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Developing An Effective Microbial Community As Bioinoculant For Enhanced Productivity Of Tomato (*Lycopersicon Esculantum* Mill.) With Improved Soil Fertility

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ABSTRACT

The efficacy of a consortium of seven plant growth promoting micro-organisms immobilized in a mixture of talc and gluten (3:1 ratio) was investigated for its effect on growth and productivity of Lycopersicon esculantum Mill. in a pot experiment. The shoot length (58.08 %), root length (104.8 %t), shoot fresh and dry biomass (97.4 % and 159.1 %), and root fresh and dry biomass (202.8% and 212%) were found significantly higher in carrier based bioformulation (T1) as compared to control (T5). After inoculation of this novel microbial bioformulation, it also increased fruit number (148.3%) and fruit biomass (195.3%) as compared to the untreated control. Furthermore, post-harvest study of this bioformulation treated soil showed improvement in organic matter (57.8%), available nitrogen (285%), phosphorus (972.7%), potassium (99.2%) and enzyme activities like microbial biomass carbon (224.0%) and alkaline phosphatase activity (420.6%) as compared to pre-plantation soil. The root colonization of microbes, as evident by scanning electron microscopy was also increased. Our results demonstrated that talc+gluten-based bioformulation with the consortium of seven agriculturally beneficial soil microbes significantly enhance the soil fertility and tomato yield. Therefore, the study concluded that, this bioformulation can act as a promising substitute of chemical fertilizers for the economic and ecological benefits in the tomato cultivation.

Key words: bioformulation, carrier, consortium, colonization, soil fertility

1.0. INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill., is the second-most significant vegetable crop in terms of its production value globally (Balderas-Ruiz *et al*., 2021). It is consumed in multiple forms because of its nutritional value enriched with the abundance of lycopene, folic acid, ascorbic acid, flavonoids, -tocopherol, potassium and phenolic chemicals. In order to enhance the crop productivity as well as production of valuable compounds, different synthetic fertilizers have been used to sustain the food demands of the growing world population. However, the continuous use of fertilizers for crop production has yielded a serious environmental and health concerns. Approximately, 75–90% of the synthetic fertilizers get persist in the soil, leached out in the aquafers, emitted as NOx for an extended period of time which eventually harms the soil and its microflora (Tripti *et al*., 2017). A good quantity of excessively absorbed synthetic fertilizers remained unassimilated in the plant parts in inorganic forms are toxic to the consumers (Kumar and Singh, 2021).

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Presently, the farmers are interested in integrating agro-ecological practises into the production systems for transition to organic agriculture with good yields, encourage the efficient use of water and nutrients, and to obtain products with high nutritional quality within the normative standards of good agricultural practises and safety (Zayed, 2016). Farm yard manure (FYM), Zero-Budget Natural Farming (ZBNF), Vermicompost, no-tillage farming, and application of PGPMs are seen as possible alternatives to synthetic fertilizers (Chauhan *et al*., 2020). The use of plant growth-promoting microbes (PGPMs) is one of the alluring and affordable strategy to increase crop production and yields in cost-effective and environment friendly manner (Kumar and Singh. 2021). Plant growth promoting microbes can successfully colonise the surface of the roots, improve nutrient bioavailability and bio-assimilation. Additionally, PGPMs aid in the plant's detoxification from harmful substances, shield soil infections to plants and can act as an alternative to chemical fertilizers, pesticides and transgenic plants (Bhattacharyya *et al*., 2020; Bhattacharyya *et al*., 2017). Thus, the application of these beneficial plant-associated microbial strains is a sustainable approach to enhance crop performance in agriculture. Before being employed as a commercial product, a promising microbial inoculant must first be developed into a bioformulation. A biologically active ingredient (a living microbe or spore) is combined with inert molecules, known as carriers, to create a bioformulation (Aamir *et al*., 2020). The major concerns in adoptability of the bioformulations at large scale in the biofertilizer market are the low microbial viability during storage, marketing and application. Therefore, the focus is shifting towards mixing of diverse compatible microbes and flexible carrier material that can sustain different environmental stress conditions, maintain viability, improve efficiency, crop productivity along with the fertility of the soil. Recently, Mishra and Singh (2022) observed that combination products based on PGPMs consortia are increasingly being used to take advantage of complementary or even synergistic interactions. Keeping in view the above information, we have attempted a novel approach to use talc and gluten as a carrier material along with different microbes as the competent solution to enhance effectiveness and stability of the bioformulation. The present study hypothesized that bioformulation will prove to be a better alternative to the existing organic carrier materials in agricultural industries. Therefore, developing bioformulation using the affordable carrier material and beneficial microbial community can serve as the multilevel advantageous system in elevating the crop yield, productivity and soil fertility.

2.0. Methodology:

2.1. Collection of microbial strain, estimation of PGP traits and compatibility test.

Seven pre-isolated and characterized PGPM strains (*Bacillus filamentosus* RS3B, *Bacillus pseudomycoides* RS6B, *Bacillus paramycoides* RPB3, *Alcaligenes faecalis* RS10B, *Aspergillus luchuensis* RS6F, *Aspergillus tamarii* RS8F, *and Trichoderma lixxi TvR1*) (Sachdev and Singh 2018; Maddhesiya *et al*., 2020) were obtained from departmental laboratory (research group of Prof. Rana Pratap Singh). Following the procedure outlined by Schadev and Singh (2018), microbial strains were collected and re-evaluated for the retention of PGP activities using an assay for ammonium production, phosphate solubilization, indole acetic acid (IAA), siderophore production, potassium solubilization, and nitrogen fixation. Based on the development of an inhibitory zone or overlapping growth between the paired cultures, the compatibility of microbial strains with one another was evaluated. The bacterial and fungal compatibility were tested on nutrient agar (NA) and potato dextrose agar (PDA)

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media by spot inoculation and disc placement (James and Mathew, 2017) respectively while a modified agar media (Hi-Media) was used to assess the compatibility between bacterial-fungal strains following the methods described by Mishra *et al*., (2021).

Based on PGP traits (ammonia production, IAA production, phosphate solubilization, nitrogen fixation, potassium solubilization and siderophore production), and compatibility test, a consortium of seven microbial strains was prepared and used as an inoculum for carrier-based formulation.

The carrier materials i.e., Talc and gluten for the preparation of bioformulation were purchased from G-1082 basement Sushant Lok-2 sector 57 Gurugram Haryana, 122011 IN and HiMedia Lab Pvt. Ltd, Mumbai, India. Seeds of tomato were purchased from Punjab seed bandar Alambag Lucknow.

2.2. Bioformulation preparation and its application in the Pot experiment: Firstly, the carrier materials (Talc+Gluten (3:1 ratio) was processed (dried and autoclaved at 121psi for 20 minutes) by following the method described by Tripathi *et al.,* (2015). The granular bio formulation was prepared by using freshly prepared microbial consortium ($1x10^8$CFU) in 2:1 ratio (100g carrier and 50 ml inoculum) following the methods of Kumar *et al*., (2014).

In order to evaluate the bioformulation's effect on growth and productivity of tomato, a pot study was conducted in which one carrier-based bioformulation T1 (talc+gluten (3:1 ratio) +MC) along with control treatments T2 (MC in Nutrient Broth), T3 (talc+gluten), T4 (chemical fertilizer) and T5 (soil only) were used. The experiment was conducted in triplicates in earthen pots, in the month of Jan-June 2020-21. The pots were arranged randomly in three blocks under open greenhouse conditions (Inostroza *et. al*. 2016) at Environmental Science Research Station, Babasaheb Bhimrao Ambedkar University, Lucknow, India (26° 72 E, 80° 85 N). The soil for the pot experiment was collected from the non-agricultural land of BBAU campus and different parameters such as pH, EC, organic carbon, available nitrogen, phosphorous and potassium, microbial biomass carbon and alkaline phosphatase activity were checked to examine the nutrient status of the soil according to Jackson (1973). Sandy loam soil, (pH 6.7, EC 5.52 ds/m, organic matter 1.47%, available nitrogen 40.00mg/kg, available potassium 132.63g/kg, available phosphorus 1.17mg/kg, Alkaline phosphatase 17.95µg/g, MBC 77.54µg/g) was used for the experiment. The soil was mixed thoroughly and 7 kg/pot of non-autoclaved soil filled in earthen pots(45x15x15cm). The bioformulation (0.5%/100g of soil) was added once into the soil at the time of transplantation. In this study 3 plantlets per pot were maintained. The pots were watered regularly to maintain moisture, and weed removal was done when required. The effectiveness of bioformulations for soil fertility status was evaluated by comparing the soil's fertility before and after plantation.

2.3. Effect of bioformulation on plant growth and productivity

The plants were harvested after 180 days of transplantation, rinsed with tap water and were assessed for plant growth and productivity, viz. length in cm by using meter sale, fresh weight of shoot and root in g/ plant by using weighing scale. After taking fresh weight of plant sample were kept in a dry air oven at 70˚C for 48 hours. dry biomass of shoot and root was taken after 72 hours of drying. Number of fruits were calculated by manual counting of fruits per plant and fruit fresh biomass was taken by weighing scale (Tripti *et al*., 2017).

2.4. Effect of bioformulation on root colonization

Root colonization (cfu/g of soil) was checked after 180 days of plantation by following Cavaglieri *et al*. (2009). Plants were collected, and any adherent soil with roots was

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washed off, weighed, and then put back into distilled water for resuspension. In order to plate the soil suspension on the appropriate agar media (Nutrient agar for bacteria and Potato dextrose agar for fungus; Hi-media), the soil suspension was serially diluted. CFU per/g of root were then calculated (Fatima and Arora 2021).

A scanning electron microscope (SEM) was also used to analyse the microbial colonisation of roots (JEOL, JSM 6490 LV). Before microscopic examination for colonisation experiments, roots were gently washed three times with sterile distilled water to eliminate unattached microorganisms from the root surface (Gomez *et al*., 2018). To observe colonies on the root surface, roots were sliced into transverse sections. A small portions of root samples was put on double-sided conductive carbon tab that was adhered to a regular vacuum-clean stub.

2.4. Statistical analysis:

Statistical analysis of the data was done using MS Excel and IBM SPSS statistics 20. Data were analysed by one-way analysis of variance, and mean values were compared using Duncan's multiple range test ($P<0.05$).

3.0. Results and discussion

3.1. Detection of Plant Growth Promoting traits and compatibility:

Presence of PGPMs in agricultural soil and their interaction with the cultivated plants is receiving the attention of scientific community due to their capability of solubilizing insoluble nutrients like NPK and to produce other important PGP traits which make them potent to be used as biofertilizer. In the present study, all the selected microbial strains showed the production of IAA, ammonia and phosphate solubilization (Table 1). Results showed that, RS3B and RS8F showed very high IAA production, while RS6B and RS8F showed highest ammonia production and siderophore production was observed highest in RPB3. Further, it was observed that consortium (MC) showed the highest production for all the studied traits. The positive results of IAA, ammonia, phosphate and siderophore production suggested the PGP ability of the selected microbes. Additionally, all the selected microbial strains were found to be compatible with each other. The results of this study was supported by the finding of Mishra *et al*., (2021), who also found enhanced PGP traits in the consortium as compared to single strain.

3.2. Effect of bioformulation on plant growth and productivity:

After 180 days of tomato cultivation, the obtained results showed that the applied bio formulations (T1) have highest efficacy to enhance tomato growth and productivity. The results showed that, transplants subjected to carrier-based bioformulation exhibited a remarkable visual increase in their shoot and root length as compared to the non-inoculated plants (fig.1.) At 180 days after transplantation (DAT), maximum increase in shoot length (60.8%), root length (104.8%), shoot fresh biomass (97.4%), shoot dry biomass (159.1%), root fresh biomass (202.8%) and root dry biomass 212%) was found in carrier based bioformulation (T1) over untreated control (T5). Whereas minimum percent increase in shoot length (16.7%), root length (36%), shoot fresh biomass (13.6%) and root fresh biomass (4 %) over T5 was found in non-inoculated carrier material (T3). Similarly, the maximum increase in number of fruits per-plant (148.3%) and fruit biomass (195.3%) was also found in T1 treatment over control T5, while, the minimum percent increase in fruit number and fruit

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biomass (15% &2.6%) over the control (T5) were found in T3 treatment (fig. 1). The increased plant growth and productivity upon inoculation of carrier based bioformulation might be due to higher number of viable microbes in carrier material which in turn increase their PGP activity like production of IAA, ammonia, phosphatase solubilization and siderophore production. Kushwaha *et al*., (2001) also reported phosphorus as an important nutrient for plant growth and formation of roots, which in turn helps in better yield. Other possible reason for the increased growth and productivity is the colonization capacity of the microbes inoculated in carrier material. Results are in lineation with the results of Jain *et al*. (2022), Helaly *et al*. (2020), Jeet and Baldi (2020).

3.3 Effect of experimental treatments on soil fertility status

The effect of bioformulations on fertility status of post plantation soil was assessed and results are presented in table 2. All the studied parameters showed a remarkable difference between carrier-based bioformulation and other treatments. The maximum percentage increase in OM, available nitrogen, phosphorus and potassium of post-harvest soil were about 57.8%, 285%, 972% and 99% respectively in T1 treatment as compared to control soil whereas, minimum percentage increase of 11.5%, 5%, 154% and 13.6% for OM, available nitrogen, phosphorus and potassium respectively was showed by T5 over control. Increased organic matter and nutrient might be due to the ability of the microbes for nutrient solubilization like phosphate solubilization, nitrogen fixation and potassium solubilization. The results were in lineation with the results of Kushwaha (2011). who also reported improved fertility of soil upon microbial inoculation. Similarly soil enzymes like microbial biomass carbon (MBC)and alkaline phosphatase were increased by 224% and 420% in T1 over control, while minimum increased was found in case of T3(table 2). The increment in enzymes like MBC and Alkaline phosphatase could be attributed to the increased population of PGPM in the soil resulting in improved soil fertility. Previous study by Mishra *et al*., (2021) and Mishra and Singh, (2022) also reported increased percentage of soil MBC upon inoculation of bioformulation, which in turn have a positive effect on interaction of microbes with native microbes and on colonization. Results were also supported by the findings of Yu *et al*., (2016), who reported increased enzyme activity and microbial community in soil upon inoculation of bioformulation consisting of mushroom residue and *pseudomonas sp.*

3.4. Effect of bioformulation on microbial population in rhizosphere and root colonization

The results further showed that inoculation of bioformulation helps in the microbial colonization in the rhizosphere. T1 showed maximum enhanced population density of 7.6$\log_{10}$ cfu/g of soil for bacteria, whereas T4 showed minimum bacterial population (7.24 $\log_{10}$ cfu/g of soil) in post plantation soil as compared to pre- plantation soil. Further T1 also showed maximum fungi population (6.57$\log_{10}$ cfu/g of soil) while, T4 shows minimum fungal population as compared to pre-plantation soil. Similar to the current work, Novinscak and Filion 2020 also found that *Pseudomonas fluorescens* and *P. synxantha's* talc and peat-based bioformulation improve rhizospheric colonisation. The results further indicated that carrier-based bioformulation helped in maintaining the population of microbes in the rhizosphere (fig. 3.). Further it was evident from the results that carrier based bioformulation enhanced root colonization potential of microbes, as maximum colonies of microbes were present in T1 followed by T2 as evident from SEM analysis. In other selected treatment there were very less or no colonization on the root surface (Fig. 3 b). The most possible reason for root colonization by carrier-based formulation might be the protective niche provided by the

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carrier material, which will help in slow release of microbes in the soil, and in turn helped the microbes to colonize, and work efficiently. The results were supported by the findings of Jain *et al*., (2022).

4.0. CONCLUSION

The study indicated that the novel bioformulation (T1) prepared from compatible communities of seven beneficial microbes isolated long back has a positive effect on growth and yield of tomato plant which was reflected by increase in its vegetative and reproductive parameters. In addition to promoting plant growth, the microbial bioformulation enhances soil fertility by increasing the soil microbial biomass carbon, population of soil microbes and accessibility of crucial nutrients for plant development. Overall, the findings of the study showed that bioformulation of competent microbes in carrier material is a viable alternative to traditional, non-sustainable synthetic chemical-based methods of crop production. Therefore, in order to maintain soil fertility and plant growth, it can be inferred that tomato seeded with microbial bioformulation can readily reduce input cost of crop production, reliance on chemical-based fertiliser and eventually enhance the soil health and nutritional quality of crop.

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Declaration of Interest Statement: We confirm there is no conflict of interest.

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ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, S Iss 3, Dec 2022

Table: 1. PGP traits of the microbial strains and their consortium

Microbial strain	Indole acetic acid production	Ammonia production	Phosphatase solubilization	Potassium solubilization	Nitrogen fixation	Siderophore production
***Bacillus filamentosus* RS3B**	++++	++	+	-	+	+
***B. pseudomycoides* RS6B**	+++	++++	++	+	+	-
***B. paramycoides* RPB3**	++	+	+	-	+	++++
***Alcaligenes faecalis* RS10B**	++	++++	+	-	+	-
***Aspergillus tamarii* RS8F**	++++	++++	++	-	-	-
***Aspergillus luchuensis* RS6F**	++	++++	++	+	-	+
***Trichoderma lixxi* TvR1**	++	+++	++	+	-	++
Consortium (MC)	**+++++**	**+++++**	**++++**	**+++**	**+++**	**++++**

MC= (Bacillus filamentosus RS3B, *Bacillus pseudomycoides* RS6B, *Bacillus paramycoides* RPB3, *Alcaligenes faecalis* RS10B, *Aspergillus luchuensis* RS6F, *Aspergillus tamarii* RS8F, *and Trichoderma lixxi TvR1*), + = positive, - = negative, ++++= very high, +++= high, ++ moderate

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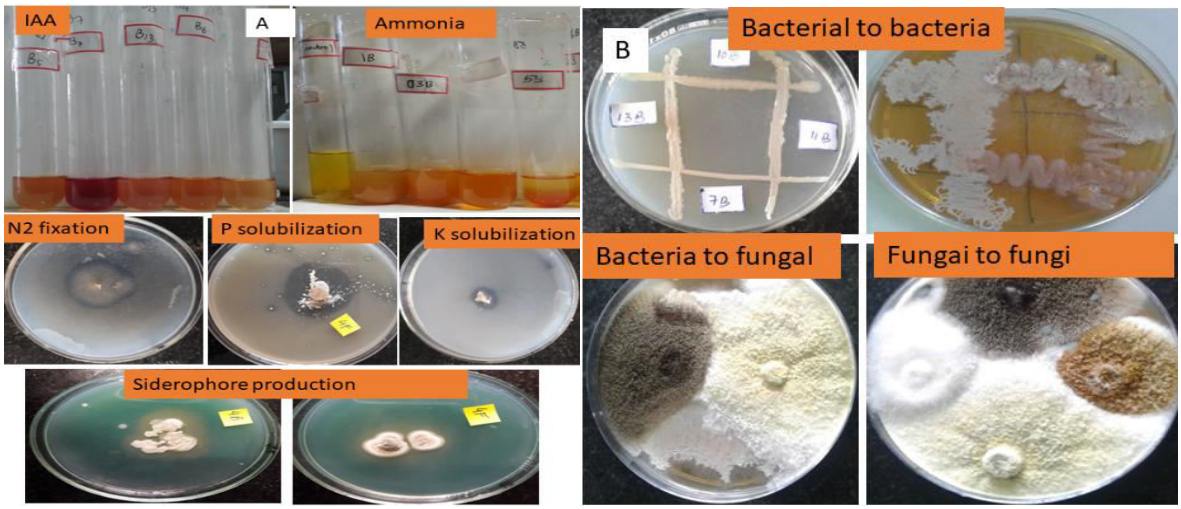

Fig: 1: PGP traits and compatibility of microbial strains A= PGP properties, B= compatibility

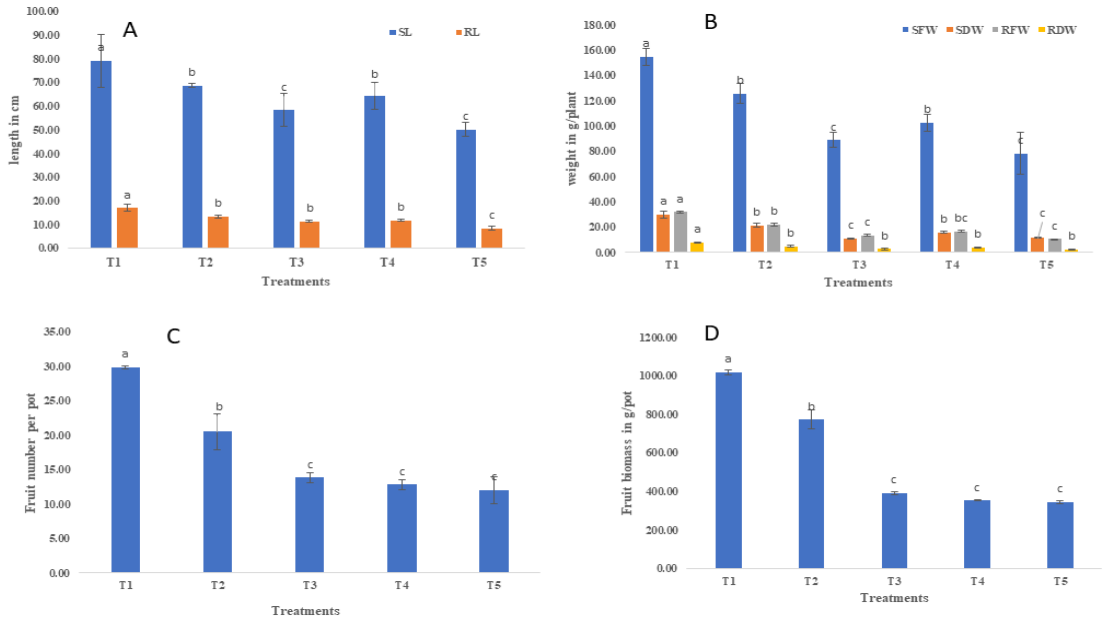

Fig. 2: Effect of experimental treatments on plant growth parameter and productivity. A=shoot and root length, B= shoot and root fresh and dry weight, C= fruit number per plant and D= fruit biomass per plant

__T1__= talc+gluten +MC, __T2__ =nutrient broth+MC, __T3__=talc+gluten, __T4__=chemical fertilizer and __T5__= soil only, __SL__=shoot length, __RL__= root length, __SFW__ shoot fresh weight, __SDW__= shoot dry weight, __RFW__= root fresh weight, __RDW__= root dry weight, Data are mean of three replicas, in duplicate determination (n=6) ± standard error of means. Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)

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Table 2: Effect of treatments on soil physio-chemical and enzymatic properties

T1*= talc+gluten +MC,* ***T2*** *=nutrient broth+MC,* ***T3****=talc+gluten,* ***T4****=chemical fertilizer and* ***T5****= soil only,* ***AN****=Available nitrogen;* ***AP****=*

	pH		EC (ds/m)	OM (%)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	ALkP(µg/g)	SMBC(µg/g)
Pre-sowing	0 days	6.77±0.0^{c}	5.52±0.02^{a}	1.47±0.0^{d}	40.00±12.96^{d}	1.17±0.00^{d}	132.63±11.25^{e}	17.95±1.98^{d}	77.54±6.59^{d}
Post-harvesting	**T1**	**7.00±0.4^{b}**	**1.26±0.02^{d}**	**2.32±0.0^{a}**	**154.17±2.36^{a}**	**11.89±1.32^{a}**	**263.04±33.74^{a}**	**93.24±0.33^{a}**	**512.09±7.36^{a}**
	T2	7.00±0.3^{b}	3.20±0.04^{c}	2.12±0.0^{a}	130.00±15.3^{b}	7.46±0.99^{b}	204.20±13.49^{b}	35.43±0.99^{b}	329.26±26.8^{b}
	T3	6.95±0.4^{c}	4.23±0.00^{b}	1.77±0.3^{b}	76.25±25.34^{c}	2.80±0.33^{cd}	178.75±22.49^{c}	27.04±0.99^{c}	154.62±13.3^{c}
	T4	7.08±0.4^{ab}	4.90±0.52^{b}	1.64±0.1^{c}	51.67±14.14^{d}	3.50±1.32^{c}	154.90±11.25^{d}	30.30±4.29^{c}	158.26±7.67^{c}
	T5	7.27±0.1^{a}	4.33±0.19^{b}	1.65±0.1^{c}	42.92±10.05^{d}	3.26±0.99^{c}	150.13±9.00^{d}	27.74±3.30^{c}	158.33±22.8^{c}

available phosphorus; ***AK*** *=available potassium;* ***OM****= organic matter;* ***ALkP=*** *alkaline phosphatase;* ***SMBC****= soil microbial biomass carbon* ***mg/kg****= milligram per kilograms;* **µg/g=** microgram/grams; **%** *=percentage;* ***EC*** *= Electrical conductivity;* ***ds/m****= desicimen per meter, Data are mean of three replicas, in duplicate determination (n=6) ± standard error of means. Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

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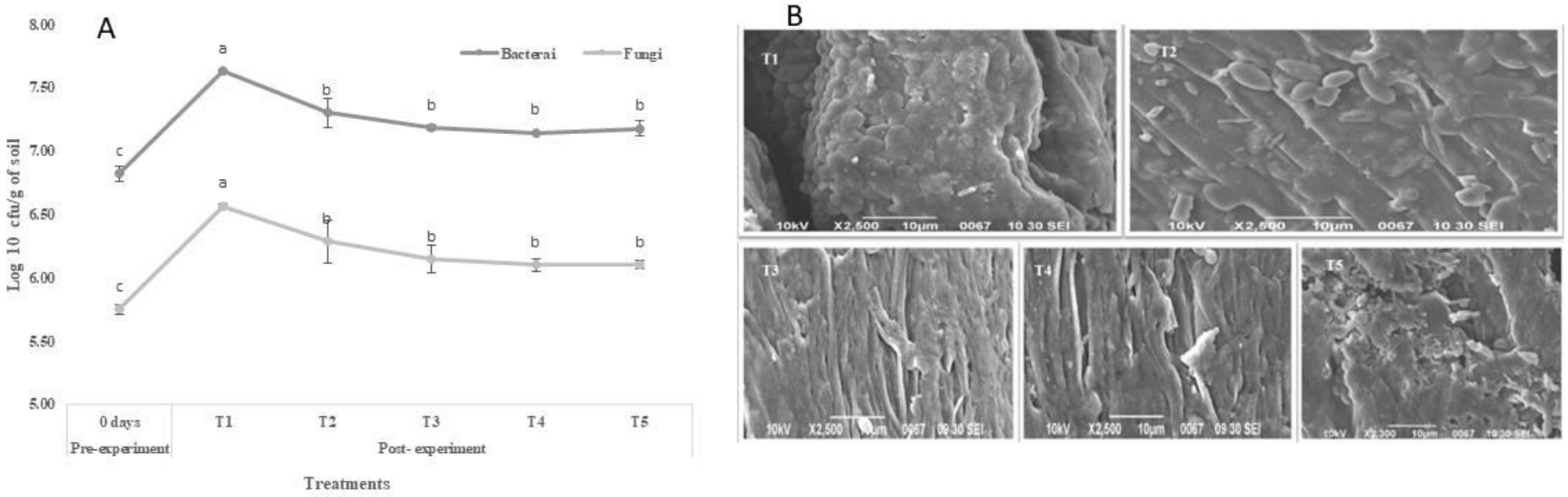

Fig. 3. Effect of treatments on microbial population in rhizosphere A= log $_{10}$ cfu/g of rhizospheric soil, B=colonization on root surface by SEM analysis.

***T1**= talc+gluten +MC, **T2** =nutrient broth+MC, **T3**=talc+gluten, **T4**=chemical fertilizer and **T5**= soil only; **CFU**= colony forming unit, Data are mean of three replicas, in duplicate determination (n=6) ± standard error of means. Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*