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Nutrient Rich Bioformulations to Enhance Colonization of Bio-inoculants in Tomato Rhizosphere, Rhizoplane and Endorhiza

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Abstract
Nutrient rich bioformulation(s) were prepared by inoculating the bio-inoculants *viz*., *Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*, in vermicompost supplemented with minimal inorganic fertilizers i.e. Diammonium phosphate and Muriate of potash, compatible with bio-inoculants. In order to enhance nutrient use efficiency and efficiently protect the root system against the attack of phytopathogenic microorganism, a bioinoculants has to establish itself in the rhizosphere and root. An experiment were conducted to evaluate efficiency of nutrient rich bioformulations on rhizosphere and root colonization in tomato. Nutrient rich bioformulations were mixed separately with soil at three dose *viz*. 25gm, 50gm and 75gm per kg soil and the population was enumerated. The results showed that all treatments having nutrient rich bioformulations at all three doses supported colonization of each bio-inoculants at rhizosphere and root of tomato. In dose-i, maximum populations of *Trichoderma* isolate (TRT-9) and *Pseudomonas fluorescens* were recorded (44 x 10^4 cfu/g soil) and (37 x 10^6 cfu/g soil), respectively, in treatment T21 i.e. Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*, and population of *Azotobacter chroococcum* (35 x 10^6 cfu/g soil) recorded in T20 i.e. Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*. In dose-ii, maximum populations of *Trichoderma* isolate (TRT-9) was recorded (51.67 x 10^4 cfu/g soil) in T21 i.e. Vermicompost + DAP (5% w/w) and MoP (2% w/w) + *Trichoderma* isolate (TRT-9) whereas, *Pseudomonas fluorescens* and *Azotobacter chroococcum* (41.33 x 10^6 cfu/g soil) and (35.67 x 10^6 cfu/g soil), respectively, in treatment T21 i.e. Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*. Similarly, in dose-iii, maximum populations of *Trichoderma* isolate (TRT-9) were recorded (56.67 x 10^4 cfu/g soil) in treatment T7 i.e. Vermicompost + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*, and *Pseudomonas fluorescens* (53.67 x 10^6 cfu/g soil), in treatment T14 i.e. Vermicompost + DAP (5% w/w) and MoP (2% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum* whereas maximum *Azotobacter chroococcum* population (39 x 10^6 cfu/g soil) recorded in T12 i.e. Vermicompost + DAP (5% w/w) and MoP (2% w/w) + *Trichoderma* isolate (TRT-9) + *Azotobacter chroococcum*. It was also confirmed that *Trichoderma* isolate (TRT-9) and *Pseudomonas fluorescens* develop epiphytic as well as endophytic plant-microorganism interaction with tomato plants. However, *Azotobacter chroococcum* is associative in nature. This particular type of plant–microorganism association consists of a natural beneficial system to be explored and reported to improve nutrient uptake and increase plant tolerance against phytopathogens.

Keywords: Bioformulations, *Trichoderma* sp., *Pseudomonas fluorescens*, *Azotobacter chroococcum*, colonization

Introduction
Nutrients are very essential for complete development of crops. In a wide range of agricultural crop systems, the limited natural nutrient supply in soil restricts plant yields therefore, crop productivity relies heavily on chemical fertilization. The massive application of inorganic fertilizers, insecticides and fungicides have increased the toxicity and polluted the total ecosystem of rhizosphere. The benefits of chemical fertilizers added to cropping systems come with well-documented high energy costs and environmental damage. The root system is not well developed which affects nutrient uptake and plants show the deficiency of nutrients. The soil adhered to such roots consists of number of harmful fungi which cause diseases in plants. In this way, developing techniques for improving nutrients use efficiency (NUE) along with

disease managements are crucial for the establishment of a sustainable agriculture and represents an important challenge of this century.

A wide range of interactions occur between plants and microorganisms. These microorganisms could be beneficial, harmful, or neutral, according to their effects on plant development (Dobbelaere *et al*., 2003) [3]. Among beneficial associations between plants and microorganisms, those of great interest are the ones related to the biological conversion of the nutrients from plant-unavailable to plant-available and related to the disease control biologically. This type of plant–microorganisms interaction is another major source of nutrients and diseases control inputs in agriculture. Represent a promising alternative to chemical fertilizers and chemical fungicides. Microorganisms have the ability to develop different types of root associations with plants. Several of these are found on the root surface, where they are usually designated associative microorganisms. Also, there are some microorganisms that can be detected inside surface-sterilized plants, called endophytic N-fixing bacteria, and one of their traits is that it is located inside the plant and do not cause any visible harmful effects (Reinhold-Hurek and Hurek, 1998) [7]. Some bacteria live in the rhizosphere and are called rhizobacteria (Kloepper and Beauchamp, 1992) [5].

Keeping these facts in view, the combined application of biological control agents *viz. Trichoderma* spp. and *Pseudomonas fluorescens*, and biofertilizer *viz*. *Azotobacter chroococcum* with minimum dose of inorganic fertilizers and required dose of organic manure tested in the present investigation under tomato crop.

Materials and Methods

Fungal and bacterial bio-inoculants

The *Azotobacter chroococcum* was obtained from the Department of Agricultural Microbiology, College of Agriculture, Raipur, Chhattisgarh. The *Pseudomonas fluorescens* was obtained from Biocontrol laboratory, Department of Plant Pathology, College of Agriculture, Raipur, Chhattisgarh. The *Trichoderma* spp. were isolate from the rhizosphere soil of healthy tomato plants and evaluated for their compatibility with *Azotobacter chroococcum* and *Pseudomonas fluorescens*.

Preparation of nutrient rich bioformulations

Three bio-inoculants i.e. *Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum* along with Inorganic fertilizers i.e. DAP and MoP, were used to prepare bioformulation(s) rich in nutrients, by using vermicompost as substrat. Nutrient rich bioformulation(s) were prepare in three lots (batch-i, batch-ii and batch-iii) where, bio-inoculants were inoculated, individually and in their possible combinations, on vermicompost, vermicompost supplemented with DAP (5% w/ w) and MoP (2% w/ w) and vermicompost supplemented with DAP (10% w/ w) and MoP (3% w/ w), respectively in batch-i, batch-ii and batch-iii.

Effect of nutrient rich bio-formulations on colonization of bio-inoculants

A pot test was carried out for the colonization effect of bioinoculancy in rhizosphere and rhizoplane of nutritionally rich bioformulation(s). For the study, 25gm, 50gm and 75gm of bioformulation(s) were mixed with 1 kg of soil separately, followed by 2 kg of PVC pots sterilized, and incubated overnight. 25 tomato seeds were seeded in each pot after the incubation and sterilized water was applied regularly. Treatment having vermicompost, vermicompost supplemented with DAP (5% w/w) and MoP (2% w/w), vermicompost supplemented with DAP (10% w/ w) and MoP (3% w/w) and inorganic fertilizers were serve as control for batch-i, batch-ii, batch-iii and overall experiments, respectively. The pots were placed in the ambient light and temperature.

Assessment for colonization bio-inoculants on the tomato rhizosphere

For the study, tomato plants were gently and carefully uprooted before drying and the soil tightly adhered to the root surface was collected in butter paper bags followed by air dried in shade and ground to fine powder. Population dynamics were evaluated by using serial dilution plate method (Subba Rao, 1988) [8].

For the assessment of *Trichoderma*, 1ml aliquot from 10^{-3}, 10^{-4}, 10^{-5} dilution, separately, was transferred to Petri plates, which were formerly poured with Trichoderma selective media (TSM), then spread uniformly all over the plate. For the assessment of *Pseudomonas fluorescens* and *Azotobacter chroococcum* population dynamics, 1ml aliquot from 10^{-6}, 10^{-7}, 10^{-8} dilution, separately, were transferred in Petri plates previously poured with King's B Agar media and Jensen's media respectively, then spread uniformly over the plate. Petri plates were incubated at 28±2 °C and morphologically different colony forming units appearing on the plates were counted by using colony counter (cfu/g soil).

Assessment for colonization of bio-inoculants on the tomato root

Colonization of bioinoculants on roots were assessed by adopting root imprinting technique. In these, after one month, plants were uprooted and adhered soil particles were removed by shaking, followed by dipping the roots in distilled water for ten minutes. Furthermore, the roots were cleaned with camel brush and again washed with distilled water then blotter dried and placed on Petri plates poured with *Trichoderma* Selective Medium, King's B Agar Medium and Jensen's Medium, to confirm the presence of *Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*, respectively on root surface. However, blotter dried root were place on plates after surface sterilization with 0.1% $HgCl_2$ for assessment of endophytic colonization. Plates were kept for incubation at 28±2 °C for 3 days. After 3 days the plates were examined for the growth of respective inoculants. For the confirmation of *Pseudomonas fluorescens* plate were examined under UV light.

Results and Discussion

Assessment for colonization of bio-inoculants on the tomato rhizosphere

The effect of nutrient-rich bioformulation(s) to increase bio-inoculants colonization in tomato rhizosphere was investigated. The data from table 1 indicates that *Trichoderma* populations in tomato rhizosphere are likely to be influenced by nutrient rich bioformulations. All treatments having nutrient rich bioformulations potentially increases their populations in rhizosphere. In dose-I, population dynamics range from 38.33 x 10^4 cfu/g to 43.67 x 10^4 cfu/g which is much higher than the population (9.33 x 10^3 cfu/g) in Inorganic fertilizers and population (14.67 x 10^3 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (3.00 x 10^3 cfu/g). Highest population recorded in T7 (Vermicompost + *Trichoderma* isolate (TRT-9) +

Pseudomonas fluorescens + *Azotobacter chroococcum*), followed by T5>T1, minimum in T4. In dose II, populations range from 46.67 x 10^4 cfu/g to 49.00 x 10^4 cfu/g which is much higher than the population (5.33 x 10^3 cfu/g) in Inorganic fertilizers (T25) and population (16.67 x 10^3 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (3.00 x 10^3 cfu/g). Highest population recorded in T7 (Vermicompost + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T4=T5, minimum in T1. In dose-III, populations range from 51.33 x 10^4 cfu/g to 56.67 x 10^4 cfu/g which is much higher than the population (2.33 x 10^3 cfu/g) in inorganic fertilizers (T25) and population (19.67 x 10^3 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (3.00 x 10^3 cfu/g). Highest population recorded in T7 (Vermicompost + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T5>T4, minimum in T1.

However, DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost based microbial inoculums (*Trichoderma* isolates (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually as well as in their combinations were also showing increased population similar to batch-i. In dose-I, population dynamics range from 37.33 x 10^4 cfu/g to 40.00 x 10^4 cfu/g which is much higher than the population (9.33 x 10^3 cfu/g) in Inorganic fertilizers and population (14.00 x 10^3 cfu/g) in their respective control *i.e.* DAP (5% w/w) + MoP (2% w/w) supplemented vermicompost (T23) over initial population (3.00 x 10^3 cfu/g). Highest population recorded in T8 (Vermicompost + DAP (5% w/w) + MoP (2% w/w) +*Trichoderma* isolate (TRT-9)), followed by T14>T12, minimum in T11. In dose-II, population dynamics range from 44.00 x 10^4 cfu/g to 51.67 x 10^4 cfu/g which is much higher than the population (5.33 x 10^3 cfu/g) in Inorganic fertilizers and population (17.33 x 10^3 cfu/g) of their respective control *i.e.* DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost (T23) over initial population (3.00 x 10^3 cfu/g). In dose-III, population dynamics range from 49.67 x 10^4 cfu/g to 55.67 x 10^4 cfu/g which is much higher than the population (2.33 x 10^3 cfu/g) in Inorganic fertilizers and population (19.00 x 10^3 cfu/g) of their respective control i.e. DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost (T23) over initial population (3.00 x 10^3 cfu/g)

Similarly, DAP (10% w/w) and MoP (3% w/w) supplemented vermicompost based microbial inoculums (*Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually as well as in their combinations, were also recorded increased population as similar to batch-I and II. In dose-I, population dynamics range from 38.67 x 10^4 cfu/g to 44.00 x 10^4 cfu/g which is much higher than the population (9.33 x 10^3 cfu/g) in Inorganic fertilizers and population (15.67 x 10^3 cfu/g) in their respective control *i.e.* DAP (10% w/w) and MoP (3% w/w) supplemented vermicompost (T24) over initial population (3.00 x 10^3 cfu/g). Highest population recorded in T21 (Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T18>T19, minimum in T15. In dose-II, population range from 47.67 x 10^4 cfu/g to 48.67 x 10^4 cfu/g which is much higher than the population (5.33 x 10^3 cfu/g) in Inorganic fertilizers and population (18.00 x 10^3 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP (3% w/w) supplemented vermicompost (T24) over initial population (3.00 x 10^3 cfu/g). Highest population recorded in T21 (Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T18>T19=T15. In dose-III, population dynamics range from 50.67 x 10^4 cfu/g to 53.67 x 10^4 cfu/g which is much higher than the population (2.33 x 10^3 cfu/g) in Inorganic fertilizers and population (20.04 x 10^3 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP (3% w/w) supplemented vermicompost (T24) over initial population (3.00 x 10^3 cfu/g).

Data presented in table 2 clearly evident that *P. fluorescens* population in tomato rhizosphere were potentially influenced by nutrient rich bioformulations. It was found that the population (cfu/g) of *P. fluorescens* was potentially increased when applied vermicompost based microbial inoculums (*Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually as well as in their combinations. In dose-i, population dynamics range from 32.00 x 10^6 cfu/g to 35.67 x 10^6 cfu/g which is much higher than the population (13.33 x 10^4 cfu/g) in Inorganic fertilizers (T25) and population (15.33 x 10^4 cfu/g) in their respective control *i.e.* Vermicompost (T22) over initial population (10.33 x 10^4 cfu/g). Highest population recorded in T7 (Vermicompost + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T6>T2, minimum in T4. In dose II, populations range from 36.67 x 10^6 cfu/g to 41.67 x 10^6 cfu/g which is much higher than the population (11.33 x 10^4 cfu/g) in Inorganic fertilizers (T25) and population (18.67 x 10^4 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (10.33 x 10^4 cfu/g). Highest population recorded in T7 (Vermicompost + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T4=T6, minimum in T1In dose-III, populations range from 43.00 x 10^6 cfu/g to 49.00 x 10^6 cfu/g which is much higher than the population (11.33 x 10^4 cfu/g) in Inorganic fertilizers (T25) and population (20.67 x 10^4 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (10.33 x 10^4 cfu/g). Highest population recorded in T7 (Vermicompost + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T4>T6, minimum in T1.

However, DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost based microbial inoculums (Trichoderma isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually and in their combinations were also showing increased population similar to batch-I. In dose-I, population dynamics range from 31 x 10^6 cfu/g to 34.67 x 10^6 cfu/g which is much higher than the population (13.33 x 10^4 cfu/g) in Inorganic fertilizers and population (16.67 x 10^4 cfu/g) in their respective control i.e. DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost (T23) over initial population (10.33 x 10^4 cfu/g). Highest population recorded in T14 (Vermicompost + DAP (5% w/w) and MoP (2% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T11>T13, minimum in T9. In dose-II, population dynamics range from 36.67 x 10^6 cfu/g to 41.00 x 10^6 cfu/g which is much higher than the population (11.33 x 10^4 cfu/g) in Inorganic fertilizers and population (20.67 x 10^4 cfu/g) in their respective control *i.e.* DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost (T23) over initial population (10.33 x 10^4 cfu/g). Maximum population recorded in T11 followed by T14>T13. Minimum recorded in T9. In dose-III, population dynamics range from 46.33 x 10^6 cfu/g to 53.67 x 10^6 cfu/g which is much higher than the population (11.33 x 10^4 cfu/g) in Inorganic fertilizers and population (22.00 x 10^4 cfu/g) in

their respective control i.e. DAP (5% w/w) and MoP (2% w/w) supplemented vermicompost (T23) over initial population (10.33 x 10^4 cfu/g)

Similarly, DAP (10% w/w) and MoP (3% w/w) supplemented vermicompost based microbial inoculums (Trichoderma isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually as well as in their combinations, were also recorded increased population as similar to batch-I and II. In dose-I, population dynamics range from 33.00 x 10^6 cfu/g to 37.67 x 10^6 cfu/g which is much higher than the population (13.33 x 10^4 cfu/g) in Inorganic fertilizers and population (18.00 x 10^4 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP 3% supplemented vermicompost (T24) over initial population (10.33 x 10^4 cfu/g). Highest population recorded in T21 (Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T18>T20>T16. In dose-II, population range from 40.33 x 10^6 cfu/g to 41.33 x 10^6 cfu/g which is much higher than the population (11.33 x 10^4 cfu/g) in Inorganic fertilizers and population (18.67 x 10^4 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP 3% supplemented vermicompost (T24) over initial population (10.33 x 10^4 cfu/g). Highest population recorded in T21 (Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T18>T19=T16. In dose-III, population dynamics range from 43.33 x 10^6 cfu/g to 47.33 x 10^6 cfu/g which is much higher than the population (11.33 x 10^4 cfu/g) in Inorganic fertilizers and population (21.33 x 10^4 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP 3% supplemented vermicompost (T24) over initial population (10.33 x 10^4 cfu/g).

Data presented in table 3 clearly indicate that the population (cfu/g) of *Azotobacter chroococcum* was also potentially increased when applied vermicompost based microbial inoculums (*Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually as well as in their combinations. In dose-I, population dynamics range from 21.67 x 10^6 cfu/g to 26.67 x 10^6 cfu/g which is much higher than the population (12.33 x 10^4 cfu/g) in Inorganic fertilizers (T25) and population (14.67 x 10^4 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (3.47 x 10^4 cfu/g). Highest population recorded in T3 (Vermicompost + *Azotobacter chroococcum*), followed by T6>T5>7. In dose II, populations range from 32 x 10^6 cfu/g to 26.67 x 10^6 cfu/g which is much higher than the population (11.00 x 10^4 cfu/g) in Inorganic fertilizers (T25) and population (15.00 x 10^4 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (3.47 x 10^4 cfu/g). Population recorded T6=T3>T5>T7. In dose-III, populations range from 32.67 x 10^6 cfu/g to 36.00 x 10^6 cfu/g which is much higher than the population (10.67 x 10^4 cfu/g) in Inorganic fertilizers (T25) and population (17.33 x 10^4 cfu/g) in their respective control i.e. Vermicompost (T22) over initial population (3.47 x 10^4 cfu/g). Highest population recorded in T6 (Vermicompost + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T5>T3>T7.

However, DAP 5% and MoP 2% supplemented vermicompost based microbial inoculums (*Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually and in their combinations were also showing increased population similar to batch-I. In dose-I, population dynamics range from 25.33 x 10^6 cfu/g to 31.33 x 10^6 cfu/g which is much higher than the population (12.33 x 10^4 cfu/g) in Inorganic fertilizers and population (15.33 x 10^4 cfu/g) in their respective control i.e. DAP 5% and MoP 2% supplemented vermicompost (T23) over initial population (3.47 x 10^4 cfu/g). Highest population recorded in T14 (Vermicompost + DAP (5% w/w) and MoP (2% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T12>T13>T10. In dose-II, population dynamics range from 29.67 x 10^6 cfu/g to 35.33 x 10^6 cfu/g which is much higher than the population (11.00 x 10^4 cfu/g) in Inorganic fertilizers and population (18.67 x 10^4 cfu/g) in their respective control i.e. DAP 5% and MoP 2% supplemented vermicompost (T23) over initial population (3.47 x 10^4 cfu/g). Maximum population recorded in T14 followed by T13>T12>T10. In dose-III, population dynamics range from 32.33 x 10^6 cfu/g to 39.00 x 10^6 cfu/g which is much higher than the population (10.67 x 10^4 cfu/g) in Inorganic fertilizers and population (20.33 x 10^4 cfu/g) in their respective control i.e. DAP 5% and MoP 2% supplemented vermicompost (T23) over initial population (3.47 x 10^4 cfu/g)

Similarly, DAP (10% w/w) and MoP 3% supplemented vermicompost based microbial inoculums (*Trichoderma* isolate (TRT-9), *Pseudomonas fluorescens* and *Azotobacter chroococcum*) individually as well as in their combinations, were also recorded increased population as similar to batch-I and II. In dose-I, population dynamics range from 27.00 x 10^6 cfu/g to 35.00 x 10^6 cfu/g which is much higher than the population (12.33 x 10^4 cfu/g) in Inorganic fertilizers and population (15.67 x 10^4 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP 3% supplemented vermicompost (T24) over initial population (3.47 x 10^4 cfu/g). Highest population recorded in T20 (Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T21>T17>T19. In dose-II, population range from 30.00 x 10^6 cfu/g to 35.67 x 10^6 cfu/g which is much higher than the population (11.00 x 10^4 cfu/g) in Inorganic fertilizers and population (17.67 x 10^4 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP 3% supplemented vermicompost (T24) over initial population (3.47 x 10^4 cfu/g). Highest population recorded in T21 (Vermicompost + DAP (10% w/w) and MoP (3% w/w) + *Trichoderma* isolate (TRT-9) + *Pseudomonas fluorescens* + *Azotobacter chroococcum*), followed by T20>T19=T17. In dose-III, population dynamics range from 31.33 x 10^6 cfu/g to 36.00 x 10^6 cfu/g which is much higher than the population (10.67 x 10^4 cfu/g) in Inorganic fertilizers and population (19.33 x 10^4 cfu/g) in their respective control i.e. DAP (10% w/w) and MoP 3% supplemented vermicompost (T24) over initial population (3.47 x 10^4 cfu/g).

Table 1: Effect of nutrient rich bioformulation(s) on colonization of *Trichoderma* isolate (TRT-9) in tomato rhizosphere

Treatments		Details	cfu/g soil			
			Initial	Dose-I	Dose-II	Dose-III
T1	Batch-I	VC + TRT-9	3.00 x10^3	41.33 x 10^4	44.00 x 10^4	50.67 x 10^4
T2		VC + Pf		-	-	-
T3		VC + Az		-	-	-
T4		VC + TRT-9 + Pf		38.33 x 10^4	46.67 x 10^4	51.33 x 10^4

T5		VC + TRT-9 + Az		41.67×10^4	46.67×10^4	53.00×10^4
T6		VC + Pf + Az		-	-	-
T7		VC + TRT-9 + Pf + Az		43.67×10^4	49.00×10^4	56.67×10^4
T8	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9		40.00×10^4	51.67×10^4	53.67×10^4
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf		-	-	-
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az		-	-	-
T11		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf		37.33×10^4	44.00×10^4	49.67×10^4
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Az		39.00×10^4	47.00×10^4	52.33×10^4
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az		-	-	-
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf + Az		39.67×10^4	50.33×10^4	55.67×10^4
T15	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9		38.67×10^4	47.67×10^4	51.33×10^4
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf		-	-	-
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az		-	-	-
T18		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf		43.67×10^4	48.00×10^4	52.67×10^4
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Az		39.33×10^4	47.67×10^4	50.67×10^4
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az		-	-	-
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf + Az		44.00×10^4	48.67×10^4	53.67×10^4
T22	Control	Vermicompost		14.67×10^3	16.67×10^3	18.67×10^3
T23		VC + DAP (5% *w/w*) + MoP (2% *w/w*)		14.00×10^3	17.33×10^3	19.00×10^3
T24		VC + DAP (10% *w/w*) + MoP (3% *w/w*)		15.67×10^3	18.00×10^3	20.04×10^3
T25		Inorganic Fertilizers		9.33×10^3	5.33×10^3	2.33×10^3

*VC=Vermicompost; TRT-9=*Trichoderma* isolate (TRT-9); Pf=*Pseudomonas fluorescens;* Az= *Azotobacter chroococcum;* DAP-Diammonium Phosphate; MoP- Muriate of Potash

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

Table 2: Effect of nutrient rich bioformulation(s) on colonization of *Pseudomonas fluorescens* in tomato rhizosphere

Treatments		Details	cfu/g soil			
			Initial	Dose-I	Dose-II	Dose-III
T1	Batch-I	VC + TRT-9	10.33×10^4	-	-	-
T2		VC + Pf		35.00×10^6	36.67×10^6	43.00×10^6
T3		VC + Az		-	-	-
T4		VC + TRT-9 + Pf		32.00×10^6	39.33×10^6	48.67×10^6
T5		VC + TRT-9 + Az		-	-	-
T6		VC + Pf + Az		35.67×10^6	39.33×10^6	46.33×10^6
T7		VC + TRT-9 + Pf + Az		37.33×10^6	41.67×10^6	49.00×10^6
T8	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9		-	-	-
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf		31.00×10^6	36.67×10^6	46.33×10^6
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az		-	-	-
T11		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf		33.33×10^6	41.00×10^6	50.00×10^6
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Az		-	-	-
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az		32.67×10^6	38.67×10^6	48.00×10^6
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf + Az		34.67×10^6	39.67×10^6	53.67×10^6
T15	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9		-	-	-
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf		33.00×10^6	40.33×10^6	44.00×10^6
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az		-	-	-
T18		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf		36.00×10^6	40.67×10^6	43.33×10^6
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Az		-	-	-
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az		33.67×10^6	40.33×10^6	44.00×10^6
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf + Az		37.67×10^6	41.33×10^6	47.33×10^6
T22	Control	Vermicompost		15.33×10^4	18.67×10^4	20.67×10^4
T23		VC + DAP (5% w/w) + MoP (2% w/w)		16.67×10^4	20.67×10^4	22.00×10^4
T24		VC + DAP (10% w/w) + MoP (3% w/w)		18.00×10^4	18.67×10^4	21.33×10^4
T25		Inorganic Fertilizers		13.33×10^4	11.33×10^4	11.33×10^4

*VC=Vermicompost; TRT-9=*Trichoderma* isolate (TRT-9); Pf=*Pseudomonas fluorescens*; Az= *Azotobacter chroococcum*; DAP- Diammonium Phosphate; MoP- Muriate of Potash

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

Table 3: Effect of nutrient rich bioformulation(s) on colonization of *Azotobacter chroococcum* in tomato rhizosphere

Treatments		Details	cfu/g soil			
			Initial	Dose-I	Dose-II	Dose-III
T1	Batch-I	VC + TRT-9	3.47×10^4	-	-	-
T2		VC + Pf		-	-	-
T3		VC + Az		26.67×10^6	32.00×10^6	34.33×10^6
T4		VC + TRT-9 + Pf		-	-	-

T5		VC + TRT-9 + Az		24.67×10^6	28.67×10^6	34.67×10^6
T6		VC + Pf + Az		25.67×10^6	32.00×10^6	36.00×10^6
T7		VC + TRT-9 + Pf + Az		21.67×10^6	26.67×10^6	32.67×10^6
T8	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9		-	-	-
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf		-	-	-
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az		25.33×10^6	29.67×10^6	35.67×10^6
T11		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf		-	-	-
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Az		29.67×10^6	31.00×10^6	39.00×10^6
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az		29.00×10^6	31.67×10^6	32.33×10^6
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf + Az		31.33×10^6	35.33×10^6	36.67×10^6
T15	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9		-	-	-
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf		-	-	-
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az		31.00×10^6	30.00×10^6	34.33×10^6
T18		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf		-	-	-
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Az		27.00×10^6	32.33×10^6	33.67×10^6
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az		35.00×10^6	33.33×10^6	31.33×10^6
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf + Az		34.67×10^6	35.67×10^6	36.00×10^6
T22	Control	Vermicompost		14.67×10^4	15.00×10^4	17.33×10^4
T23		VC + DAP (5% *w/w*) + MoP (2% *w/w*)		15.33×10^4	18.67×10^4	20.33×10^4
T24		VC + DAP (10% *w/w*) + MoP (3% *w/w*)		15.67×10^4	17.67×10^4	19.33×10^4
T25		Inorganic Fertilizers		12.33×10^4	11.00×10^4	10.67×10^4

*VC=Vermicompost; TRT-9=*Trichoderma* isolate (TRT-9); Pf=*Pseudomonas fluorescens*; Az= *Azotobacter chroococcum*; DAP- Diammonium Phosphate; MoP- Muriate of Potash

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

Table 4: Effect of nutrient rich bioformulation(s) on colonization of bio-inoculants on tomato rhizoplane

Treatments		Details	Dose-I			Dose-II			Dose-III		
			TRT-9	Pf	Az	TRT-9	Pf	Az	TRT-9	Pf	Az
T1	Batch-I	VC + TRT-9	+	-	-	+	-	-	+	-	-
T2		VC + Pf	-	+	-	-	+	-	-	+	-
T3		VC + Az	-	-	+	-		+	-	-	+
T4		VC + TRT-9 + Pf	+	+	-	+	+	-	+	+	-
T5		VC + TRT-9 + Az	+	-	+	+		+	+	-	+
T6		VC + Pf + Az	-	+	+	-	+	+	-	+	+
T7		VC + TRT-9 + Pf + Az	+	+	+	+	+	+	+	+	+
T8	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9	+	-	-	+	-	-	+	-	-
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf	-	+	-	-	+	-	-	+	-
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az	-	-	+	-	-	+	-	-	+
T11		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf	+	+	-	+	+	-	+	+	-
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Az	+	-	+	+	-	+	+	-	+
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az	-	+	+	-	+	+	-	+	+
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf + Az	+	+	+	+	+	+	+	+	+
T15	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9	+	-	-	+	-	-	+	-	-
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf	-	+	-	-	+	-	-	+	-
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az	-	-	+	-	-	+	-	-	+
T18		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf	+	+	-	+	+	-	+	+	-
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Az	+	-	+	+	-	+	+	-	+
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az	-	+	+		+	+		+	+
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf + Az	+	+	+	+	+	+	+	+	+

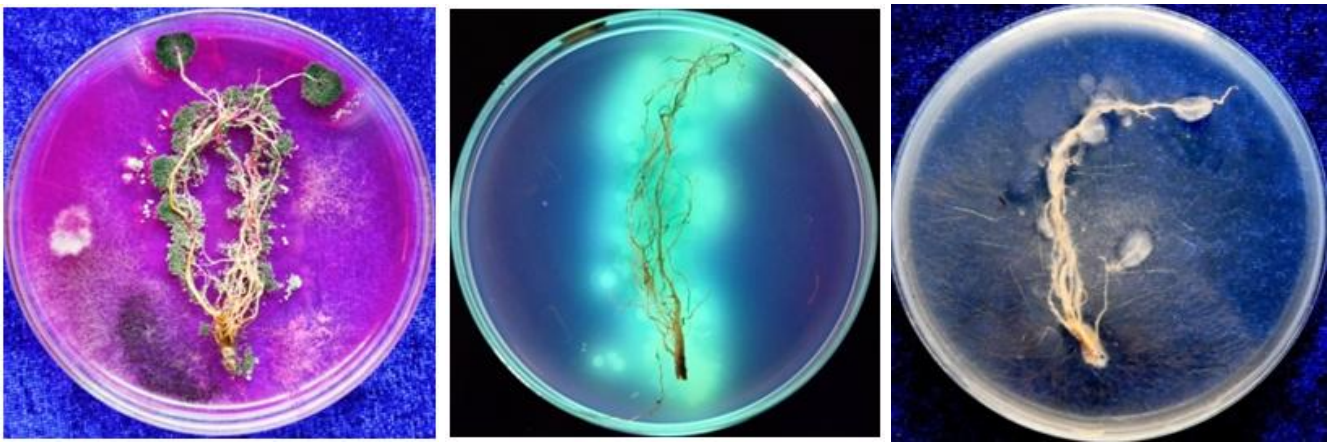
*VC=Vermicompost; TRT-9=*Trichoderma* isolate (TRT-9)*;* Pf=*Pseudomonas fluorescens*; Az= *Azotobacter chroococcum*; DAP- Diammonium Phosphate; MoP- Muriate of Potash (+): Present (-): Absent

Table 5: Effect of nutrient rich bioformulation(s) on colonization of bio-inoculants on tomato endorhiza

Treatments		Details	Dose-I			Dose-II			Dose-III		
			TRT-9	Pf	Az	TRT-9	Pf	Az	TRT-9	Pf	Az
T1	Batch-I	VC + TRT-9	+	-	-	+	-	-	+	-	-
T2		VC + Pf	-	+	-	-	+	-	-	+	-
T3		VC + Az	-	-	-	-		-	-	-	-
T4		VC + TRT-9 + Pf	+	+	-	+	+	-	+	+	-
T5		VC + TRT-9 + Az	+	-	-	+		-	+	-	-
T6		VC + Pf + Az	-	+	-	-	+	-	-	+	-
T7		VC + TRT-9 + Pf + Az	+	+	-	+	+	-	+	+	-
T8	Batch-II	VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9	+	-	-	+	-	-	+	-	-
T9		VC + DAP (5% w/w) + MoP (2% w/w) + Pf	-	+	-	-	+	-	-	+	-
T10		VC + DAP (5% w/w) + MoP (2% w/w) + Az	-	-	-	-	-	-	-	-	-
T11		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf	+	+	-	+	+	-	+	+	-

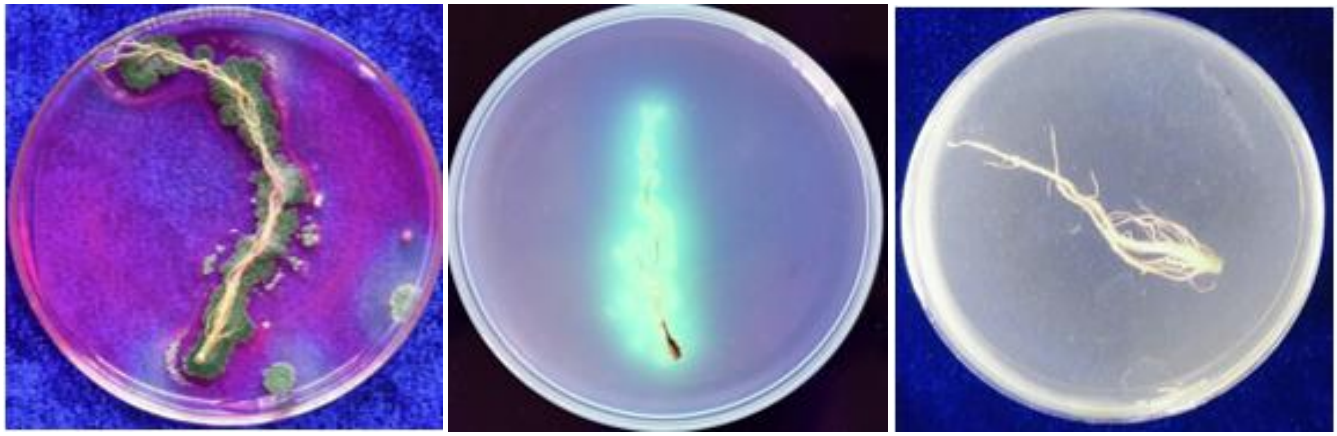
T12		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Az	+	-	-	+	-	-	+	-	-
T13		VC + DAP (5% w/w) + MoP (2% w/w) + Pf + Az	-	+	-	-	+	-	-	+	-
T14		VC + DAP (5% w/w) + MoP (2% w/w) + TRT-9 + Pf + Az	+	+	-	+	+	-	+	+	-
T15	Batch-III	VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9	+	-	-	+	-	-	+	-	-
T16		VC + DAP (10% w/w) + MoP (3% w/w) + Pf	-	+	-	-	+	-	-	+	-
T17		VC + DAP (10% w/w) + MoP (3% w/w) + Az	-	-	-	-	-	-	-	-	-
T18		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf	+	+	-	+	+	-	+	+	-
T19		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Az	+	-	-	+	-	-	+	-	-
T20		VC + DAP (10% w/w) + MoP (3% w/w) + Pf + Az	-	+	-		+	-		+	-
T21		VC + DAP (10% w/w) + MoP (3% w/w) + TRT-9 + Pf + Az	+	+	-	+	+	-	+	+	-

*VC=Vermicompost; TRT-9=*Trichoderma* isolate (TRT-9); Pf=*Pseudomonas fluorescens*; Az= *Azotobacter chroococcum*; DAP- Diammonium Phosphate; MoP- Muriate of Potash (+): Present (-): Absent



Trichoderma sp. (Colony developed from tomato rhizoplane) | *Pseudomonas fluorescens* (Colony developed from tomato rhizoplane) | *Azotobacter chroococcum* (Colony developed from tomato rhizoplane)

Plate 1: Effect of nutrient rich Bioformulation (s) on colonization of bio-inoculants on tomato rhizoplane



Trichoderma sp. (Colony developed from tomato endorhiza) | *Pseudomonas fluorescens* (Colony developed from tomato endorhiza) | *Azotobacter chroococcum* (No colony developed from tomato endorhiza)

Plate 2: Effect of nutrient rich Bioformulation (s) on colonization of bio-inoculants on tomato endorhiza

Assessment for colonization of bio-inoculants on the tomato root

It is clearly evident from the table 4 and plate 1 that nutrient rich bioformulation(s) were potentially influence the colonization of bioinoculants. All three bio-inoculants used in nutrient rich bioformulation(s) were prominently colonise itself on root surface alone as well as in combination with other inoculants that indicate bio-inoculants make effective interaction with tomato root surface i.e. epiphytic nature. However, table 5 and plate 2 indicate presence of *Trichoderma* isolate (TRT-9) and *Pseudomonas fluorescens* inoculums inside root tissue that shown endophytic interaction with tomato root in all nutrient rich bio formulations but *Azotobacter chroococcum* did not shown any kind of endophytic interaction by lacking colony on Petri plates.

Rhizosphere competence and establishment on the root surface has been recognised as a fundamental ability of *Trichoderma* spp. which stimulates plant growth and activates plant defences (Contreras-Cornejo *et al.*, 2016) [2]. Several *Trichoderma* spp. are known to mediate the solubility of nutrients, including micronutrients at the root surface and soils (Adams *et al.*, 2007) [1]. The aggressive nature of the PGPR in colonizing all parts of the potato rhizosphere indicate that they are natural root colonizing bacteria and are vigorous rhizosphere competitors (Kloepper *et al.* 1980) [6]. Our study supports colonization of tomato rhizosphere and root by bio-inoculants were potentially enhanced by nutrient rich bio-formulations.

References

1. Adams P, Lynch JM, de Leij FAAM. Desorption of zinc by extracellularly produced metabolites of *Trichoderma*

harzianum, *Trichoderma reesei* and *Coriolus versicolor*. J Appl. Microbiol. 2007; 103:2240-7.
2. Contreras-Cornejo HA, Macıas-Rodrıguez L, del-Val E, Larsen J. Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. FEMS Microbiol. Ecol. 2016; 92(4):fiw036.
3. Dobbelaere S, Vanderleyden J, Okon Y. Plant growth promoting effects of diazotrophs in the rhizosphere. CRC Crit Rev Plant Sci. 2003; 22:107-149.
4. Jayant R. Response of *Azotobacter*, *Pseudomonas* and *Trichoderma* on Growth of Apple Seedling. International Conference on Biological and Life Sciences (IPCBEE) (2012) © (2012) IACSIT Press, Singapore, 2012, 40.
5. Kloepper JW, Beauchamp CJ. A review of issues related to measuring colonization of plant roots by bacteria. Can. J Microbiol. 1992; 38:1219-1232.
6. Kloepper JW, Schroth MN, Miller TD. Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. The American Phyto pathological society. 1980; 70(11):1078-1082.
7. Reinhold-Hurek B, Hurek T. Life in grasses: Diazotrophic endophytes. Trends in Microbiology. 1998; 6:139-144.
8. Subba Rao NS. Biological Nitrogen fixation. Oxford and I.B.H. Pub. Co., New Delhi, 1988.