KAVAKA 59(1): 56-74 (2023) DOI: 10.36460/Kavaka/59/1/2023/56-74

Preparation of Nutrient Enriched Bio-Formulation(s) Using Vermi-Compost as a Carrier Material for Sustainable Agriculture

Praful Kumar*, Sandhya Sahu, and K.P. Verma

Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur- 492 012, Chhattisgarh, India.
**Corresponding author Email:prafulsoni127@gmail.com*

(Submitted on November 08, 2022; Accepted on February 24, 2023)

ABSTRACT

A nutrient enriched bio-formulation(s) was prepared by inoculating the combination of bio-inoculants viz., *Trichoderma* sp., *Pseudomonas fluorescens* and *Azotobacter chroococcum* in vermicompost supplemented with minimal inorganic fertilizers i.e., Diammonium phosphate and Muriate of potash, which is compatible with bio-inoculants. Twenty four *Trichoderma* isolates were isolated from different locations of Dhamtari, Rajnandgaon, and Kabirdham District of Chhattisgarh. All the 24 *Trichoderma* isolates were evaluated for compatibility with *P. fluorescens* and *A. chroococcum*. Three *Trichoderma* isolates were found compatible with both the bacterial bio-inoculants. *Trichoderma* isolates TRT2, TRT-9, and TRT-12 found potentially able to produce IAA, siderophore, HCN, cellulase, chitinase, and phosphatase. Similarly, *P. fluorescens* and *A. chroococcum* individually having potential to produce IAA, siderophore, HCN, cellulase, chitinase, and phosphatase. *Trichoderma* isolate TRT-2 did not produce HCN and *A. chroococcum* did not produce cellulase enzymes. *Trichoderma* isolates i.e. TRT-2, TRT-9, and TRT-12, *P. fluorescens* and *A. chroococcum* individually were further evaluated for sensitivity with inorganic fertilizers i.e., DAP, MoP, SSP, urea, and complex fertilizer 28:28:00. TRT-2, TRT-9, and TRT-12 were inhibited by urea and complex fertilizer (28:28:00) at all concentrations. However, SSP could not inhibit the growth at any concentration, whereas inhibition percentages in DAP were ranged, 0.00-42.10%, 0.0-0.20%, and 0.37-19.11% for TRT-2, TRT-9, and TRT-12, respectively. Successive increases in concentration up to 5% of fertilizer could not affect the growth of isolate TRT-9 that leads to resistance. Based on the above mentioned findings, three formulations (1) Vermicompost enriched with three bio-inoculants i.e. *Trichoderma* isolates, *P. fluorescens,* and *A. chroococcum*, (2) DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost enriched with three bio-inoculants i.e. *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*, and (3) DAP (10% w/w) and MoP (3% w/w) supplemented vermicompost enriched with three bio-inoculants i.e. *Trichoderma* isolates, *P. fluorescens,* and *A. chroococcum*, were constituted and evaluated.

Keywords: *Trichoderma* isolates; *Pseudomonas fluorescens*; *Azotobacter chroococcum*; Nutrient Enrichment; Bio-formulation(s).

INTRODUCTION

Now a days, application of chemical fertilizers and pesticides is highly practiced by farmers for higher yield to meet the ever-increasing demand of growing population, where the need is turning to greed day by day that led farmers to bring in the use of additional dose of synthetic chemicals such as chemical fertilizers, fungicides and pesticides for higher yield and crop protection of vegetable crops irrespective of the harm it causes to the environment. Although synthetic chemicals are credited and gain a reputation of enhancement of crop productivity and checking out or elimination of various plant disease causing organisms but are also proved to cause distress to the environment as the damage due to agro-chemicals are prominent. In the last few years, numerous long-term experiments have been conducted to check the impact of chemical fertilizers on soil fertility. Studies showed that, although, the use of chemical fertilizers is obligatory and its constant application increases the concentration of available nutrients in the soil compared to the initial level, continuous use of chemical fertilizers is accountable for the decline in soil quality and productivity.

Chemical fertilizers used to compensate for the deficiency of nutrients in the soil and pesticides that are used for the control of plant diseases are being accumulated in the environment causing pollution, spreading disease, and degrading ecosystems (Gerhardson, 2002). For reducing the accumulation of such pollutants in agro-ecosystems we need to minimize the use of toxic agrochemicals especially synthetic fertilizers and pesticides in agricultural processes. So, there is a rising need to develop a greener and safer alternative so that the food for the growing population and disease and pest control can be gained without causing any harmful effect on the environment. It can be achieved by use of microbe-

based bio-formulations for achieving the goal of sustainability. The importance of microbes in sustainable agriculture has provided a new understanding of agro-economy, and one of the immediate advantages is reduction in the use of agrochemicals like fertilizers and pesticides

A vital area of plant pathological research all over the world is the control of soil-borne fungal plant pathogens by the species of *Trichoderma* (Mukhopadyay, 1987). Use of *Trichoderma* spp. as a biological control agent for a variety of economically important soil- borne fungal plant pathogens has been known (Champawat and Sharma, 2003). Several plant pathogens are known to be successfully controlled by Rhizobacteria *Pseudomonas fluorescens* (Jayashree *et al*., 2000). *Azotobacter chroococcum* is a beneficial microorganism used for seed or soil application to enhance the soil fertility and increases the number and biological activity of desired microorganisms in the root environment, thereby promoting plant growth while decreasing the N fertilizer dose and allows to alleviate the environmental deterioration related to N pollution (Romero-Perdomo *et al*., 2017).

The advancement in the understanding of microbial consortium (bioformulation) has emerged as a better alternative for promoting plant growth and disease eradication. Microbial consortiums are viable microbial products that contain more than one beneficial microbial strain in economical carrier materials. This green strategy is facing hurdles at socio-economic level, and is yet to match up to their competitors, the synthetic chemical fertilizers and pesticides.

Search for effective bio-inoculants for the management of plant diseases and nutrient management has been intensified to reduce the dependency on ecologically hazardous chemicals. However, bio-inoculants must be compatible with each other as well as commonly used agrochemicals so that they can be integrated into the production system effectively (Prasanna *et al*., 2002; Tripathi and Johri, 2002; Sharma and Dureja, 2004). In fact, the combined usage of beneficial microbes (N_2-fixing (NF), P-solubilizing, P-mobilizing, and Biocontrol agents) and mineral resources is an emerging research area that aims to design and develop efficient microbial formulation which are highly compatible with mineral inputs, with positive impacts on both crops and environment.

In such an approach Disease cum Nutrient Management concepts have an ultimate goal of complete crop care with the use of various beneficial microorganisms and vermicompost enriched with a lesser amount of inorganic fertilizers for achieving best possible results. Addition of lesser amounts of inorganic nutrients (NPK) along with beneficial microbes in vermicompost contain nutrients in available form with increased microbial activity that increases aeration in the soil for further improvement of plant root development and increased plant growth and yield.

This study, therefore, was conducted to prepare vermicompost based nutrient enriched bio-formulation(s) with the combination of biocontrol agents and biofertilizer, to be used with a lesser amount of commonly used chemical fertilizers without any hazardous effect on antagonists.

MATERIAL AND METHODS

Collection of fungal and bacterial bio-inoculants
Pure culture of *A. chroococcum* was obtained from the Department of Agricultural Microbiology, College of Agriculture, Raipur, Chhattisgarh. Pure culture of *P. fluorescens* was obtained from Biocontrol laboratory, Department of Plant Pathology, College of Agriculture, Raipur, Chhattisgarh. *Trichoderma* spp. were isolated from the rhizosphere soil of healthy tomato plants collected from tomato growing fields at different locations of Dhamtari, Rajnandgaon and Kabirdham district, Chhattisgarh. Soil samples were brought to the laboratory and stored at 4 °C until used. Up to five-fold serial dilutions of each soil samples were prepared in sterilized distilled water and 1 ml suspension of 10^{-3}, 10^{-4}, and 10^{-5} dilution was transferred to sterile petri plates, having 15 ml *Trichoderma* Selective Medium (TSM) and incubated at 28±2 °C. Three days old colonies of *Trichoderma* isolates were purified and maintained at 4 °C for further studies.

Assessment for compatibility of *Trichoderma* isolates with *P. fluorescens* and *A. chroococcum*

Under *in vitro* by plate method: *In vitro* compatibility of *Trichoderma* isolates with *P. fluorescens* and *A. chroococcum* was studied by co-culturing them on Potato Dextrose Agar medium (Manjula *et al*., 2004). Sterilized Petri plates were poured with 15ml Potato Dextrose Agar medium without antibiotics and allowed to solidify. Two days old cultures of *P. fluorescens* and *A. chroococcum* were inoculated perpendicular to each other and incubated at 28±1 °C for 24 hours. Then, five days old cultures of *Trichoderma* isolates were

placed at the center of both the bacterial streaks and allowed to incubate for 5 days. Incubated plates were observed for zones of inhibition (if any) produced by cultures at regular intervals. The test was performed in three replications.

Under *in vivo* by soil methods: Compatible *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*, tested under *in vitro* by plate method were again evaluated for their compatibility in the soil condition using a method proposed by Mishra *et al.* (2013) with slight modification. Non-sterilized air-dried soil sieved through 2 mm screens was inoculated with vermicompost based formulations of fungal and bacterial bio-inoculants, individually and in combinations @ 50 g kg^{-1} soil (approximately $x10^8$ cfu g^{-1} of each inoculant). Pots inoculated with individual fungal and bacterial formulation were maintained as control. These pots were irrigated with sterilized water and kept in an incubator at 27±2 °C. Population dynamics of bio-inoculants were estimated in each pot at 30 days after inoculation by using serial dilution plate technique.

Assessment for sensitivity of *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* with inorganic fertilizers under *in vitro:* Three compatible *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* were evaluated for their sensitivity with the commonly used inorganic fertilizers *viz.* Urea, Diammonium phosphate (DAP), Single Superphosphate (SSP), Muriate of potash (MoP), and Complex fertilizer (28:28:0) by using poison food technique.

Sensitivity of *Trichoderma* isolates: Potato Dextrose Agar medium supplemented with different concentrations (1%, 2%, 3%, 4%, and 5%) of each inorganic fertilizer separately, were used to evaluate the sensitivity of *Trichoderma* isolates. Five mm fresh culture disc of each *Trichoderma* isolates grown in Potato Dextrose Agar medium was placed at the centre of Petri plates poured with 15 ml of Potato Dextrose Agar medium supplemented with inorganic fertilizer, separately and incubated for seven days at 27±2 °C. Plates having Potato Dextrose Agar medium without fertilizers were maintained as control. Per cent inhibition (I) of the mycelial growth of *Trichoderma* isolates over the control was calculated by using the following formula:

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition
C = Colony diameter of *Trichoderma* in control.
T = Colony diameter of *Trichoderma* in treatment.

Sensitivity of *P. fluorescens* and *A. chroococcum*

Sensitivity of the bacterial inoculants i.e., *P. fluorescens* and *A. chroococcum*, were evaluated by using Nutrient Agar medium supplemented with different concentrations (1%, 2%, 3%, 4%, and 5%) of each inorganic fertilizer separately. A loopful of active bacterial culture was streaked on individual Nutrient Agar (NA) medium plates amended with appropriate concentrations of fertilizers and incubated for 48 h at 27±2 °C. Plates having Nutrient Agar (NA) medium without fertilizers were maintained as control. To measure the compatibility, growth of bacterial culture on fertilizer amended medium was compared with control and rated as + + + (Good growth); + + (Moderate growth); + (Poor growth); and – (No growth) (Hanuman and Madhavi, 2018).

Assessment for the ability to produce plant growth promoting hormones and antifungal enzymes by *Trichoderma* isolates, *P. fluorescens,* and *A. chroococcum*

Siderophore production test: Selected compatible *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* were screened for their ability to produce siderophores. Potato Dextrose Agar medium and Nutrient Agar medium separately supplemented with Chrome Azurol Sulfonate solution in the ratio of 10:1 (Medium: CAS solution) were used for fungal and bacterial bio-inoculants, respectively. CAS solution was added in an autoclaved molten medium just prior to pouring in Petri plates.

For the fungal inoculant, *Trichoderma* isolates were cultured on Potato Dextrose Agar medium. Sterilized Petri plates were poured with Potato Dextrose Agar medium supplemented with CAS (Chrome Azurol Sulfonate) solution. Five mm discs of freshly grown culture of *Trichoderma* isolates were inoculated separately on the center of plates followed by incubation for 3 days at 27±1 °C.

For the bacterial inoculants, *P. fluorescens* and *A. chroococcum* were cultured on their respective medium broth. Five mm disc of Whatman's filter paper, dipped in freshly grown bacterial inoculants culture was inoculated on Nutrient Agar medium supplemented with CAS solution followed by incubation for 3 days at 26±1 °C.

After the incubation period the colour change in the medium from blue to orange or formation of yellow to light orange halo surrounding the colony confirmed the production of siderophores.

Phosphate Solubilization test

Selected compatible *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* were screened for their phosphate Solubilization potential. Tests were performed by using Pikovskaya's agar medium containing Tri-calcium phosphate as a phosphate source and Bromocresol purple (BCP) as a pH indicator for acidification. Five mm discs of actively growing *Trichoderma* culture and five mm disc of Whatman's filter, dipped in *P. fluorescens* and *A. chroococcum* culture suspension were placed in the center of separate petri plates poured with the medium. Plates were then incubated at 26±2 °C for 48 h. In the zones of acidification, the medium showing colour changes from purple to yellow confirmed the phosphate solubilization.

Hydrogen cyanide (HCN) production test:

Production of HCN was tested on Tryptic Soy Agar (TSA) supplemented with glycine (4.4 g l^{-1}). After inoculation with fresh cultures of *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*, white filter papers soaked in picric acid solution (0.5% picric acid in 2% (w/v) sodium carbonate in 1 L of distilled water) were placed on the upper lid of Petri plates. The plates were sealed with Parafilm and incubated for seven days at 28±2 °C. After incubation, HCN production was confirmed by the colour change of the filter paper from yellow to light brown or reddish brown which indicated the production of HCN (Meera and Balabaskar, 2012).

Chitinase production test

Chitinase activity was detected using the colloidal chitin supplemented medium. Prepared medium supplemented with colloidal chitin was poured in petri plates followed by inoculation of fresh culture of *Trichoderma* isolates, *P. fluorescens*, and *A. chroococcum* and incubated at 26±2 °C for 48 h. The chitinase activity was identified by the formation of purple-coloured zones on the inoculated medium.

Cellulase production test

Cellulose hydrolysis activity was determined by using Congo red (indicator) supplemented medium, in which phosphoric acid swollen cellulose (PASC) was added as a substrate. *i.e.,* Mandel and Weber's medium. Petri plates were poured with autoclaved Mandel and Weber's medium and inoculated with freshly grown *Trichoderma* isolates, *P. fluorescens*, and *A. chroococcum* culture and incubated for 72 h at 28±2 °C thereafter kept for incubation at 50 °C for 18 h. Cellulolytic potential isolates were screened on the basis of diameter of the hydrolysed zone (light halo area) surrounding the colonies.

IAA production test:

IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR. The isolates were tested for their ability to produce indole acetic acid in pure culture. From the edge of actively growing culture of *Trichoderma* isolates *i.e.,* TRT-2, TRT-9, and TRT-12, were separately inoculated in DF salts minimal media (20 ml) amended with L-tryptophan (IAA precursor) at concentration of 1.02 g/L, in 50 ml capacity conical flasks and incubated for one week at 26 °C. Similarly, *P. fluorescens* and *A. chroococcum* were separately grown for 72 h on the nutrient broth medium amended with L-tryptophan (IAA precursor) at 28 °C on rotary shaker. Stock of L-tryptophan prepared in warm distilled water. After the incubation period the fully grown cultures were centrifuged at 10,000 rpm for 15 min. From the culture supernatant 1 ml aliquot was taken and mixed with salkowski's reagent @ 4 ml and kept for 20 min. at room temperature. The blank was prepared using 1 ml of uninoculated DF salts, minimal media, and nutrient broth medium individually with 4 ml of salkowski's reagent. Pink colour developed, indicating IAA production.

Assessment for nitrogen fixing capacity of *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*

Non-sterilized air-dried soil sieved through 2 mm screens was inoculated with vermicompost based formulation of *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* @ 50g kg^{-1} soil, approximately $x10^8$ cfu g^{-1} of each inoculant. One kg capacity plastic pots were filled with soil mixture. Pots were kept under open condition to facilitate continuous airflow for 90 days. The moisture content of inoculated soil was adjusted up to approximately the optimum level for aerobic microbial activity. The non-inoculated soil mixture (50 g vermicompost in 1 kg soil) was used as a control. The soil moisture was kept at the same level (50% water holding capacity (WHC) by adding sterile distilled water at regular

intervals throughout the incubation period. There were three replicates per treatment. At the end of the incubation period, the content of Nitrogen in each soil was quantified by digestion and subsequent measurement by the Kjeldahl method (Kizilkaya, 2009).

Mass multiplication of fungal and bacterial bio-inoculants

Mass multiplication of *Trichoderma* isolate: Five mm disc of five days old *Trichoderma* culture was transferred to sterilized Potato Dextrose Broth aseptically and incubated at 27±1ºC as a stationary culture for seven days. The fungal mats of *Trichoderma* culture were picked carefully from the culture medium after seven days of incubation. *Trichoderma* mats were ground in a mixer for a few seconds separately and collected in flasks.

Mass multiplication of *P. fluorescens*

Mass multiplication of *P. fluorescens* was done on King's B Broth. A loopful of *P. fluorescens* grown on King's B Agar medium was aseptically transferred to 500 ml sterilized King's B broth and incubated at 27±1ºC for five days then the culture was harvested and thoroughly mixed.

Mass multiplication of *A. chroococcum*

A. chroococcum was grown in Jensen's Broth medium (N-free broth medium) in a one litre flask containing 500 ml broth. A loopful of two days old *A. chroococcum* culture grown on Jensen's agar medium was aseptically transferred to flask and incubated at 27±1 ºC for 5 days then the culture was harvested and thoroughly mixed.

Nutrient (NPK) enrichment of vermicompost

Two water soluble inorganic fertilizers *viz.* Diammonium phosphate and Muriate of potash, with which fungal and bacterial bio-inoculants showed compatibility, were used for nutrient (NPK) enrichment of well-prepared vermicompost. For nutrient enrichment, fertilizers were used in two different quantities i.e., Diammonium phosphate (5% w/w) i.e., 50 g and Muriate of potash (2% w/w) i.e., 20 g, and Diammonium phosphate (10% w/w) i.e., 100 g and Muriate of potash (3% w/w) i.e., 30 g, were dissolved in 100 ml distilled water, separately. The prepared fertilizer solution was well mixed with one kg vermicompost. Essential nutrients (NPK) present in fertilizer solution were absorbed by vermicompost.

Preparation of nutrient enriched bio-formulation(s)

Well grown microbial culture was harvested aseptically and collected in flasks, separately. Culture suspension was prepared by adding distilled water in culture broth to obtain desired number of microbial cells per ml of broth i.e., $x10^8$ cells ml^{-1} for *Trichoderma* culture and $x10^{11}$ cells ml^{-1} for *P. fluorescens* and *A. chroococcum* culture. Culture suspension 75 ml (75 ml of each culture suspension) was taken out for mono inoculant formulation as well as bi- and tri-inoculant formulation and distilled water was added to make up total volume of 250 ml followed by mono-, bi- and tri-inoculants combinations, were mixed with one kilogram of normal vermicompost and nutrient enriched vermicompost, separately. The substrate was manually mixed and the polybags were covered properly and incubated 1 day for multiplication of the consortia. The nutrient enriched bio-formulation(s) was stored for further experiments.

Nutrient enriched bio-formulation(s) were prepared in three batches (Batch-I, Batch-II, and Batch-III) to easily understand the effect of inorganic nutrient amendment in vermicompost based formulations. Where, batch-I consisted of vermicompost enriched with beneficial microorganisms (*Trichoderma* isolate, *P. fluorescens* and *A. chroococcum*), individually and their combinations. Batch-II comprised Diammonium phosphate (5% w/w) and Muriate of potash (2% w/w) supplemented vermicompost enriched with *Trichoderma* isolates, *P. fluorescens*, *A. chroococcum*, individually and in their combinations. Similarly, batch-III comprised Diammonium phosphate (10% w/w) and Muriate of potash (3% w/w) supplemented vermicompost enriched with *Trichoderma* isolates, *P. fluorescens*, *A. chroococcum*, individually and in their combinations.

Assessment for viability of fungal and bacterial bio-inoculants in nutrient enriched bio-formulation(s) at different days after storage under room temperature

The formulated nutrient enriched bio-formulation(s) were stored under room temperature at 15-35 °C and assessed for their shelf-life at different intervals up to 360 days after storage. The bioformulation(s) were air dried in shade and ground to fine powder. Viability was evaluated by using serial dilution plate technique. Up to eight-fold serial dilutions of each sample were prepared in sterilized distilled water.

For the assessment of *Trichoderma* sp., 1ml aliquot from 10^{-3}, 10^{-4}, and 10^{-5} dilution, separately, were transferred in Petri plates previously poured with TSM, then spread uniformly all over the plate. Petri plates were incubated at 28±2 °C and observed everyday up to 5 days. Morphologically different colonies appearing on the plates were counted by using colony counters (cfu g^{-1}).

For the assessment of *P. fluorescens* and *Azotobacter*, 1ml aliquot from 10^{-6}, 10^{-7}, and 10^{-8} dilution, separately, were transferred in Petri plates previously poured with King's B Agar medium and Jensen's medium, respectively, then spread uniformly all over the plate. Petri plates were incubated at 28±2 °C and observed everyday up to 3 days and colonies appearing on the plates were counted by using colony counters (cfu g^{-1}).

RESULTS AND DISCUSSION

Results

In the present investigation, 24 isolates of *Trichoderma* spp. were isolated from different locations of Dhamtari, Rajnandgaon, and Kabirdham District of Chhattisgarh given in **Table 1**.

Table 1: Isolates of *Trichoderma* spp. collected from different location of Chhattisgarh

S. No.	Districts	Isolates code
1.	Dhamtari	TRT-18
2.		TRT-19
3.		TRT-20
4.		TRT-12
5.		TRT-15
6.	Rajnandgaon	TRT-4
7.		TRT-5
8.		TRT-9
9.		TRT-11
10.		TRT-13
11.		TRT-21
12.		TRT-22
13.		TRT-23
14.	Kabirdham	TRT-1
15.		TRT-2
16.		TRT-3
17		TRT-6
18.		TRT-7
19.		TRT-8
20.		TRT-10
21.		TRT-14
22.		TRT-16
23.		TRT-17
24.		TRT-24

Assessment for compatibility of *Trichoderma* isolates with *P. fluorescens* and *A. chroococcum*

Under *in vitro* by plate method: On the basis of results obtained from the experiments of *in-vitro* compatibility presented in **Table 2** and **Figure 1** showing that 24 *Trichoderma* isolates were inoculated with *P. fluorescens* and *A. chroococcum*, that of 12 different *Trichoderma* isolates, designated as TRT-1, TRT-3, TRT-4, TRT-5, TRT-6, TRT-7, TRT-8, TRT-10, TRT-11, TRT-13, TRT-14, and TRT-16 were found to be compatible with *A. chroococcum* when grown together and these all were showing incompatible result with *P. fluorescens*, on other hand of this, TRT-20 showed compatible growth with *P. fluorescens* and incompatible with *A. chroococcum.* Eight *Trichoderma* isolates named TRT-15, TRT-17, TRT-18, TRT-19, TRT-21, TRT-22, TRT-23, and TRT-24 were potentially suppressed by both the bacterial inoculants that lead to incompatible relation with them.

Table 2: Assessment for compatibility of *Trichoderma* isolates with *Pseudomonas fluorescens* and *Azotobacter chroococcum* under *in vitro* by plate method.

S. No.	Isolates	*P. fluorescens*	*A. chroococcum*
1	*P. fluorescens*	+	+
2	TRT-1	-	+
3	TRT-2	+	+
4	TRT-3	-	+
5	TRT-4	-	+
6	TRT-5	-	+
7	TRT-6	-	+
8	TRT-7	-	+
9	TRT-8	-	+
10	TRT-9	+	+
11	TRT-10	-	+
12	TRT-11	-	+
13	TRT-12	+	+
14	TRT-13	-	+
15	TRT-14	-	+
16	TRT-15	-	-
17	TRT-16	-	+
18	TRT-17	-	-
19	TRT-18	-	-
20	TRT-19	-	-
21	TRT-20	+	-
22	TRT-21	-	-
23	TRT-22	-	-
24	TRT-23	-	-
25	TRT-24	-	-

(-) = Zone of inhibition present (Incompatible), (+) = Zone of inhibition absent (Compatible)

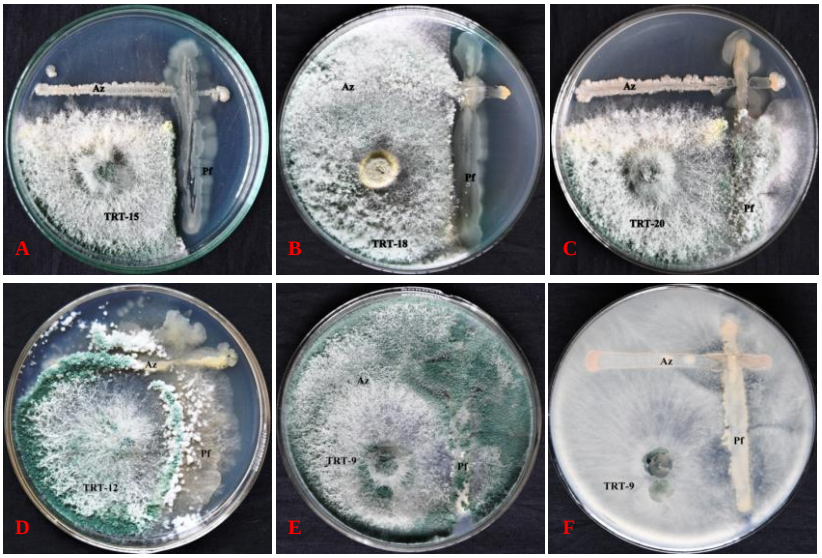


Figure 1: Assessment for compatibility of *Trichoderma* isolates with *P. fluorescens* and *A. chroococcum.* A, Incompatible with both *Azotobacter* sp. and *P. fluorescens*; B, Compatible with *Azotobacter* sp. but incompatible with *P. fluorescens*; C, Incompatible with *Azotobacter* sp. but compatible with *P. fluorescens*; D, Compatible with both *Azotobacter* sp. and *P. fluorescens* but *Trichoderma* growth slow down after coming contact with bacterial inoculants; E, Compatible with both *Azotobacter* sp. and *P. fluorescens*; F, back view of (E).

Among all the 24 isolates, only three *i.e.*, TRT-2, TRT-9 and TRT-12 gave good compatibility with both bacterial inoculants with potential to grow together. They were compatible with each other and they could be used together as a consortium for further studies.

Under *in vivo* by soil method

Based on *in vitro* evaluation, three compatible *Trichoderma* isolates (TRT-2, TRT-9, and TRT-12) with *P. fluorescens* and *A. chroococcum* were selected for further evaluation of their compatibility in soil under pot conditions. All the three compatible *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* were inoculated in non-sterilized soil individually as well as in combination.

Data presented in **Table 3** showed that isolate TRT-2 increased the population up to 21.7 x 10^4 cfu g^{-1} and 24.3 x 10^4 cfu g^{-1} soil in its individual and in mixed consortia inoculation, respectively. Similarly, population seen in isolate TRT-9 *i.e.*, 28.2 x 10^4 cfu g^{-1} and 30.1 x 10^4 cfu g^{-1} soil in its individual and in mixed consortia inoculation, respectively and population of isolate TRT-12 was recorded 27.9 x 10^4 cfu g^{-1} and 31.8 x 10^4 cfu g^{-1} in its individual and in mixed consortia, respectively, which was approximately ten times higher than population in pre-inoculated soil (21.4 x 10^3 cfu g^{-1} soil).

Table 3: Assessment for compatibility of *Trichoderma* isolates with *P. fluorescens* and *A. chroococcum* under *in vivo* by soil method

Treatments	Detail	cfu g^{-1} soil		
		***Trichoderma* Isolate**	***P. fluorescens***	***A. chroococcum***
T1	Pre-inoculated soil	21.4 x 10^3	32.5 x 10^5	8.6 x 10^5
T2	Soil + TRT-2	21.7 x 10^4	-	-
T3	Soil + TRT-9	28.2 x 10^4	-	-
T4	Soil + TRT-12	27.9 x 10^4	-	-
T5	Soil + Pf	-	23.6 x 10^7	-
T6	Soil + Az	-	-	22.3 x 10^7
T7	Soil + TRT-2 + Pf + Az	24.3 x 10^4	30.1 x 10^7	18.4 x 10^7
T8	Soil + TRT-9 + Pf + Az	30.1 x 10^4	37.6 x 10^7	15.3 x 10^7
T9	Soil + TRT-12 + Pf + Az	31.8 x 10^4	29.4 x 10^7	17.1 x 10^7

Pseudomonas fluorescens population dynamics was recorded up to 37.1 x 10^7, 30.6 x 10^7 and 29.4 x 10^7 cfu g^{-1} soil, in soil inoculated with microbial consortia i.e., Soil + TRT-2 + Pf + Az (T7), Soil + TRT-9 + Pf + Az (T8) and Soil + TRT-12 + Pf + Az (T9), respectively. *Pseudomonas fluorescens* increased their population in consortia similar to soil inoculated with *P. fluorescens* individually (23.6 x 10^7 cfu g^{-1} soil). Whereas, population in pre-inoculated soil was 35.5 x 10^5 cfu g^{-1} soil.

Azotobacter chroococcum population dynamics was recorded up to 18.4 x 10^7, 15.3 x 10^7 and 17.1 x 10^7 cfu g^{-1} soil, in soil inoculated with microbial consortia *i.e.*, Soil + TRT-2 + Pf + Az (T7), Soil + TRT-9 + Pf + Az (T8) and Soil + TRT-12 + Pf + Az (T9), respectively. *Azotobacter chroococcum* increased its population in consortia as similar to soil inoculated with *A. chroococcum* individually (22.3 x 10^7 cfu g^{-1} soil), whereas, population in pre-inoculated the microbial analysis of soil after the experiment has shown improvement in microbial inoculants (alone and in their consortia) in soil, due to synergistic relation among microbial inoculants compared to its initial microbial population. These results clearly show a synergistic association among biocontrol agents (*Trichoderma* isolates and *P. fluorescens*) and biofertilizer (*A. chroococcum*) in the soil.

Assessment for sensitivity of *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* with inorganic fertilizers under *in vitro*

Sensitivity of *Trichoderma* isolates: The data regarding percent growth inhibition of selected *Trichoderma* isolates are shown in **Table 4** and **Figure 2**, **3,** and **4**. Results revealed that all three *Trichoderma* isolates were significantly inhibited by urea and complex fertilizer (28:28:00) at all the concentrations. However, SSP did not inhibit the growth at any concentration.

Table 4: Assessment for sensitivity of *Trichoderma* isolates with inorganic fertilizers under *in vitro*

Treatments	**Percent inhibition**														
Trichoderma isolate	**TRT-2**					**TRT-9**					**TRT-12**				
Concentration	**1 %**	**2 %**	**3 %**	**4 %**	**5 %**	**1 %**	**2 %**	**3 %**	**4 %**	**5 %**	**1 %**	**2 %**	**3 %**	**4 %**	**5 %**
Diammonium Phosphate	0.12	0.62	16.05	20.62	42.10	0.00	0.00	0.20	0.20	0.20	0.37	1.73	5.59	14.26	19.11
Muriate of Potash	0.00	0.00	0.00	37.24	68.04	0.00	0.00	0.00	0.00	22.68	0.00	0.12	2.72	19.75	59.14
Single Superphosphate	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.12	0.37	0.00	0.00	0.00	0.00	5.31
Urea	0.07	32.15	82.00	86.40	100	7.25	17.78	60.46	100	100	17.00	31.98	93.21	100.00	100.00
Complex fertilizer (28:28:00)	0.00	6.63	11.68	25.85	34.94	22.00	51.53	55.74	62.01	91.11	75.93	85.56	90.99	94.32	100.00
Control			0.00					0.00					0.00		
SE (m)	**0.85**	**0.93**	**0.50**	**0.55**	**0.38**	**0.19**	**0.49**	**0.49**	**0.43**	**0.43**	**0.54**	**0.61**	**0.29**	**0.25**	**0.27**
CD	**2.64**	**2.90**	**1.56**	**1.70**	**1.18**	**1.17**	**2.98**	**2.99**	**2.66**	**2.61**	**1.68**	**1.91**	**0.89**	**0.79**	**0.85**

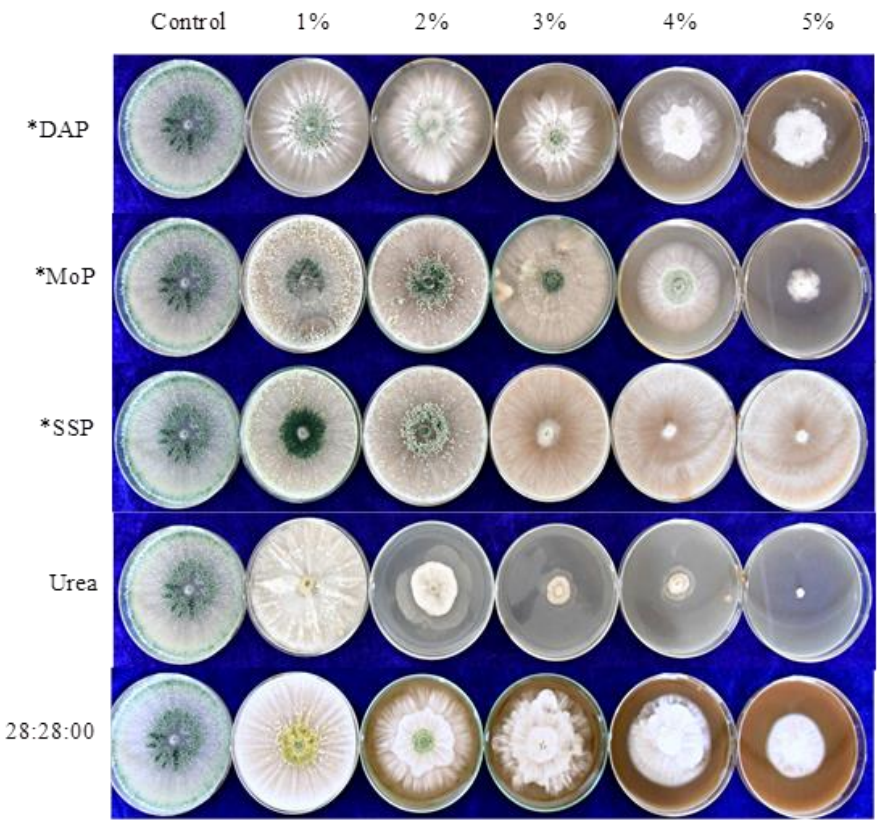


Figure 2: Sensitivity of *Trichoderma* isolate (TRT-2) with inorganic fertilizers.

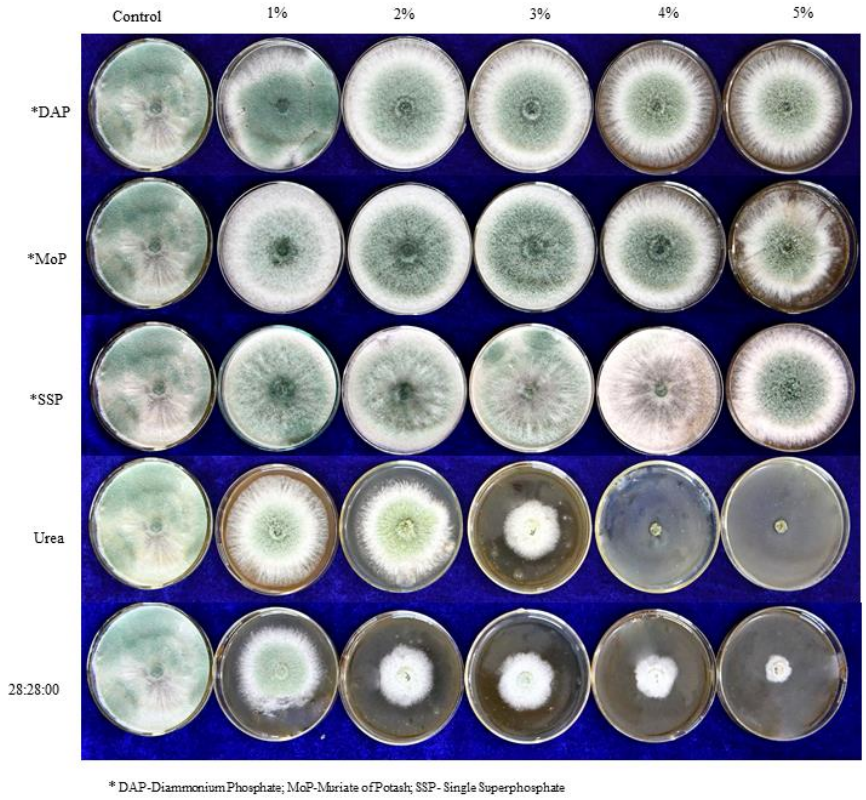


Figure 3: Sensitivity of *Trichoderma* isolate (TRT-9) with inorganic fertilizers.

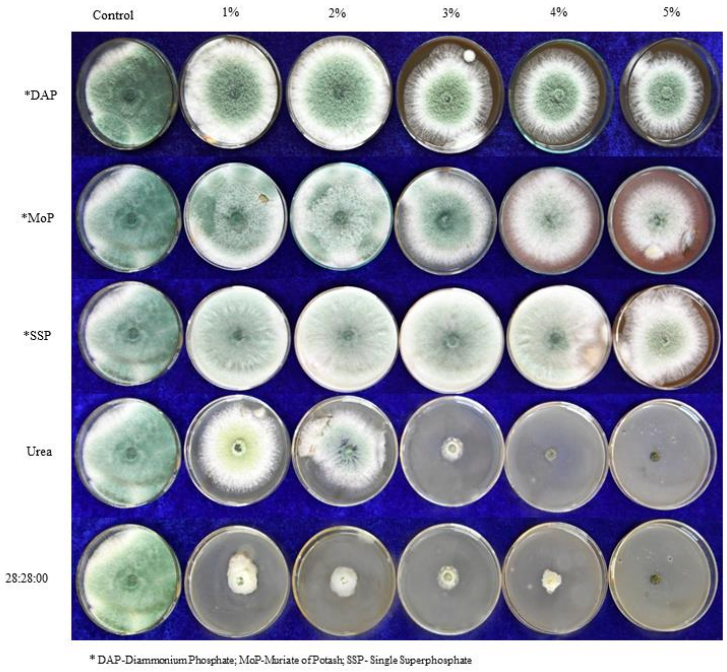


Figure 4: Sensitivity of *Trichoderma* isolate (TRT-12) with inorganic fertilizers.

With DAP, minimum growth inhibition of TRT-2 was recorded at all the five concentrations i.e., 1%, 2%, 3%, 4%, and 5%. Inhibition percent ranged from 0.00-42.10%, 0.0-0.20%, and 0.37-19.11% for TRT-2, TRT-9, and TRT-12, respectively. Successive increase in concentration up to 5% of fertilizer could not affect the growth of isolate TRT-9 that leads to resistant, and for isolate TRT-12 this was little for up to 2% while there was no tolerance shown by isolate TRT-2 therefore, inhibition was shown from 1% to 5% level of concentration of fertilizer application**.**

With Muriate of Potash, mycelial growth inhibition of *Trichoderma* isolates ranged from 0.00 to 68.04%, 0.0 to 22.68%, and 0.0 to 59.14% for TRT-2, TRT-9, and TRT-12, respectively. TRT-2 and TRT-9 recorded high resistivity up to 3% of MoP concentration, by showing no growth inhibition on agar plate, while on other hand isolate TRT-12 showed very little tolerance (2.72%) at 3% of MoP concentration.

With Single superphosphate, mycelial growth inhibition percent ranged from 0.00% to 0.04%, 0.00%, to 0.37%, and 0.00% to 5.31% for TRT-2, TRT-9, and TRT-12, respectively. Data revealed that concentration of SSP could influence the mycelium growth of *Trichoderma* isolates. TRT-12 showed 5.31% inhibition at 5% concentration.

With Urea, inhibition percent ranged from 0.07% to 100%, 7.25 to 100% and 17.00 to 100% for TRT-2, TRT-9, and TRT-12 respectively. Any concentration of urea significantly inhibited the growth of *Trichoderma* isolates. Inhibition percent of TRT-12 was significantly higher at all the concentrations. Successive increase in urea concentration caused prominent cessation of growth.

With 28:28:00 complex fertilizer, inhibition percent of TRT-2 ranged from 0.00 to 34.94%. TRT-9 was showing high growth inhibition, inhibition ranged from 22 to 91.11%. Growth inhibition of TRT-12 was significantly highest in all the concentration as compared to other isolates. Inhibition percent ranged from 75.93% to 100%.

Sensitivity of *P. fluorescens*

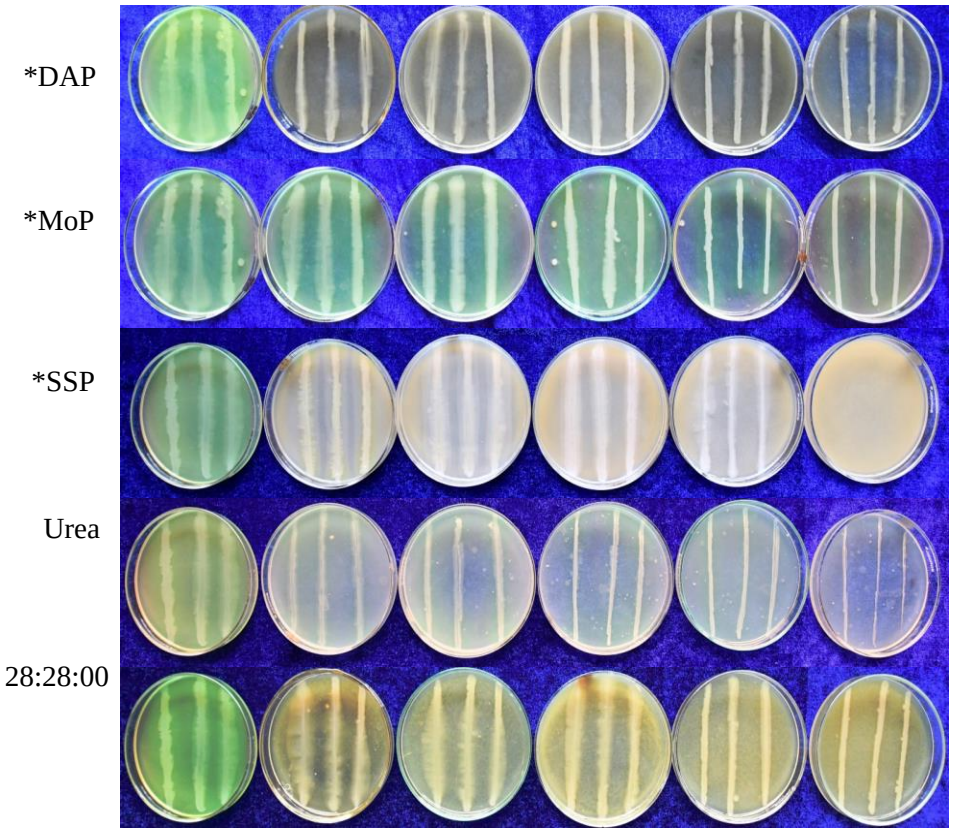
The data regarding compatibility of *P. fluorescens* are shown in **Table 5** and **Figure 5**. Results revealed that *P. fluorescens* showing good growth on medium supplemented with DAP up to 4% concentration, at 5% concentration moderate growth was noticed. MoP supplemented medium supported good growth in 1% and 2% concentration, moderate growth in 3%, poor growth was recorded in 4% and 5% concentration. SSP amended medium gave good growth on 1%, 2% and 3% concentration however 4% showed poor growth and no growth recorded on 5%. Urea gave good growth on 1% and 2%, moderate growth on

3% and 4% but 5% gave poor growth. 28:28:00 fertilizer gave good growth on 1% and 2%, moderate on 3% and poor growth recorded in 4% and 5%.

Table 5: Assessment for sensitivity of *P. fluorescens* with inorganic fertilizers under *in vitro*

Treatments	Growth of *P. fluorescens*				
	1 %	**2 %**	**3 %**	**4 %**	**5 %**
Diammonium Phosphate	+++	+++	+++	+++	++
Muriate of Potash	+++	+++	++	+	+
Single Superphosphate	+++	+++	+++	+	-
Urea	+++	+++	++	++	+
Complex fertilizer (28:28:00)	+++	+++	++	+	+
Control			+++		

(+++) = Good growth, (++) = Moderate growth, (+) = Poor growth, (-) = No growth



*DAP-Diammonium Phosphate; MoP-Muriate of Potash; SSP- Single Superphosphate

Figure 5: Sensitivity of *P. fluorescens* with inorganic fertilizers.

Sensitivity of *A. chroococcum*

The data regarding compatibility of *A. chroococcum* are shown in **Table 6** and **Figure 6**. Results revealed that *A. chroococcum* showed good growth on DAP amended medium in all the concentrations, MoP medium provided good growth in 1%, 2%, 3%, and 4% concentrations, and 5% concentration showed moderate growth. SSP amended medium gave good growth on lower concentration 1%, moderate on 2% and good growth on 3% concentration however 4% and 5% recorded no growth. Urea gave moderate growth in 1%, 2% and 3% concentrations. Poor growth was recorded in 4% and no growth was recorded in 5%. 28:28:00 complex fertilizer gave good growth on 1%, 2%, 3%, and 4% but moderate growth on 5%.

Table 6: Assessment for sensitivity of *A. chroococcum* with inorganic fertilizers under *in vitro*

Treatments	Growth of *A. chroococcum*				
	1 %	2 %	3 %	4 %	5 %
Diammonium Phosphate	+++	+++	+++	+++	+++
Muriate of Potash	+++	+++	+++	+++	++
Single Superphosphate	+++	++	+	-	-
Urea	++	++	++	+	-
Complex fertilizer (28:28:00)	+++	+++	+++	+++	++
Control			+++		

(+++) = Good growth, (++) = Moderate growth, (+) = Poor growth, (-) = No growth

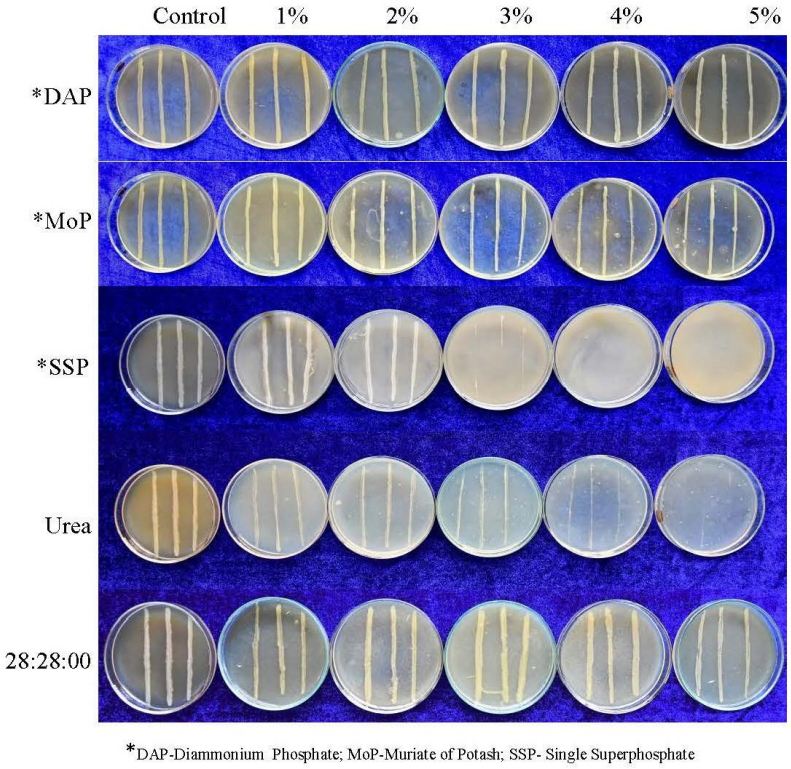


Figure 6: Sensitivity of *Azotobacter* sp. with inorganic fertilizers.

Assessment for the ability to produce plant growth promoting hormones and antifungal enzymes by *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*

Selected compatible *Trichoderma* isolates (TRT-2, TRT-9, and TRT-12), *P. fluorescens* and *A. chroococcum* were evaluated for their ability to produce essential hormones and enzymes which are responsible for plant growth promotion and antifungal properties.

Indole Acetic Acid production test

Three *Trichoderma* isolates (TRT-2, TRT-9 and TRT-12), one *P. fluorescens* and one *A. chroococcum* were used in the present investigation to evaluate the IAA production potential by using L-tryptophan as precursor. The isolates TRT-2, TRT-9 and TRT-12 were identified as the producer of indole acetic acid along with *P. fluorescens* and *A. chroococcum* thus indicating their ability to synthesize IAA from L-tryptophan as precursor *in vitro* (**Table 7**).

Table 7: Assessment for the ability to produce plant growth promoting hormones and antifungal enzymes by *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*

Treatments*	IAA	PO_4 Solubilisation	Siderophore	Chitinase	Cellulase	HCN
TRT-2	+	+	+	+	+	-
TRT-9	+	+	+	+	+	+
TRT-12	+	+	+	+	+	+
Pf	+	+	+	+	+	+
Az	+	+	+	+	-	+

(+) = Present, (-) = Absent

Siderophore production test

In the present investigation regarding siderophore production ability of selected fungal and bacterial bio-inoculants clearly indicated that all the three *Trichoderma* isolates (TRT-2, TRT-9, and TRT-12) have potential to produce siderophore in iron deficient condition. *P. fluorescens* and *Azotobacter* also confirmed their potential to produce siderophore by the formation of yellow hollow surrounding the colony of bacteria (**Table 7**)

Cellulase production test

Information given in **Table 7** regarding cellulase activity clearly indicated that all three *Trichoderma* isolates and *P. fluorescens* potentially produced cellulase. *Azotobacter chroococcum* was not recorded in cellulose hydrolization in the growing medium.

Chitinase production test

The chitinase production ability was clearly indicated in **Table 7** that all three *Trichoderma* isolates (TRT-2, TRT-9, and TRT-12) have potential to produce chitinase enzyme. *Pseudomonas fluorescens* and *A. chroococcum* are also able to produce chitinase.

Phosphate Solubilization test

In vitro phosphate solubilization efficacy of selected compatible *Trichoderma* isolates (TRT-2, TRT-9, and TRT-12), *P. fluorescens* and *A. chroococcum* were evaluated by using pikovskaya medium supplemented with tri-calcium phosphate. Information given in **Table 7** regarding Phosphate solubilization ability was clearly indicated that all three *Trichoderma* isolates showed phosphate solubilization potential by using tri-calcium phosphate as phosphate source. Both bacterial inoculants i.e., *P. fluorescens* and *A. chroococcum* also showed positive results.

Hydrogen cyanide (HCN) production test

In the present investigation three *Trichoderma* isolates (TRT-2, TRT-9, and TRT-12), one *P. fluorescens* and one *A. chroococcum* were evaluated for their ability to produce hydrogen cyanide using glycine as its precursor molecule. Information given in **Table 7** regarding Hydrogen cyanide (HCN) production ability clearly indicated that among all three *Trichoderma* isolates only two isolates (TRT-9 and TRT-12) have potential to produce Hydrogen cyanide (HCN) remaining one isolate (TRT-2) did not show positive result of hydrogen cyanide production. *Pseudomonas fluorescens* and *A. chroococcum* both are potentially able to produce Hydrogen cyanide (HCN).

Assessment for nitrogen fixing capacity of *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*

Data presented in **Table 8** showed that all the three *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* increased the nitrogen concentration in inoculated soil as compared to initial nitrogen concentration. Maximum nitrogen concentration was recorded in soil inoculated with *A. chroococcum* and 23.30 kg ha-1 nitrogen increased over initial concentration, whereas minimum recorded 1.20 kg ha-1 in soil inoculated with *P. fluorescens.*

Preparation of nutrient enriched bio-formulation(s)

Previously conducted experiments regarding clearly indicated that *P. fluorescens* and *A. chroococcum* are compatible with each other, among 24 isolates of *Trichoderma*, only three isolates were found to be compatible with *P. fluorescens* and *A. chroococcum*. However, isolate TRT-2 and TRT-12 recorded slow growth rate with *P. fluorescens*. *Trichoderma* isolate TRT-9 showed positive compatibility without losing their growing potential.

Table 8: Assessment for nitrogen fixing capacity of *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*

Treatments	Nitrogen (kg ha^{-1})		
	Initial N_2	N_2 after incubation	N_2 increase over initial
TRT-2		195.43	3.20
TRT-9		195.01	2.78
TRT-12	192.23	195.23	3.00
P. fluorescens		193.43	1.20
A. chroococcum		215.53	23.30

All three *Trichoderma* isolates recorded ability to produce all essential hormones and enzymes i.e., Siderophores, IAA, HCN, cellulase, chitinase, phosphate solubilisation and nitrogen fixing ability, which are responsible for plant growth promotion and plant disease control (except TRT-2 unable to produce HCN). Both bacterial inoculants are also able to produce these hormones and enzymes (except *A. chroococcum* able to produce cellulase enzymes).

Among three *Trichoderma* isolates, only isolate TRT-9 showed tolerance to inorganic fertilizers that provide NPK i.e., DAP and MoP. Both bacterial inoculants also showed positive compatibility with DAP and MoP.

Based on the above-mentioned findings, the *Trichoderma* isolate (TRT-9), *P. fluorescens* and *A. chroococcum* along with inorganic fertilizers i.e., DAP and MoP, were used for the preparation of nutrient enriched bio-formulation(s).

Nutrient enriched bio-formulation(s) were prepared in three batches (Batch-I, Batch-II, and Batch-III) to easily understand the effect of inorganic nutrient amendment in vermicompost based formulations.

Assessment for viability of fungal and bacterial bio-inoculants in nutrient enriched bio-formulation(s) at different days after storage under room temperature

Viability of *Trichoderma* isolate: An experiment was conducted to assess the shelf life with respect to viability of *Trichoderma* isolate in nutrient enriched bio-formulation(s). The data showed that the viability of *Trichoderma* in terms of cfu g^{-1} was recorded up to 360 days after storage (**Table 9**). The population of *Trichoderma* increased prominently up to initial 60 days and reached its maximum population in all nutrient enriched bio-formulation(s) which comprise *Trichoderma*, alone and in combination with other bio-inoculants, thereafter the growth of *Trichoderma* decreased gradually up to 360 days after storage.

When vermicompost and vermicompost supplemented with DAP and MoP at different concentrations were enriched with *Trichoderma*, the spore load of *Trichoderma* recorded was 101.33 x 10^5 cfu g^{-1} of formulation in T1 (VC + TRT), 93.33 x 10^5 cfu g^{-1} of formulation in T8 (VC + DAP (5% w/w) + MoP (2% w/w) + TRT) and 88.33 x 10^5 cfu g^{-1} of formulation in T15 (VC + DAP (5% w/w) + MoP (2% w/w) + TRT) 60 days after storage.

Similarly, when vermicompost and Vermicompost supplemented with DAP and MoP at different concentrations were enriched with all three bio-inoculants viz. *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*, the spore load of *Trichoderma* recorded was 86.00 x 10^5 cfu g^{-1} of formulation in T7 (VC + TRT + Pf + Az), 94.00 x 10^5 cfu g^{-1} of formulation in T14 (VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf + Az) and 92.33 x 10^5 cfu g^{-1} of formulation in T21 (VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf + Az) 60 days after storage.

Table 9: Assessment for viability of *Trichoderma* isolate in different nutrient enriched bio-formulation(s) at different days after storage under room temperature

Treatments		Details	x10^4 cfu g^{-1}									
			0 day	7 days	15 days	30 days	60 days	90 days	120 days	150 days	180 days	360 days
T1	Batch-I	VC + TRT	45.00	57.33	76.33	89.67	101.33	74.00	34.33	23.00	12.00	5.04
T2		VC + Pf	-	-	-	-	-	-	-	-	-	-
T3		VC + Az	-	-	-	-	-	-	-	-	-	-
T4		VC + TRT + Pf	43.00	51.33	68.33	77.67	90.00	58.33	33.67	23.33	14.33	4.66
T5		VC + TRT + Az	42.67	49.67	62.00	78.67	87.33	65.00	36.00	22.67	14.00	4.12
T6		VC + Pf + Az	-	-	-	-	-	-	-	-	-	-
T7		VC + TRT + Pf + Az	41.00	49.33	62.33	76.00	86.00	66.67	39.00	23.33	15.00	4.02
T8		VC + DAP (5% w/w) + MoP (2% w/w) + TRT	44.33	54.67	66.67	83.33	93.33	69.00	32.33	24.67	13.33	3.77
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf	-	-	-	-	-	-	-	-	-	-
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az	-	-	-	-	-	-	-	-	-	-
T11	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf	41.00	52.67	60.33	75.33	94.00	70.67	31.33	23.00	12.33	4.31
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Az	40.67	49.67	65.00	85.00	95.33	71.33	32.00	21.33	11.33	3.24
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az	-	-	-	-	-	-	-	-	-	-
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf + Az	42.00	58.33	69.33	89.67	94.00	66.67	33.67	25.00	13.33	3.98
T15		VC + DAP (10% w/w) + MoP (3% w/w) + TRT	30.67	38.33	66.33	76.00	88.33	64.00	32.00	18.33	11.67	1.67
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf	-	-	-	-	-	-	-	-	-	-
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az	-	-	-	-	-	-	-	-	-	-
T18	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf	38.67	46.00	63.33	73.67	89.00	59.67	32.33	19.00	11.33	2.00
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Az	43.33	56.67	64.00	75.33	87.00	58.33	31.00	18.00	10.33	0.38
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az	-	-	-	-	-	-	-	-	-	-
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf + Az	51.67	64.33	72.33	86.33	92.33	50.33	29.33	21.33	13.67	0.82

*VC=Vermicompost; TRT=*Trichoderma* isolate*;* Pf=*P. fluorescens;* Az=*A.;* DAP- Diammonium Phosphate; MoP- Muriate of Potash

Dose-I: Formulation(s) @ 25gm per plant, or Inorganic fertilizers @ 75% RDF (150:100:75 N:P:K kg ha^{-1}); **Dose-II:** Formulation(s) @ 50gm per plant, or Inorganic fertilizers @ 100% RDF (150:100:75 N:P:K kg ha^{-1}); **Dose-III:** Formulation(s) @ 75gm per plant, or Inorganic fertilizers @ 125% RDF (150:100:75 N:P:K kg ha^{-1})

Viability of *P. fluorescens*

The shelf life was assessed with respect to viability of *P. fluorescens* in nutrient enriched bio-formulation(s). Viability of *P. fluorescens* in terms of cfu g^{-1} was recorded up to 360 days after storage. The data presented in **Table 10** revealed that the population of *P. fluorescens* increased prominently up to initial 60 days and reached its maximum population in all nutrient enriched bio-formulation(s) which comprised *P. fluorescens*, alone and in combination with other bio-inoculants, thereafter the growth of *P. fluorescens* decreased gradually up to 360 days after storage.

When vermicompost and vermicompost supplemented with DAP and MoP at different concentrations were enriched with *P. fluorescens*, the population of *P. fluorescens* recorded was 118.33 x 10^8 cfu g^{-1} of formulation in T2 (VC + Pf), 105.33 x 10^8 cfu g^{-1} of formulation in T9 (VC + DAP (5% w/w) + MoP (2% w/w) + Pf) and 102.33 x 10^8 cfu g^{-1} of formulation in T16 (VC + DAP (10% w/w) + MoP (3% w/w) + Pf) at 60 days after storage.

Similarly, when vermicompost and vermicompost supplemented with DAP and MoP at different concentrations were enriched with all three bio-inoculants viz. *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*, the population of *P. fluorescens* recorded was 119.00 x 10^8 cfu g^{-1} of formulation in T7 (VC + TRT + Pf + Az), 109.00 x 10^8 cfu g^{-1} of formulation in T14 (VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf + Az) and 107.00 x 10^8 cfu g^{-1} of formulation in T21 (VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf + Az) at 60 days after storage.

Table 10: Assessment for viability of *P. fluorescens* in different nutrient enriched bio-formulation(s) at different days after storage under room temperature

Treatments		Details*	x10^8 cfu g^{-1}									
			0 day	7 days	15 days	30 days	60 days	90 days	120 days	150 days	180 days	360 days
T1		VC + TRT	-	-	-	-	-	-	-	-	-	-
T2		VC + Pf	44.33	56.67	91.33	117.33	118.33	90.00	62.67	48.00	26.33	7.92
T3		VC + Az	-	-	-	-	-	-	-	-	-	-
T4	Batch-I	VC + TRT + Pf	48.00	71.00	78.33	96.67	113.67	71.67	51.33	44.33	21.00	5.81
T5		VC + TRT + Az	-	-	-	-	-	-	-	-	-	-
T6		VC + Pf + Az	41.00	52.67	91.00	103.33	114.00	78.67	48.67	38.33	27.67	6.08
T7		VC + TRT + Pf + Az	49.00	55.67	92.00	109.00	119.00	92.00	64.00	50.00	29.00	6.00
T8		VC + DAP (5% w/w) + MoP (2% w/w) + TRT	-	-	-	-	-	-	-	-	-	-
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf	36.33	48.00	82.67	90.33	105.33	64.00	38.00	27.33	21.33	5.22
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az	-	-	-	-	-	-	-	-	-	-
T11	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf	40.67	51.67	80.67	87.00	98.33	54.33	44.67	36.67	27.33	4.99
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Az	-	-	-	-	-	-	-	-	-	-
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az	38.00	52.00	80.68	86.00	97.00	52.00	37.33	35.33	26.33	5.89
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf + Az	42.67	53.67	88.67	95.00	109.00	66.00	48.00	37.00	28.00	6.21
T15		VC + DAP (10% w/w) + MoP (3% w/w) + TRT	-	-	-	-	-	-	-	-	-	-
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf	41.00	51.67	78.33	89.33	102.33	60.67	39.00	32.67	23.67	6.34
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az	-	-	-	-	-	-	-	-	-	-
T18	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf	35.67	42.33	73.33	85.00	101.00	63.67	38.67	30.33	18.67	5.91
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Az	-	-	-	-	-	-	-	-	-	-
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az	34.00	50.00	81.00	87.00	96.00	52.67	42.67	26.33	21.67	4.81
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf + Az	32.33	59.00	65.00	88.00	107.00	84.00	44.00	35.00	26.00	5.06

*VC=Vermicompost; TRT=*Trichoderma* isolate*;* Pf=*P. fluorescens;* Az=*A.;* DAP- Diammonium Phosphate; MoP- Muriate of Potash

Dose-I: Formulation(s) @ 25gm per plant, or Inorganic fertilizers @ 75% RDF (150:100:75 N:P:K kg ha^{-1}); **Dose-II:** Formulation(s) @ 50gm per plant, or Inorganic fertilizers @ 100% RDF (150:100:75 N:P:K kg ha^{-1}); **Dose-III:** Formulation(s) @ 75gm per plant, or Inorganic fertilizers @ 125% RDF (150:100:75 N:P:K kg ha^{-1})

Viability of *A. chroococcum*

The viability of *A. chroococcum* in terms of cfu g^{-1} was assessed and found that the *A. chroococcum remained* viable up to 360 days after storage. The data presented in **Table 11** revealed that the population of *A. chroococcum* increased prominently up to initial 60 days and reached its maximum population in all nutrient enriched bio-formulation(s) which comprised *A. chroococcum* alone and in combination with other bio-inoculants, thereafter the growth of *A. chroococcum* decreased gradually up to 360 days after storage.

When vermicompost and vermicompost supplemented with DAP and MoP at different concentrations were enriched with *A. chroococcum* The population of *A. chroococcum* recorded was 110.33 x 10^8 cfu g^{-1} of formulation in T3 (VC + Az), 105.67 x 10^8 cfu g^{-1} of formulation in T10 (VC + *DAP (5% w/w)* + MoP (2% w/w) + Az) and 107.67 x 10^8 cfu g^{-1} of formulation in T17 (VC + DAP (10% w/w) + MoP (3% w/w) + Az) at 60 days after storage.

Similarly, when vermicompost and vermicompost supplemented with DAP and MoP at different concentrations enriched with all three bio-inoculants viz. *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum*, the population of *A. chroococcum* recorded was 119.33 x 10^8 cfu g^{-1} of formulation in T7 (VC + TRT + Pf + Az), 106.33 x 10^8 cfu g^{-1} of formulation in T14 (VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf + Az) and 108.67 x 10^8 cfu g^{-1} of formulation in T21 (VC +

DAP (10% w/w) + MoP (3% w/w) + TRT + Pf + Az) at 60 days after storage.

Table 11: Assessment for viability of *A. chroococcum* in different nutrient enriched bio-formulation(s) at different days after storage under room temperature

Treatments		Details	x10^8 cfu g^{-1}									
			0 day	7 days	15 days	30 days	60 days	90 days	120 days	150 days	180 days	360 days
T1	Batch-I	VC + TRT	-	-	-	-	-	-	-	-	-	-
T2		VC + Pf	-	-	-	-	-	-	-	-	-	-
T3		VC + Az	46.00	60.00	84.00	100.00	110.33	72.00	47.00	36.67	25.33	2.31
T4		VC + TRT + Pf	-	-	-	-	-	-	-	-	-	-
T5		VC + TRT + Az	48.33	73.33	80.33	97.67	114.00	73.00	51.00	43.33	20.67	1.37
T6		VC + Pf + Az	45.33	54.67	93.00	103.67	115.00	80.00	48.33	38.33	29.00	2.89
T7		VC + TRT + Pf + Az	41.00	57.33	93.33	107.67	119.33	91.33	62.33	46.33	29.33	1.79
T8	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT	-	-	-	-	-	-	-	-	-	-
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf	-	-	-	-	-	-	-	-	-	-
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az	38.67	50.00	84.67	96.33	105.67	65.33	37.67	25.67	20.33	0.89
T11		VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf	-	-	-	-	-	-	-	-	-	-
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Az	42.33	55.00	82.67	88.00	98.67	55.67	44.33	35.67	25.67	1.49
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az	39.67	55.33	88.00	81.67	97.33	53.33	37.00	34.33	24.67	1.98
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT + Pf + Az	43.67	51.00	86.67	98.00	106.33	65.00	41.67	24.67	25.67	0.98
T15	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT	-	-	-	-	-	-	-	-	-	-
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf	-	-	-	-	-	-	-	-	-	-
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az	41.33	55.00	79.33	90.33	107.67	62.00	38.67	31.33	23.67	0.73
T18		VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf	-	-	-	-	-	-	-	-	-	-
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Az	40.00	45.67	75.33	86.00	101.33	65.00	38.33	29.33	18.67	1.01
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az	39.33	62.33	81.00	88.00	95.33	54.00	42.33	27.33	21.33	0.33
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT + Pf + Az	42.00	59.00	79.00	92.00	108.67	69.00	35.33	32.00	24.00	0.59

*VC=Vermicompost; TRT=*Trichoderma* isolate*;* Pf=*P. fluorescens;* Az=*A.;* DAP- Diammonium Phosphate; MoP- Muriate of Potash

Dose-I: Formulation(s) @ 25gm per plant, or Inorganic fertilizers @ 75% RDF (150:100:75 N:P:K kg ha^{-1}); **Dose-II:** Formulation(s) @ 50gm per plant, or Inorganic fertilizers @ 100% RDF (150:100:75 N:P:K kg ha^{-1}); **Dose-III:** Formulation(s) @ 75gm per plant, or Inorganic fertilizers @ 125% RDF (150:100:75 N:P:K kg ha^{-1})

DISCUSSION

Rising global population, posing a significant challenge in ensuring food security, as soil nutrients and fertility are limited and decreasing with time. To fulfil the expanding population's demand for food, agricultural production must be raised. The soil is a complex and enriched medium that aids in plant production. However, the soil environment is affected by various factors that impede plant growth. Several chemical based products have been used to suppress the effects of these harmful factors and boost plant productivity, but these chemicals are dangerous to the environment. Therefore, there is a growing need to create a greener, safer alternative so that we can provide food for a growing population while also preventing disease and controlling pests. For the purpose of achieving sustainability, microbe-based bio-formulations can be used. A new understanding of the agro-economy has been made possible by the significance of microorganisms in sustainable agriculture, and one of the immediate benefits is a decrease in the usage of agrochemicals like fertilisers and pesticides. A better option for encouraging plant growth and the eradication of disease has developed as a result of the development in our understanding of microbial consortium (bioformulation). Microbial consortiums are functional microbial products made of several advantageous microbial strains in cost-effective carrier materials. The search for efficient bio-inoculants for the control of plant diseases and nutrient management has been stepped up to lessen the reliance on environmentally risky pesticides. However, in order to be successfully incorporated into the production system, bio-inoculants must be compatible with one another and widely used agrochemicals. With such an approach, the concept of disease and nutrient management can be improved by using a variety of beneficial microbes and vermicompost, enriched with lesser amount of inorganic fertilizers to achieve the best possible results. Adding lesser amount of inorganic nutrients (NPK) along with beneficial microbes to vermicompost contains nutrients in available form, increases microbial activity, increases soil aeration, and promotes plant growth. Recently, beneficial soil microbes have been reported to produce some volatile organic compounds, which are beneficial to plants, and the amendment of these microbes with vermicompost to formulate biofertilizers and

biopesticides to increase plant productivity. This was due to the fact that vermicompost was more effective in maintaining a higher level of microorganisms (bio-inoculants) population. The nutrient content was more and its pH was also near neutrality, which made it more suitable for microorganisms. These properties might have offered protection for *Trichoderma* isolates, *P. fluorescens* and *A. chroococcum* to survive during storage. This clearly supports the findings of earlier studies of Debroy and Sharma (2010) where, vermicompost can excellently be used as carrier material for *A. chroococcum* (strain SDSA-I12/2, MTCC 9788) as it provides suitable micro-environment for better survival of the diazotroph strain. This result is in harmony with the findings of Giraddi (1997) that the survival of *Trichoderma viride* was better in vermicompost based formulations, compared to that of lignite-based formulations. This was due to the fact that vermicompost is a rich source of micro and macro nutrients which increased fungal activity very effectively.

REFERENCES

Champawat, R.S. and Sharma, R.S. 2003. Integrated management of nursery diseases in brinjal, chilli, cabbage, and onion. *Journal of Mycology and Plant Pathology*, **33:**290-291; doi: 10.5539/jas.v5n1p17.

Debroy, B. and Sharma, G.D. 2010. Evaluation of carrier-based inoculant of *Azotobacter chroococcum* strain SDSA-I12/2 in improving growth and yield of summer (ahu) rice *cv*. IR-36. *Biofrontiers*, **1:**36-40.

Gerhardson, B. 2002. Biological substitutes for pesticides. *Trends in biotechnology*, **20(8)**:338-343; doi: 10.1016/s0167-7799(02)02021-8.

Giraddi, R.S. 1997. Vermicompost and its role in agriculture, In: Proceedings of course on use of bio-fertilizers training programme for the officers State department of agriculture, Karnataka. Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, pp.50-54.

Hanuman, L.N. and Bindu Madhavi, G. 2018. Compatibility of *Pseudomonas fluorescens* with Pesticides in vitro. *International Journal of Current Microbiology and Applied Sciences*, **7(03)**:3310-3315; doi: 10.20546/ijcmas.2018.703.381.

Jayashree, K., Shanmugam, V., Raguchander, T., *et al*., 2000. Evaluation of *Pseudomonas fluorescens* (pf1) against blackgram and sesame root-rot disease. *Journal of Biological Control*, **14:**55-61; doi: 10.18311/jbc/2000/4163.

Kizilkaya, R. 2009. Nitrogen fixation capacity of *A.* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. *Journal of Environmental Biology*, **30(1):**73-82; doi: 10.1098/rsbl.2021.0612.

Manjula, K., Kishore, G.K., Girish, A.G. *et. al*., 2004. Combined Application of *Pseudomonas fluorescens* and *Trichoderma viride* has an improved biocontrol activity against stem Rot in Groundnut. *Plant Pathology Journal*, **20(1)**:75-80; doi: 10.5423/PPJ.2004.20.1.075.

Meera, T. and Balabaskar, P. 2012. Isolation and characterization of *Pseudomonas fluorescens* from rice fields. *International Journal of Food Agriculture and Veterinary Science*, **2(1)**:113-120.

Mishra, D.S., Kumar, A., Prajapati, C.R. *et. al*., 2013. Identification of compatible bacterial and fungal isolate and their effectiveness against plant disease. *Journal of Environmental Biology*, **34:**183-189.

Mukhopadyay, A.N. 1987. Biological control of soil-borne plant pathogens by *Trichoderma* spp. Presidential address delivered at the 9th Annual meeting of the Society of Mycology and Plant Pathology. *Indian Journal of Mycology and Plant Pathology*, **17:**1-10.

Prasanna, A.R., Nargund, V.B., Bheemanna, M. *et al*., 2002. Compatibility of thiomethoxam with *Trichoderma harzianum. Journal of Biological Control*, **16(2)**:149-152; doi: 10.18311/jbc/2002/4125.

Romero-Perdomo, F., Abril, J., Camelo, M., *et. al*., 2017. *Azotobacter chroococcum* as a potentially useful bacterial biofertilizer for cotton (*Gossypium hirsutum*): Effect in reducing N fertilization. *Revista Argentina de Microbiologia*, **49(4)**:377-383; doi: 10.1016/j.ram.2017.04.006.

Sharma, P. and Dureja, P. 2004. Evaluation of *T. harzianum* and *T. viride* isolates at BCA Pathogen Crop Interface. *Journal of Mycology and Plant Pathology*, **34(1)**:47-55.

Tripathi, M. and Johri, B.N. 2002. In vitro antagonistic potential of fluorescent pseudomonads and control of sheath blight of maize caused by *Rhizoctonia solani*. *Indian Journal of Microbiology*, **42**:207-214.