Original Research Article

Effect of Cell Free Microbial Bio-stimulant on Growth, Yield and the Microbial Activity of Tomato (*Solanum lycopersicum* L.) Grown Soil

Abstract

A field experiment was conducted in *vertisol* to find out the effect of a cell-free microbial biostimulant on growth, yield, and the microbial activity of tomato (*Solanum lycopersicum* L.) grown in a field during the *rabi* season (December–April) of 2021–22 at the Instructional Cum Research Farm, College of Agriculture, IGKV, Raipur, Chhattisgarh, India . Pant-3 variety of tomato was used for the experiment, which was spaced in 45×60 cm. The experiment consists of seven treatments, viz., six levels of cell-free microbial biostimulant (control, 0.125 L ha^{-1}, 0.25 L ha^{-1}, 0.50 L ha^{-1}, 0.63 L ha^{-1}, 1.25 L ha^{-1}, and 2.50 L ha^{-1}), in combination with 100% RDF, and was laid out in a randomised block design (RBD) with three replications. Data regarding the height of the plant, numbers of fruits ($plant^{-1}$), yield (kg ha^{-1}), dehydrogenase, and microbial activity were observed. The experimental result revealed that the application of a cell-free microbial biostimulant with a combination of 100% RDF was found to have a significant impact on the microbial population and dehydrogenase activity of soil, but that plant height, fruit number, and yield were not significantly affected. According to the performance of the crop and analysis of rhizosphere soil, it can be concluded that the cell-free microbial biostimulant @ 2.50 L ha^{-1} with soil application along with 100% RDF (120:80:80 kg ha^{-1} N:P:K) increases the soil microbial population, i.e., bacteria, actinomycetes, and fungi, and also improves the quality of the soil.

Keywords: Biostimulant, cell free microbial, microbial biostimulant, tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important, popular, and widely grown vegetables worldwide. The tomato and its products continue to grow in popularity as a good source of vitamins, minerals, and antioxidants essential for human health. They are rich in vitamins A and C and are important because they contain lycopene, a food component known to reduce the risk of prostate cancer, heart disease, and age-related diseases. Modern horticulture faces significant problems like declining soil fertility and pollutant contamination of soil and water (Schwarz et al., 2010). There is a need for vegetable crop cultivation in unfavourable environments in the context of global climate change and food security, as well

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as for the sustainable use of precious and limited natural resources through the protection of biodiversity (Szparaga et al., 2019, Postel, 2000, Del Buono, 2021). Bio stimulants are a unique and sustainable method to crop development, especially under biotic and abiotic stressors, that has been suggested as one of several farming systems over the years (Bertrand et al., 2021, Del Buono, 2021, Bulgari et al., 2014). Application of bio stimulants is a viable and sustainable method of supplementing the nutrition of crops, and it may help to solve the environmental issues brought on by excessive fertilisation (Bulgari et al., 2014, Halpern et al., 2015). Du Jardin (2015) define bio-stimulant as "any substance or microorganism that applied to plants, regardless of its nutrients content, is able to enhance nutrition efficiency and also abiotic stress tolerance and quality traits. The main characteristic of bio-stimulants, especially those based on a single microorganism or microbial consortium, is their ability to reduce fertilizer application while increasing the yield and quality of horticultural crops. The positive influence of beneficial microorganisms with well-known agronomic techniques can increase the sustainability of tomato processing yields per hectare if cultivated in organic farming systems (Ronga et al., 2019). Specifically, the microbial bio stimulants may advance plant development both straightforwardly and by implication, biofertilization, feeling of root development, resistance to establish stressors and rhizoremediation are a couple of instances of direct impacts on plant development advancement (Massa et al., 2018, Lugtenberg and Kamilova, 2009, De Vries et al., 2020) while controlling plant microorganisms and improving the enzymatic movement of plants may in a roundabout way prompt plant development (Pérez-Montaño et al., 2014, Ahemad and Kibret, 2014). Additionally, biostimulatory substances may benefit soil biology and are recognised as an effective method for restoring semi-arid regions and damaged ecosystems (Askari-Khorasgani et al., 2019, Karapouloutidou and Gasparatos, 2019, Calvo et al., 2014). Many study studies have examined several bio stimulants. Among the most frequently researched bio stimulants, *Ascophyllum nodosum* extracts have varied effects on a variety of crops, including spinach yield and nutritional quality (Pereira et al., 2019, Fan et al., 2013, Rouphael et al., 2018), increased drought tolerance in tomato plants (Goñi et al., 2018), or reduced the effects of water stress on common beans (Petropoulos et al., 2020, Galvão et al., 2019). Bio stimulants in view of plant development advancing microorganisms incorporate microbial inocula from microscopic organisms and parasites of different genera and have additionally found commonsense applications in agricultural yields, either alone or in mix with one another (Sani et al., 2020). Caradonia et al. (2020) reported that biostimulant which is used in tomato seedling increases yield and quality of tomato fruit in sustainable farming systems. Therefore,

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the present investigations were undertaken to study the effect of cell free microbial bio-stimulant on growth, yield and microbial activity of tomato (*Solanum lycopersicum* L.) grown field by different level of microbial bio-stimulants.

Materials and Methods

The field trials was conducted during the *rabi* season (December–April) of 2021–22 at the Instructional Cum Research Farm, College of Agriculture, IGKV, Raipur, Chhattisgarh, India which is located at an altitude of 298.58 m above the mean sea level (MSL) at 21°16″ N latitude and 81°36″ E longitude. Soil of the trial plots was *vertisol*, Alkaline (7.9) in soil reaction, low in organic carbon (0.54 dS m^{-1}), low in available nitrogen (188.23 kg ha^{-1}), medium in phosphorous (24.19 kg ha^{-1}), high in potassium (505.90 kg ha^{-1}) and EC was 0.24 dS m^{-1}. The seed of tomato, variety Pant-3 was collected from college of Agriculture, IGKV Raipur, (C.G.). Seeds were sown on nursery beds on 5th December 2021 and the soil of seed bed was covered with organic mulching (grass) to protect the young seedlings from adverse climatic condition and keep soil moist. Covering materials were removed from the bed after seed germination (5 days after sowing) for optimum growth of seedlings. 30 days old healthy seedlings with uniform growth were transplanted on 5th January 2022 at a spacing of 45×60 cm accommodating 81 plants $plot^{-1}$ (4×5 m^2 plot size). The experiment was laid out in randomized block design with seven treatments and three replications. The treatments include T_1 (100% RDF+Foliar application of cell free microbial bio-stimulant @ 0.125 L ha^{-1}), T_2 (100% RDF+Foliar application of cell free microbial bio-stimulant @ 0.25 L ha^{-1}), T_3 (100% RDF+Foliar application of cell free microbial bio-stimulant @ 0.50 L ha^{-1}) and T_4 (100% RDF+Soil application of cell free microbial bio-stimulant @ 0.63 L ha^{-1}), T_5 (100% RDF+Soil application of cell free microbial bio-stimulant @ 1.25 L ha^{-1}), T_6 (100% RDF+Soil application of cell free microbial bio-stimulant @ 2.50 L ha^{-1}) and T_7 (Control, only 100% RDF). The recommended dose of fertilizer 120:80:80 kg NPK ha^{-1} was applied through the urea, single super phosphate and murate of potash to all the plots. Full dose of phosphorus, potassium and half dose of nitrogen was applied as basal dose before transplanting of the seedlings, while the remaining half dose of the nitrogen was used in split doses at 15, 30 and 45 days after transplanting (DAT) as top dressing. According to the treatments cell free microbial bio-stimulant was applied by two different methods, first as foliar application in T_1, T_2 and T_3 treatment and second as soil application (drenching) in T_4, T_5 and T_6 treatment, respectively. Cell-free microbial bio-stimulant was applied three

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different times through a knapsack sprayer: the first application of microbial bio-stimulant was between 25 and 30 days after transplanting, the second was between 15 and 20 days after the first application, and the third was between 15 and 20 days after the second application. The plant was given the correct irrigation for optimum growth and development, and all crucial intercultural procedures and plant protection measures were implemented. Observations on three randomly selected plants in each treatment were tagged properly for recording various observations viz. height of plant (cm), fruits number (plant^{-1}), yield (q ha^{-1}). Microbial population in rhizosphere soil was analyzed at 30, 60 and 90 days after transplanting (DAT) by serial dilution plating method as describe by Subba Rao (1982). For the isolation of total bacteria, actinomycetes and fungus all three media, nutrient agar, Kenknight‟s and Rose Bengal Agar media were used, respectively. Plating of each plot soil sample was done in triplicate and mean values were worked out for each sample. One Control was also incorporated with each set of plating and colony forming unit was observed. Dehydrogenase activity of rhizosphere soil was analyzed at 45 DAT as method described by Klein et al. (1971). A 15 ml airtight screw-capped test tube was used to hold a 1 g. air-dried soil sample to saturate the soil in each tube, 0.2 ml of a 3% TTC (Triphenyl tetrazolium chloride) solution was applied. Each tube also had 0.5 ml of distilled water. To create a water seal above the soil, gently tap the tube's bottom to release any trapped oxygen. After 24 h of incubation at 37°C, 10 ml of methanol was added to the tubes. Shake it overwhelmingly and permitted to represent 6 hrs. Clear pink shaded supernatant was pull out and readings were taken with a spectrophotometer. All glassware's which are used in this experiment were cleaned with detergent powder and washed under tap and distilled water. The dried glassware was sterilized in hot air oven at 160°C for 2 h. The inoculation needle was sterilized by dipping them in alcohol and heating over the flame of spirit lamp before using. Sterilization of media was done by autoclaving at 15 lb pressure for 20 m and all the isolation and inoculation work from soil sample were carried out in laminar air flow. All observations recorded from this experimental study were tabulated systematically for proper interpretation. The observations were statistically analyzed using analysis of variance (ANOVA) and p value<0.05 were considered as statistically significant as described by Panse and Sukhatme (1967). Statistical analysis was done by taking the mean value of observed data.

Results and Discussion

The data on average plant height were recorded at three different stages (30, 60, and 80 DAT), which are presented in Table 1, and they clearly indicate that no significant difference

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was observed due to the application of cell-free microbial bio-stimulant with a combination of 100% RDF at different growth stages. Similarly, the number of fruits and yield were not affected significantly (Table 1). The results of the present investigation showed that the application of a cell-free microbial biostimulant in combination with 100% RDF had significant effects on the microbial population and dehydrogenase activity of soil. The microbial population in rhizosphere soil was analysed at 30, 60, and 90 days after transplanting (DAT). Data obtained (Table no. 2) thus revealed that no statistical difference was observed in the early stage of crop growth for the population of total bacteria at 30 DAT, but at 60 and 90 DAT, observed data showed that the highest population of total bacteria (118.67 and 99.00) was found significantly in treatment T_6 due to the application of a cell-free microbial bio-stimulant at 2.50 L ha^{-1} with soil application along with 100% RDF, whereas the lowest population of total bacteria at 60 and 90 DAT (88.00 and 50.67) was found from treatment T_7 (Control, only 100% RDF). The present findings are supported with the result of Tejada et al. (2011), who mentioned that the soil amended with biostimulant had the highest soil enzymatic activities and bacterial and fungal biomass. Additionally, the use of bio stimulants improved the biological characteristics of the soil and encouraged the growth of vegetation that will shield the soil from erosion and aid in its restoration. Similar to this, Sani et al. (2020) reported that the application of bio stimulants based on *Trichoderma* and bio stimulants extracted from seaweed increased soil fertility and nutrient availability as a result of an abundance of bacterial and fungal microbial populations in the rhizosphere. The data regarding actinomycetes population in soil at 30, 60 and 90 DAT as influenced by different treatments are presented in table 2. Population of actinomycetes in early stage of crop growth (30 DAT) found non-significant effect due to application of cell free microbial bio-stimulant. Population of actinomycetes increase significantly at 60 and 90 DAT. observed data revealed that the maximum actinomycetes population (62.33 and 98.33) was found from treatment T_6 due to the application of cell free microbial bio-stimulant @ 2.50 L ha^{-1} with soil application along with 100% RDF and minimum population (45.67 and 47.67) was observed in treatment T_7 (Control, only 100% RDF). These findings are supported with the result of Tejada et al. (2014). Fungal population in soil at 30, 60 and 90 DAT obtained data revealed that no statistical difference was observed in early stage of crop growth (Table no.2) for fungal population at 30 DAT but at 60 and 90 DAT fungal population found significantly. The highest population of fungal (13.05 and 9.32) was found in treatment T_6 due to the application of cell free microbial bio-stimulant @ 2.50 L ha^{-1} with soil application along with 100% RDF and lowest fungal population (6.20 and 5.33) was observed in

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treatment T_7 (Control, only 100% RDF). Finding was also correlate with work of Baroja-Fernández et al. (2021) who revealed that soil application of fungal based cell free biostimulant promoted similar changes in the soil microbiota, and promoted the proliferation of the same beneficial microbial taxa. Collectively, his finding indicated that cell-free microbial culture filtrates (CFs) as biostimulant can be used to activate the beneficial soil and plant-associated microbiota without significant changes in the relative abundance of populations of pathogenic microbial species. Sani et al. (2020) also mentioned that fungal communities are increase in foliar and soil applications of different bio-stimulant. The result on Dehydrogenase activity (DHA) as affected by different treatments is recorded at 45 DAT which is presented in table no.1. The maximum dehydrogenase activity (21.60 µg TPF $ha^{-1}g^{-1}$ soil) was found from treatment T_6 due to the application of cell free microbial bio-stimulant @ 2.50 L ha^{-1} with soil application along with 100% RDF and lowest dehydrogenase activity (15.27 µg TPF ha^{-1} g^{-1} soil) was recorded in treatment T_7 (Control, only 100% RDF). Chen et al. (2003) also mentioned that the soil dehydrogenase activity increased with addition of biostimulant in soil and the enzyme activity was maximum at flowering compared to harvest. This could be due to higher root exudates from the plant roots at flowering over harvesting stage. García-Martínez et al. (2010) detailed that use of biostimulant in soil, increments soil microbial action, which can work on the soil physical and chemical attributes. In particular, the expanded bioactivity in the soil causes a speedier breakdown of organic matter, which changes organic supplements into plant-accessible mineral structures. The expansion in dehydrogenase movement in soil could likewise be good to increase in microbial population.

Table 1: Effect of cell free microbial bio-stimulant on plant growth, yield and dehydrogenase activity of soil

Treatment details	Plant height (cm)			Number of fruits ($plant^{-1}$)	Total yield (q ha-1)	Dehydrogenase activity (µg TPF ha^{-1} g^{-1} soil)
	30 DAT	60 DAT	80 DAT	At harvest	At harvest	45 DAT
T_1	24.33	58.13	69.53	23.60	241.95	16.47
T_2	24.41	60.53	70.37	24.92	256.92	18.90
T_3	23.35	61.27	71.87	24.95	260.79	19.73
T_4	23.35	61.87	71.00	23.74	244.59	18.30

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T_5	23.41	62.27	71.91	25.04	261.30	20.20
T_6	24.07	62.50	72.43	25.11	263.04	21.60
T_7	23.17	57.53	66.00	23.00	232.55	15.27
SEm±	0.58	2.04	2.55	0.74	12.35	0.67
CD (*p*=0.05)	NS	NS	NS	NS	NS	2.07

Table 2: Effect of cell free microbial bio-stimulant on microbial activity of tomato grown soil

Treatment details	Total bacteria (10^6 g^{-1} soil)			Actinomycetes (10^5 g^{-1} soil)			Fungi (10^3 g^{-1} soil)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_1	45.33	101.67	67.00	34.33	47.00	57.67	3.78	9.90	7.54
T_2	45.33	108.33	71.00	37.33	48.33	83.67	3.95	11.16	7.89
T_3	43.33	111.67	90.33	36.33	54.00	87.00	3.92	11.18	8.32
T_4	54.67	104.67	69.00	37.33	48.00	67.67	3.93	10.10	7.67
T_5	52.67	112.67	92.33	38.00	61.67	94.67	3.90	12.21	8.46
T_6	54.33	118.67	99.00	38.33	62.33	98.33	3.98	13.05	9.32
T_7	47.33	88.00	50.67	36.67	45.67	47.67	3.87	6.20	5.33
SEm±	2.13	3.25	3.36	1.46	3.65	4.25	0.17	0.80	0.48
CD (*p*=0.05)	NS	10.01	10.36	NS	11.23	13.09	NS	2.46	1.47

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Conclusion

The plant height, fruit number, and yield were not affected significantly, but the application of the recommended dose of fertilizer (120:80:80 kg ha^{-1} $N:P_2O_5:K_2O$) with soil application of cell-free microbial bio-stimulant @ 2.50 L ha^{-1} increased the soil microbial population, i.e., bacteria, actinomycetes, and fungi, and also improved the quality of the soil.

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