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Utilizing bioformulation to increase root colonization, soil fertility, and productivity of chilli (*Capsicum annuum* L.)

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Abstract

Microbes have the capability to colonize the rhizosphere of the plants and trigger its growth by different mechanisms. This study investigated the ability of microbial bioformulations application in pot culture study for better colonization in the root zone, that increase soil fertility which enhance the growth of chilli (*Capsicum annuum* L.). Pot experiments containing exogenously applied microbial inoculates immobilized in talc+gluten-based carrier material was conducted for the study at two different locations. Among all the treatments bioformulation containing microbial consortia immobilized in carrier material showed enhanced shoot length (52%), root length (128.55%), shoot biomass (144.95%), root biomass (134.1%) and productivity in terms of fruit number (148%) and fruit biomass (60.8%) over non-treated control plants. Results further depicted that microbial colonization (8.4 log_{10} bacteria and 7.4 log_{10} fungi) and enzymatic activity in rhizospheric soil were found increased i.e., 89.6% for alkaline phosphatase and 542% for microbial biomass carbon by the application of bioformulation as compared to the control. The soil physio-chemical attributes like organic matter (69.2%), available nitrogen (221%), phosphorus (788%) and potassium (15.7%) were also increased post-harvest over the pre-sowing soil by application of this bioformulation. The study suggests that carrier based microbial bioformulation can improve the growth and productivity of chilli along with higher rhizospheric colonization of the beneficial microbes and soil health. Hence, it can be a promising substitute of chemical fertilizers with multiple economic and ecological benefits during the chilli cultivation.

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Introduction

In the agricultural bioeconomy, the application of biofertilizers could be fully used to assure food production and inclusion in crop breeding programs (Uzoh and Babalola, 2018; Fasusi *et al.*, 2021). It is believed that using biofertilizers in agriculture is a safe and environment friendly beneficial practices that can substitute agrochemicals without endangering the ecosystem (Glick, 2010; Kumar; Singh, 2021). The growing demand for biologically based natural fertilizers as an alternative to agrochemicals fascinates sustainable agriculture (Parveen *et al.*, 2023). However, the inability of microbial inoculants to adapt to adverse environmental conditions prevents monocultures from being inoculated successfully in the field (Mishra *et al.*, 2021). Whereas, when utilized in the form of consortium, microbial isolates have greater competence and a broader range of action without genetic engineering toward crop productivity and plant health (Mishra and Singh, 2022; Parveen *et al.*, 2023).

The low microbial viability during storage, marketing and application are major concerns regarding their widespread adoption and consequently, the biofertilizer market. After PGPMs is applied to the rhizospheric niche, its population begins to decline because of the ever-changing soil environment (Roy *et al.*, 2010). Therefore, using carrier-based formulations to inoculate microorganisms could be a promising strategy for reaping the benefits of microbial inoculants (Parveen *et al.*, 2022a &b). Bioformulations inoculated with PGPMs have the potential to boost plant growth and yield. It was reported that bioformulations of *Bacillus cereus* and *Pseudomonas rhodesiae* with a variety of plant growth promoting properties increased the yield of several vegetable crops (Kalita *et al.*, 2015).

Capsicum annum L. (Chilli) is one of the most important, cultivated cash crops worldwide, while India stands on the first position for the production and export of dried chilli all over the world (Saxena *et al.*, 2016; Olatunji *et al.*, 218). Chilli crop is enriched with nutritionally important components like proteins, vitamins, fibres and mineral elements that is important for boosting immunity and to lower the cholesterol level (Sanati *et al.*, 2018). Maintaining production levels and increasing crop yields have become increasingly important because of their greater utilization for daily necessities. To meet the increasing demand for chilli, various chemical fertilizers have also been utilized in the cultivation for increasing the quality and yield of this economically important crop (Sanchez-Roque *et al.*, 2016). However, this excessive utilization of synthetic fertilizers would cause many negative impacts on human health and degrade environment (Savci *et al.,* 2012; Sanchez-Roque *et al.,* 2016). To overcome the detrimental effects of these chemical fertilizers there must be some alternative for the cultivation of chilli. One of the most possible alternatives to chemical fertilizers is the use of plant growth promoting organisms as biofertilizers.

Considering the above information, the current study aimed to assess the efficacy of talc+gluten based bioformulations containing seven compatible microbial strains as a single microbial consortium for their effect on chilli plant growth and productivity as well as the rhizospheric colonization and soil fertility status after harvesting of crop.

Materials and methods

Preparation of granular bioformulation

Granular bioformulation have been prepared using seven microbial strains (*Bacillus filamentosus* RS3B*, Bacillus pseudomycoides* RS6B*, Bacillus paramycoides* RPB3*, Alcaligenes faecalis* RS10B) as a single consortium and carrier material talc+gluten in 3: 1 ratio by followed the process described in our previous study Parveen *et al.* (2023).

Pot experiment

Effect of bioformulation and other treatments were evaluated on the growth and productivity of chilli in pot experiments which were conducted from August - November 2020 and 2021 at research station BBAU Lucknow and SKAUST- Jammu Chatha (J & K).

Experiments were conducted in replicas of six in earthen pots which were randomly arranged in three blocks in open green house (Inostroza *et. al.* 2016). Non-agricultural soil was collected for the experiment to analyse the physio-chemical parameters and enzymatic properties (pre-sowing and post harvesting) by following the standard methods of Jackson (1973). Soil having average pre- sowing soil properties {pH 6.8±0.1, EC 5.52±0.1 ds/m, organic matter 1.53±0.01%, available nitrogen 30.83±1.4mg/kg, available potassium 140.59±5.1g/kg, available phosphorus 1.17±0.8mg/kg, alkaline phosphatase 19.35±0.8µg/g, soil microbial biomass carbon (SMBC) 82.88±0.87µg/g} was used for the experiment. While conducting pot experiments in our study, non-sterile soil was utilized to minimize the differences between pot and field results Jimenez *et al.* (2020).

Earthen pot having dimension 50x20x20cm were filled with 7 kg of non- autoclave soil which was mixed thoroughly before the pot experiment**.** Chilli seeds were surface sterilized pre-sowing using 3% hydrogen peroxide solution (Bakhsh *et al.*, 2016). After sterilization 10 seeds per pot were sown and after germination 4 plantlets per pots were maintained for homogeneity. Bioformulation (T1) (0.5%/g of soil after optimization described in our previous study Parveen *et al.*, 2023), liquid microbial inoculum (T2), non- inoculated carrier material (T3) and recommended dose of chemical fertilizer (T4) was added once into the pot soil and mixed properly before seed sowing. To maintain moisture level in the pots, they were irrigated regularly and weeding was done, when required. The effectiveness of bioformulations for soil fertility status was evaluated by comparing the soil's property before seed sowing and after crop harvesting.

Efficacy of bioformulation and other treatments on plant growth and productivity

The chilli plants were harvested after 120 days of sowing. After harvesting the plants were taken into the laboratory and washed with running tap water to remove the adhered soil. Then the root and shoot were separated and assed for root and shoot length by using a meter scale and biomass by using a weighing scale. Number of fruits was calculated by manual counting of fruits per plant and fruit fresh biomass was taken by weighing scale (Tripti *et al.*, 2017).

Bioformulation efficacy for promoting root colonization and microbial population in rhizosphere

Microbial population in rhizospheric soil was checked by taking soil adhered to roots of chilli by washing with distilled water. Then solution was weighted and resuspended in distilled water and serially diluted upto 10^{-11} with distilled water. In order to assess the microbial cfu/g of rhizospheric soil, the dilutions were spreaded on nutrient agar media for bacteria and potato dextrose agar for fungi and kept for incubation at 28˚C for 48-72 hours. After incubation number of colonies were counted by using digital colony counter and expressed as cfu/g of soil (Fatima and Arora, 2021). Furthermore microbial colonization with roots of chilli was also checked by scanning electron microscope (SEM) (JEOL, JSM 6490 LV). Roots of chilli were gently washed with distilled water for microscopic examination (Gomez *et al.*, 2018). A small part of chilli root sample was taken and put on double sided conductive carbon tab that was adhered to regular vacuum clean stub to examine the colonization on root surface.

Statistical analysis

Data was analysed statistically by MS excel, metaboanalyst 5.0 and IBM SPSS statistics 20. One way analysis of variance was done to compare the mean values by Duncan's multivariate range test ($P<0.05$).

Results

Efficacy of bioformulation on plant growth

In this experimental study five distinct (T1-T5) treatments were given to the plants to assess the impact of the selected bioformulation. Different plant growth parameters, such as root and shoot biomass and length were recorded to determine how different treatments affected plant growth (Fig. 1).

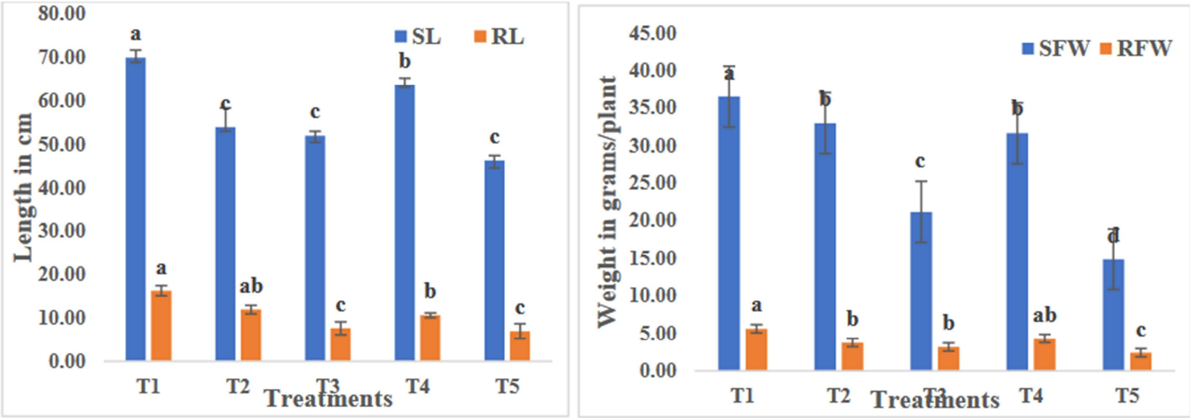

Fig. 1. Effect of bioformulation on chilli growth a- length of shoot and root; b- Fresh weight of shoot and root per plant T1= talc+gluten based Bioformulation; T2= broth based bioinoculant; T3= talc+gluten; T4= chemical fertilizer; T5= non-inoculated control. Data are represented by mean value (n=6).

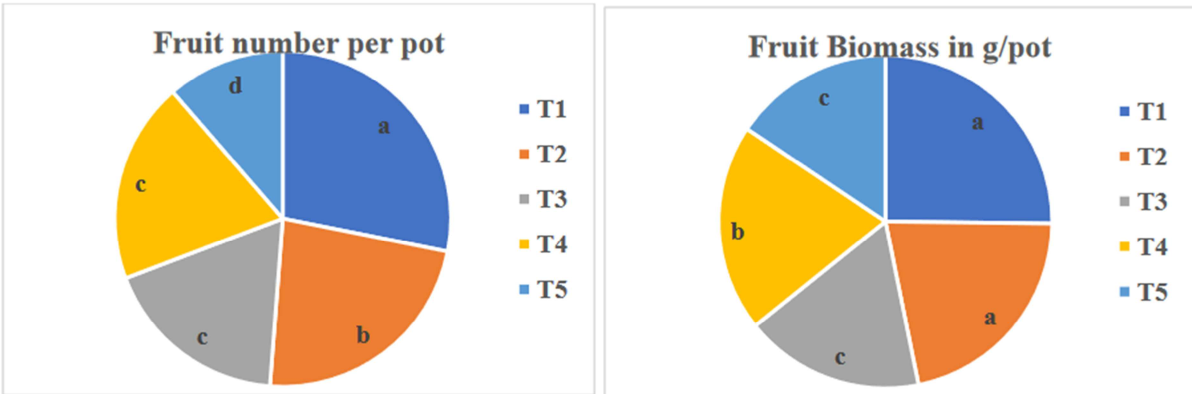

Fig. 2. Effect of bioformulation on production of chilli fruits a- number of fruits/pots; b- fresh fruit biomass in g/pot T1= talc+gluten based Bioformulation; T2= broth based bioinoculant; T3= talc+gluten; T4= chemical fertilizer; T5= non-inoculated control; Data are represented by mean value (n=6).

When compared to plants that were not treated, the results showed that the addition of carrier-based bioformulation resulted in a distinct improvement in the growth of chilli. The greatest increases in plant length and biomass were observed in plants treated with the T1 bioformulations. The results showed that the maximum percentage increase in shoot length (52.17%), root length (128.5%), shoot biomass (144.9%) and root biomass (134%) was for T1 as compared to untreated control (T5) at 120 days after sowing (DAS).

Effectivity of bioformulation on production of chilli fruits

In this experimental study, the number of fruits in the chilli plant per pot (148.1%) and fruit fresh biomass per pot (60.8%) increased as compared to untreated control (T5). Furthermore the results showed that this bioformulation was more effective than chemical fertilizers as its productivity was 25.1% increased than chemical fertilizers treatment (T4). Therefore, the results confirmed that T1 set of treatment was highly effective and have prominent impact on chilli growth and productivity (Fig. 2).

Effect of bioformulation inoculation on root colonization and rhizospheric microbial population

The results indicated that carrier-based bioformulation helped in maintaining the population of microbes in the rhizosphere. As it was observed that, maximum colonies of microbes were present in T1 followed by T2 as evident from SEM analysis. There were very less or no colonization on the root surface of other plants (Fig. 3). The results further showed that soil microbial population can be influenced by the application of bioformulation. The highest bacterial population was recorded for T1 which was 40.1% over control followed by 21% for T2. The highest fungal population was also observed for T1 which was 48% higher over control followed by 26% for T2 over control (pre sowing soil).

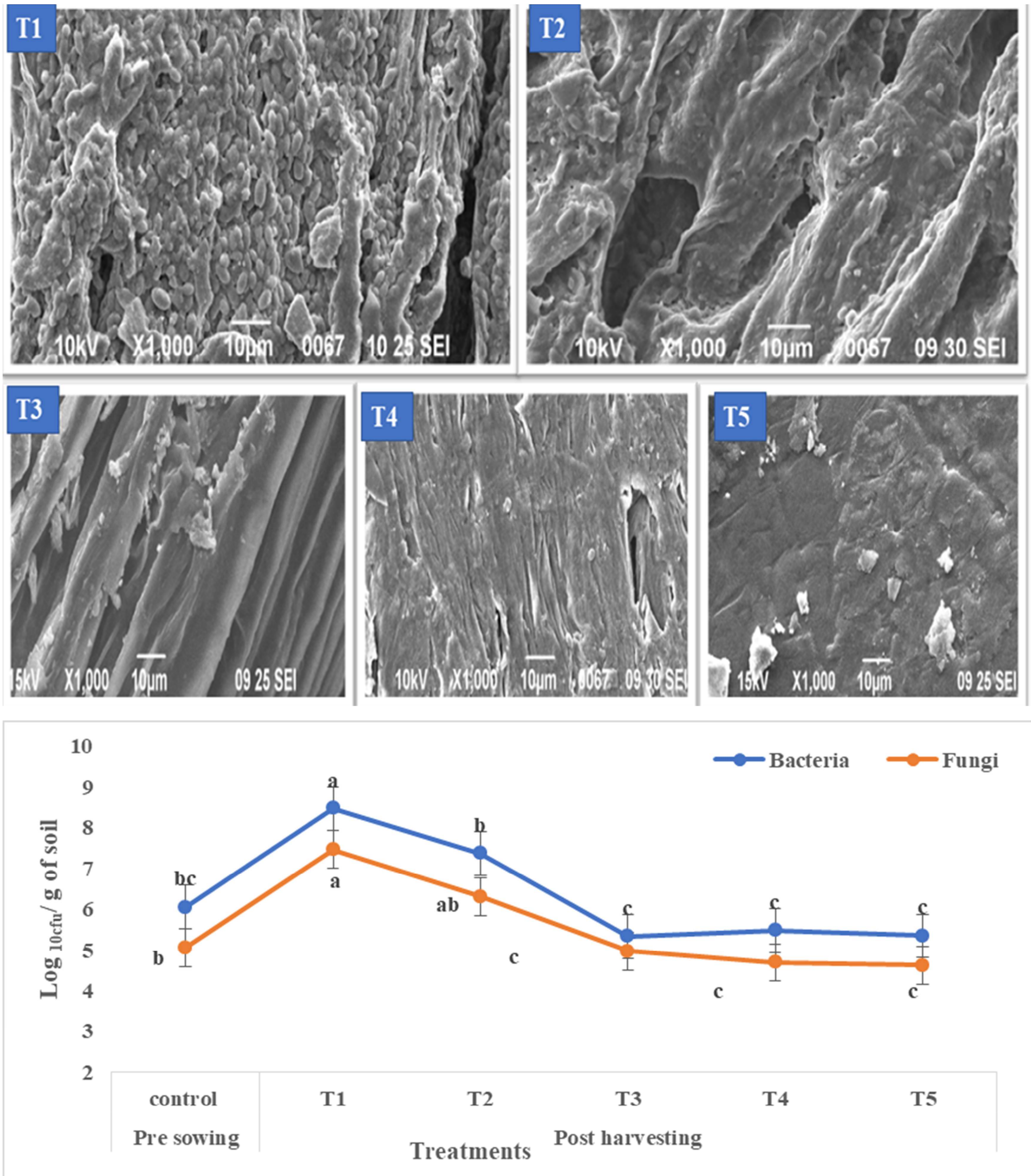

Fig. 3. Efficacy of bioformulation in enhancing microbial colonization in rhizosphere; a- SEM analysis of chilli roots showing colonization.; b- log_{10} cfu of bacteria and fungi in post harvested soil of chilli rhizosphere
T1= talc+gluten based Bioformulation; T2= broth based bioinoculant; T3= talc+gluten; T4= chemical fertilizer; T5= non-inoculated control; Data are represented by mean value (n=6).

Effect of bioformulation on soil enzymatic and physio-chemical properties

Soil is the reservoir of nutrients that helps plants grow and develop. Soil health is determined by the quantity of nutrients and their capacity to hold them. As a result, various physio-chemical and enzymatic attributes of soil collected prior to sowing and after crop harvest was analyzed for the nutritional evaluation. The highest percentage increase of alkaline phosphatase activity was observed for T1 (89.6%) followed by T2 (62.9%) over pre sowing control soil. The results of microbial enzymes in the soil were well correlated with the microbial population in the rhizosphere.

The soil microbial biomass carbon (SMBC) was also significantly increased for all treatment set. It was recorded highest for T1 (542.2%) followed by T2 (406%) and the lowest increased was for T3 (157.3%) over control (pre- sowing).

Table 1. Effect of treatments on pot soil physico-chemical properties cultivated with Chilli pre sowing and post harvesting of chilli

	Treatments	ALkP(µg/g)	SMBC(µg/g)	pH	EC (ds/m)	OM (%)	AP (mg/kg)	AK (mg/kg)	AN (mg/kg)
Pre-sow	0 day	19.35 ± 0.81^{b}	82.88 ± 0.87^{d}	6.80 ± 0.10^{b}	5.50 ± 0.10^{a}	1.53 ± 0.01^{b}	1.17 ± 0.81^{c}	140.59 ± 5.51^{b}	30.83 ± 1.44^{c}
Post harvesting	T1	36.60 ± 2.14^{a}	531.16 ± 1.38^{a}	6.60 ± 0.17^{b}	1.16 ± 0.01^{d}	2.59 ± 0.02^{a}	10.47 ± 1.15^{a}	162.85 ± 5.51^{a}	99.17 ± 2.89^{a}
	T2	31.47 ± 2.80^{a}	415.52 ± 1.48^{b}	6.47 ± 0.21^{c}	2.40 ± 0.04^{c}	1.50 ± 0.02^{b}	3.92 ± 0.18^{b}	147.40 ± 9.54^{b}	75.00 ± 4.33^{b}
	T3	19.81 ± 16.57^{b}	211.32 ± 1.43^{c}	7.17 ± 0.12^{a}	3.24 ± 0.03^{bc}	1.33 ± 0.01^{b}	3.14 ± 0.58^{b}	105.60 ± 5.51^{c}	33.33 ± 5.77^{c}
	T4	4.43 ± 1.61^{c}	217.48 ± 0.48^{c}	6.70 ± 0.20^{b}	5.65 ± 0.11^{b}	1.29 ± 0.08^{c}	1.89 ± 0.50^{c}	73.79 ± 11.02^{d}	65.83 ± 5.20^{b}
	T5	7.69 ± 1.40^{c}	218.08 ± 1.08^{c}	6.77 ± 0.06^{b}	4.47 ± 0.25^{b}	1.07 ± 0.02^{c}	0.73 ± 0.09^{c}	54.71 ± 5.51^{d}	28.33 ± 1.44^{c}

T1= talc+gluten based Bioformulation; T2= broth based bioinoculant; T3= talc+gluten; T4= chemical fertilizer; T5= non-inoculated control; EC= electrical conductivity; OM= Organic matter; AN= available nitrogen; AP= Available phosphorus; AK= Available Potassium; ALkP= Alkaline phosphatase; SMBC=Soil Microbial biomass Carbon; ds/m= desicimen per meter; % =percentage; mg/kg= milligram per kilogram; (µg/g) = microgram per gram. Data are represented by mean value (n=6).

The results further showed that a slight change in pH was occurred after cropping of chilli as compared to pre-sowing data for all treatment set including control**.** The EC of the soil was highly reduced for T1, T2 and T3 treatment 5.5ds/m to 1.1, 2.4, 3.2 ds/m respectively. The organic matter was enhanced only for bioformulation treatment set after cropping chilli. It was increased 69.2% for T1 and 1.96% for T2 after harvesting of crop. Further the available nitrogen, phosphorus, and potassium are the major soil parameters that were highly influenced with application of bioformulation. As can be seen in Table 1, the maximum percentage increases in available nitrogen was 221% for T1 followed by 150% for T2, 116% for T4 and 10% for T3 as compared to pre sowing control soil. However, in T5 treatment set showed a fall in nitrogen level of soil. An increase in the available phosphorus and potassium was also recorded for T1 and T2. It was increased by 788% and 15.7% for T1 and 233% and 5% for T2 respectively as compared to pre sowing control.

Discussion

Efficacy of bioformulation for plant growth promotion

As per the experimental investigation in agriculture, the relationships between PGPMs that help plants growth are very important. Microorganisms present in soil are the dynamic architects of soil. Through the production of necessary growth regulators and the availability of nutrients, they create the conditions necessary for plant growth. Furthermore, inoculation of microbes in the form of bioformulation could help in the enhancement of plant growth and productivity. The results of the present study showed that the plants of chilli treated with bioformulation showed an enormous increase in the plant growth and productivity. These incremental changes in the root and shoot length and their fresh and dry biomass were due to the application of bioformulation containing beneficial microbial strains which can promote plant growth by different mechanism like root colonization, growth hormone production and nutrient solubilization as described in our previous study Parveen *et al.* (2023). The applied bioformulation was able to produces IAA, fix atmospheric nitrogen and mobilize soil minerals that possibly connected with promoted growth of chilli crops. Our results are supported by the findings of Mishra and Singh (2022) who also observed the similar phenomenon by the application of a bioformulation containing microbial consortium (*Bacillus pseudomycoides*, *B. firmus*, *Aspergillus luchuensis,* and *A. tamarii)* on chilli growth and productivity.

Influence of bioformulation on crop productivity

The productivity is the goal of the research. It has been demonstrated in several studies that bioformulations have excellent potential to increase the crop yield. Based on the findings of this study, it may be hypothesized that an increase in the solubility of potassium and phosphorus and the release of growth-promoting substances at the root interface, which stimulated root development, contributed to the improvement in fruit yield and quality. Results are supported by the findings of Parewa *et al.* (2014) who reported a 23% increase in the average yield of chilli per plant with the application of a consortium (*Azotobacter* + *Azospirillum* + PSB).

Enhanced root colonization by the application of bioformulation

Carrier based bioformulation helps in providing a habitat or a medium which protect the microbes from the changing environmental conditions might be the reason for higher root colonization. The results were supported by the findings of Parveen *et al.* (2022 a & b). Due to the rise in the microbial population in the rhizospheric soil after crop harvesting might be associated with elevated level of solubilized minerals and accelerated growth of the chilli plants. This increase in the microbial population reflects that bioformulation have wide impact on microbial population that alter the soil properties and influence the growth and productivity of the crops which it has been applied (Liu and Xu, 2022; Xu *et al.*, 2019). The ability to colonize the root system is essential for rhizobacteria to be effective plant growth promoters and results indicated that PGPMs can colonize well in the rhizosphere of chilli and therefore showed a positive impact on chilli plants. These findings are in lineation with the findings of Maity *et al.* (2019).

Increased post harvesting soil fertility by the application of bioformulation

The results of the current study also showed that an increase in the fertility status of the post-harvest soil. The alkaline phosphatase activity, enzyme involved in phosphate solubilization from organic phosphate complexes, was increased with application of bioformulation. The biological activity of the soil, particularly its nutrient cycling process, is reflected in its enzymes. The present study found that bioformulation-treated soil had better phosphatase activity than control soil, indicating that treated soil had more microbes activated, which was consistent with previous research (Kang *et al.*, 2013; Rai *et al.,* 2021; Parveen *et al.*, 2023).

As, treated plants had higher root colonization of microbes in the rhizosphere than control plants which is supported by the findings of Rai *et al.* (2021). Through increased nutrient availability, the host plants benefit from the increased microbial activity and improved soil enzymatic values (Schoebitz *et al.*, 2016). This increase in the alkaline phosphatase was associated with increased in the level of phosphate in soil that influenced with bioformulation treatment (Behera *et al.*, 2019; Devi *et al.*, 2022). Increase in soil SMBC might be associated with increase in the microbial population of the soil. The results were supported by the findings of Mishra and Singh (2022); Parveen *et al.* (2022 a & b).

The results of the current study showed great reduction in the EC of the soil. The reduction in EC of the soil might be associated with accelerated assimilation of soluble minerals by fast growing chilli plant influenced by the applied bioformulation (Parveen *et al.*, 2023). As shown in the results, increase in nitrogen level in the soil was associated with microbial fixation of atmospheric nitrogen by microbes applied as bioformulation (Zegeye *et al.,* 2019; Wang *et al.*, 2021). While decrease in nitrogen level might be due to assimilation of nitrate by plant. The phosphate solubilizing microbes of bioformulation played crucial role in phosphate solubilization (Gupta and Kumar, 2017; Verma *et al.*, 2017). The increase in the phosphate possibly associated with the microbial mobilization of phosphate anions from the complex compound. The increase in the alkaline phosphatase also indicated the microbial action on solubilization of phosphate (Chauhan and Bagyaraj, 2015; Gupta and Kumar, 2017; Verma *et al.*, 2017). Elevation in available potassium might be possibly associated with solubilization of potassium from their complexes through microbial action applied as bioformulation (Schoebitz and Vidal, 2016). Bioinoculant application is thought to be good for nutrient mobilization, native microflora, and soil health because it increases soil enzyme activity. The current investigation's findings, which were confirmed by the significant increase in total microbial activity and nutrient mobilizers upon bioinoculant application, reflect the fact that enhanced microbial activity is also responsible for the enhancement of soil enzymes and nutrients. In addition, increased levels of microorganisms and nutrient solubilizers improved soil nutrients, resulting in flourishing plant growth and productivity.

Conclusion

It can be concluded that an efficient bioformulation with multiple potentials for promoting plant growth using bioformulation prepared with microbial consortia and carrier material. Based on the experiments, this can be concluded that this bioformulation enhanced chilli growth, yield, and nutrient content by influencing a variety of physiological characteristics. In addition, the study suggests that the PGPMs used in this bioformulation have potential to be utilized as biofertilizers following a rhizospheric colonization and enhanced soil fertility. This bioformulation can be used in a variety of climates because these microbial strains are resistant to several harsh environments.

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