time chloride accumulation in the surface due to capillary action can be accountable for the decrease in electrical conductivity as mentioned by Prasad [38].

3.3 Organic Carbon (%)

Organic carbon induces changes in microbial activities that affect the transformation and availability of nutrients, organic matter and overall soil health. Data revealed that highest organic carbon (0.85%) was observed in treatment T_7 (75% Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM) whereas, minimum organic carbon was obtained in treatment T_0 (Control) after harvesting the crop (Table 1). This might be due to inoculation by *Bacillus subtilis* and *Pseudomonas fluorescens* as plant growth promoting rhizobacteria strains which are required for maximizing the plant yield and improved soil quality by increasing the organic carbon as reported by [26]. Similar findings are reported by Prasad [39].

3.4 Available Nitrogen (kg/ha)

The data for available nitrogen is presented in Table 2. Maximum available nitrogen (257.79 kg/ha) was recorded in treatment T_7 (75% Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM) and minimum available nitrogen (188.86 kg/ha) was observed in treatment T_0 (Control) after harvesting the crop. Breakdown of complex nitrogenous compounds to nitrate due to action of micro-organisms might have increased nitrogen and enhanced nitrogen uptake in plants as reported by Ramesh et al. [42]. Moreover, free-living nitrogen fixing PGPR like *Bacillus* attaches to the root and efficiently colonizes root surfaces as stated by [2]. Whereas, [45] reported that it might be due to the use of mixture of plant growth promoting rhizobacteria including *Bacillus spp.* which fix the atmospheric nitrogen and excretes growth promoters that increase nitrogen content in soil.

3.5 Available Phosphorus (kg/ha)

An examination of data regarding available phosphorus presented in Table 2 and depicted that maximum available phosphorus (26.14 kg/ha) in soil after harvesting of crop was recorded in treatment T_7 (75% Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM) while, minimum available phosphorus (14.87 kg/ha) was recorded in treatment T_0 (Control). Increase in available phosphorus over initial value might be ascribed to the role of *Bacillus* which solubilizes the insoluble phosphorus compounds and increases the plant growth as reported by [11]. Increase in phosphorous could also be due to greater solubilization of native phosphorous from the soil due to action of various organic acids liberated due to decomposition of organics as stated by [42].Whereas, [26] mentioned that inoculation with isolates of *Pseudomonas* had a stimulatory effect on plant growth and produces indolic compounds and siderophores, to solubilize phosphate. Plant growth promoting rhizobacteria enhance phosphorous uptake by releasing organic acids such as fumeric acid, butyric acid, citric acid etc. which improves microbial activity and increases phosphorous availability as reported by [50].

3.6 Available Potassium (kg/ha)

Maximum available potassium (174.59 kg/ha) in soil after harvesting was recorded in treatment T_{12} (100% Recommended dose of NPK + FYM) whereas, minimum available potassium (142.13 kg/ha) was recorded in treatment T_0 (Control) after harvesting the crop (Table 2). Overall mean for available potassium was 157.76 kg/ha. Increase in available potassium could be ascribed to the improved soil properties due to the action of organics, leading to better penetration of roots, thereby resulting to greater uptake of potassium as reported by [42]. Similar results on conjunctive application of both organic and inorganic fertilizers were documented by [41]. Greater availability of nutrients from inorganic sources might have increased available potassium in soil. Similar results are in conformity with [33, 10].

3.7 Available Sulphur (kg/ha)

Data pertaining to available sulphur (Table 2) depicted that maximum available sulphur (53.45 kg/ha) was recorded in treatment T_{11} (100% Recommended dose of NPK + 40 kg S/ha + FYM) however, minimum available sulphur (29.31 kg/ha) was recorded in treatment T_0 (Control) in soil after harvesting of crop. Overall mean value was 42.66 kg/ha. Sulphur application

Table 1. Effect of integrated nutrient management on pH, EC (dSm^{-1}) and organic carbon (%) in soil

Treatment Code	Treatment Details	Soil pH	EC (dSm^{-1})	Organic carbon (%)
T_0	Control	7.07	0.202	0.61
T_1	*Bacillus subtilis* + FYM (250 q/ha)	7.01	0.197	0.73
T_2	*Pseudomonas fluorescens* + FYM (250 q/ha)	7.03	0.198	0.75
T_3	75 % Recommended dose of NPK+ *Bacillus subtilis* + FYM (250 q/ha)	7.00	0.194	0.77
T_4	75 % Recommended dose of NPK + *Pseudomonas fluorescens* + FYM (250 q/ha)	7.02	0.195	0.74
T_5	50 % Recommended dose of NPK + *Bacillus subtilis* + FYM (250 q/ha)	7.03	0.194	0.76
T_6	50 % Recommended dose of NPK + *Pseudomonas fluorescens* + FYM (250 q/ha)	7.02	0.196	0.78
T_7	75 % Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha)	6.94	0.186	0.85
T_8	75 % Recommended dose of NPK + 40 kg S/ha + *Pseudomonas fluorescens* + FYM (250 q/ha)	6.96	0.189	0.83
T_9	50 % Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha)	6.98	0.192	0.80
T_{10}	50 % Recommended dose of NPK + 40 kg S/ha + *Pseudomonas fluorescens* + FYM (250 q/ha)	6.99	0.193	0.79
T_{11}	100 % Recommended Dose of NPK + 40 kg S/ha	7.04	0.198	0.70
T_{12}	100 % Recommended Dose of NPK (125:75:60 kg/ha)	7.05	0.199	0.71
	Mean	7.00	0.194	0.75
	$CD_{(0.05)}$	0.01	0.002	0.01
	SE(m)	0.00	0.00	0.00
	C.V	0.16	0.51	1.33

Table 2. Effect of integrated nutrient management on available N (kg/ha), P (kg/ha), K (kg/ha) and S (kg/ha) in soil

Treatment Code	Treatment Details	Available N in soil (kg/ha)	Available P in soil (kg/ha)	Available K in soil(kg/ha)	Available S in soil (kg/ha)
T_0	Control	188.86	14.87	142.13	29.31
T_1	*Bacillus subtilis* + FYM (250 q/ha)	215.46	17.57	148.01	36.62
T_2	*Pseudomonas fluorescens* + FYM (250 q/ha)	212.53	16.41	145.77	35.68
T_3	75 % Recommended dose of NPK+ *Bacillus subtilis* + FYM (250 q/ha)	240.52	19.50	156.12	42.76
T_4	75 % Recommended dose of NPK + *Pseudomonas fluorescens* + FYM (250 q/ha)	237.27	18.17	154.18	41.36
T_5	50 % Recommended dose of NPK + *Bacillus subtilis* + FYM (250 q/ha)	228.76	17.95	153.01	40.70
T_6	50 % Recommended dose of NPK + *Pseudomonas fluorescens* + FYM (250 q/ha)	226.53	17.71	150.61	39.55
T_7	75 % Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha)	257.79	26.14	167.89	48.72
T_8	75 % Recommended dose of NPK + 40 kg S/ha + *Pseudomonas fluorescens* + FYM (250 q/ha)	254.54	24.70	165.74	46.59
T_9	50 % Recommended dose of NPK + 40 kg S/ha + *Bacillus subtilis* + FYM (250 q/ha)	251.42	23.82	162.91	44.42
T_{10}	50 % Recommended dose of NPK + 40 kg S/ha + *Pseudomonas fluorescens* + FYM (250 q/ha)	249.24	22.66	159.78	43.86
T_{11}	100 % Recommended Dose of NPK + 40 kg S/ha	246.38	21.82	170.17	53.45
T_{12}	100 % Recommended Dose of NPK (125:75:60 kg/ha)	242.52	20.53	174.59	41.64
	Mean	234.75	20.14	157.76	41.89
	$CD_{(0.05)}$	1.35	1.14	0.71	0.52
	SE(m)	0.46	0.39	0.24	0.17
	C.V	0.34	3.35	0.26	0.73

enhances the population of sulphur consuming microorganisms leading to oxidation of S to SO_4^{2-} producing sulfuric acid that reduces the soil pH and increase the concentration of available sulphur in soil. The results are in line with the findings of [34, 27, 47].

4. CONCLUSION

Combined application of 75% Recommended dose of NPK, 40 kg S/ha, *Bacillus subtilis* and farm yard manure resulted in improved soil available nutrients level in onion.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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