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Research Article

Exploring the Effects of Biocontrol and Plant Growth-Promoting *Trichoderma* spp. on Initial Root Growth and Nodulation in *Cicer arietinum*

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Abstract

In nowadays leguminous plant production in all over the world is high, and farmers are also used various types of chemical fertilizers, which reduce soil fertility and are harmful to soil microbes. In this research, we used the Trichoderma strains like *Trichoderma longibranchium, Trichoderma ressie, Trichoderma harzinium*, and *Trichoderma viride* used as biofertilizer and biocontrol agents. A soil survivability test was performed to analyze how much time Trichoderma live ups into the soil, a Root colonization assay was performed for the detection of Trichoderma strains that colonized over the root or not, and further compatibility test isolated Rhizobium pusense PR4 and Trichoderma for capable to each other on the same environment or not. Nodule formation effect checked those plants with treated different organisms. in the plant treated with dual culture PR4+T10, at least one nodule formation occurs, otherwise only plant treated with Rhizobium PR4, four nodules are observed. In this paper, we analyzed how *Trichoderma* affected the chickpea plant (*Cicer arietinum* L.), the nodulation process when applied in soil, and reduced the number of nodules by indirectly inhibiting or declining the growth of nodulation bacteria. Plant growth is facilitated by Trichoderma strain, but nitrogen fixation is insufficient in leguminous plants..

Keywords: *Trichoderma* spp; *Rhizobium* spp; Nodules; Compability, Leguminous Plant

Introduction

The chickpea is a significant food grain legume that is crucial to crop rotations all over the world [7]. Due to their nutritional value and bioactive makeup, chickpeas play a significant role in the human diet. The chickpea (*Cicer arietinum* L.), which is primarily grown in the warm regions of India, Pakistan, Iran, Ethiopia, Mexico, and the Mediterranean region, is the second-largest legume in the world by total production after the soybean and bean. They have abundant fiber, fats, carbohydrates, and protein [7]. It is a rich source of B-complex vitamins and minerals, complex carbs (60–65 g/100 g), and protein (19–29 g/100 g). The second-most significant pulse crop, the chickpea, provides farming systems with several advantages, including improved soil quality, a reduction in the use of synthetic nitrogen, and improved livestock and human health due to the chickpea's superior nutritional quality, which includes valuable proteins, minerals, vitamins, and fibers [3]. A key source of protein, chickpeas are a cool-season crop. With an annual yield of 12 Mt, chickpeas are farmed in over 54 countries across the world on 12.7 Mha of land. Currently, the most widely grown legumes are the chickpea, with global production expected to reach 15,083,871 tons in 2020, respectively [5]. Due to climate change, its annual production has fluctuated over the past five years. Production and productivity of chickpeas are now significantly challenged by climate change [2].

Trichoderma strains are utilized as biocontrol agents (BCAs) to prevent a variety of plant infections. In addition to controlling diseases, *Trichoderma* species also improve plant growth and root development (bio-fertilizer) and activate the defense mechanisms of plants [1]. *Trichoderma* species are frequently found in decaying wood and vegetable material. Several strains of *Trichoderma* are the largest producers of industrial enzymes [12]. *Trichoderma ressie, Trichoderma harzianum* produces the enzymes cellulases, hemicellulases, and pectinases. *Trichoderma* fungi are employed in the brewing, winemaking, and animal feed industries to manufacture lytic enzyme [4]. It has been demonstrated that some *Trichoderma* strains may penetrate the epidermis and create strong and long-lasting colonization of the root surface. It's the versatility of *Trichoderma* that increases its value as a biofertilizer [16].

Rhizobium bacteria are classified as belonging to the Rhizobiaceae family, class Alphaproteobacteria, Pseudomonadota phylum, and Hyphomicrobiales Order and domain is bacteria they are mostly found in soil [9]. A genus of nitrogen-fixing Gram-negative soil bacteria is termed *Rhizobium. Rhizobium* species associate with the roots of (mostly) legumes and other flower plants for fixed nitrogen. A genus of bacteria called *Rhizobium* is linked to the development of root nodules in plants [6]. The interaction between plants

and rhizobia is symbiotic (favorable), as one organism benefits from the other and provides something in return. In a field trial, it was discovered that the native, symbiotic N_2-fixing bacteria *Rhizobium leguminosarum* considerably improved nodulation, plant development, and yield of chickpeas [19]. *Rhizobium* can increase phosphate uptake, supply nutrients to plants, provide mobilizing nutrients, and also function as a biocontrol pathogen [14]. *Rhizobium* inoculums can be applied to legume plants to enhance the root nodules, which are responsible for fixing nitrogen for plants. The development of leaves, stems, roots, flowers, and fruit depend heavily on nitrogen [17].

In this research, we must know the impact of *Trichoderma* on plant nodules of *Cicer arietinum* L. Because, for the last two decades and today, farmers are using *Trichoderma* as a biofertilizer and biocontrol agent. All over the world, the production of legumes seed is high. Farmers are also used *Trichoderma* as a bio-fertilizer and biocontrol agent. When applying the *Trichoderma* on legumes seed chickpea plants. So what is the important role played by *Trichoderma* Is it increasing plant nodules as compared to *Rhizobium*-treated plants? Therefore, a series of experiments like the root colonization assay for determining the growth of *Trichoderma* over the root act as biocontrol and biofertilizers to enhance plant growth. On the other hand, a compatibility test is conducted for their survival in the same surroundings. Finally, we analyzed the number of nodules produced on the root of plants treated with *Trichoderma*. At the end of the experiment, we found that *Trichoderma* reduced the number of nodules on the root and indirectly inhibited the growth of *Rhizobium pusense* PR4.

Materials and Methods

We used various types of *Trichoderma* strains such as *Trichoderma viride* T7, *Trichoderma longibranchium* T8*, Trichoderma* harzinium T9*, Trichoderma ressei* T10 which were previously isolated from leguminous rhizosphere soil. Other microbes employed for the study were modulating bacteria including *Rhizobium pusense* PR4 which were previously isolated from leguminous plant nodules. Use media like PDA, YEMA, and N - agar, N-broth, Tween 80, and glycerol.

Soil survivability test

Collection of rhizosphere soil from the village of Bakrol (Anand) Gujarat. Sand, silt, and clay are included in soil composition, along with a little amount of plant dry waste. A variety of rhizosphere soil samples with various physical and chemical properties were used. The method provided by Bennett., *et al*. served as the foundation for the soil survival assay. 200 g of sieved rhizosphere soil contained in a single-use plastic glass. Glass-containing soil was left at room temperature. The soil was inoculated with various *Trichoderma* bio formulation strains. Up to 30% to 40% of the soil moisture must be maintained. 10 ml of bio formulation was inoculated in 200g of soil. Subsequently, 1 g of soil, aseptically sampled, was taken from each soil sample. 10 ml of sterile distilled water with a soil sample added to it was vortexed to ensure optimum mixing. Take 100 µl of soil suspension and spread it on a plate of potato dextrose agar (PDA). After 7 days of incubation at 25°C, track the development of each plate and compare it to the control plate. Every ten days, this test was conducted [10].

Compatibility test

The Trichoderma isolates were plated with Rhizobium following Mortor and Strouble's (2015) dual culture method to determine how they interacted. Prepared the YEMA (yeast extract mannitol agar) plates. The middle of the plate was streaked with Rhizobium, and the two side ends of the plate were each covered with a 5 mm diameter mycelial ring from the 7-day-old colony of Trichoderma strains. This experiment combined various Rhizobium and Trichoderma strains to see how they would react to one another. Three replications of each treatment were used in the experiment's fully randomized design. *Pseudomonas aeroginosa* used as a reference. Inoculate plates were incubated at 28 °C for seven days. Take note of the inhibition **[11].**

Root colonization

According to [15] instructions, a root propagation test was carried out. A sugar tube was filled with 40 gm of sterile soil after being injected with seeds of legumes such as peas and chickpeas. In each tube, legume seeds were inserted up to 3 mm deep. Also, the control was set up, moreover, all parameters were done in triplicate. The bio formulation was injected appropriately when germination was initiated. Prepared potato dextrose agar plate. Once the roots have formed, they need to be washed with sterile distilled water. It was also cleaned two or three times with sterile distilled water. Following that cut a 1 cm root transplant onto a PDA plate. Saw the growth of Trichoderma surrounding the roots after 3–4 days, as well as the control plate.

Nodulation/pot study

The modulation effect and their check biometric parameter were performed as described by [15]. Prepared the normal saline: in 100ml distilled water required 0.8 gm NaCl. Prepared the culture of PR4: Take the single colony of PR4 inoculated in the N-broth, also prepare the control flask. All flasks are placed in an orbital shaker for shaking conditions. After 24 hrs. observe the growth and take the O.D. at 600nm and note down it. When reached cell population up to 10^6/ml then the culture of both strains was centrifuged at 5000 rpm for 10 min. Supernanted discard it and pellets are dissolved in normal saline solution in equal volume. The chickpea varieties GG5 were purchased from the Shiv Shakti Agro Center in Anand City and their germination time required 3-5 days. Collection of rhizosphere soil from the village of Bakrol (Anand) Gujarat. Sand, silt, and clay are included in soil composition, along with a little amount of plant dry waste.

The soil was sieved. The size of the plastic bag is height and width. In the 3 kg of a rhizospheric soil sample that was placed in the plastic bag. After being surface sterilized for fifteen minutes,

the chickpea plant seeds were rinsed three times in sterile distilled water. 10 seeds of the chickpea plants were planted in each pot at 10 mm depth, and they were left to grow for 1.5 -2.5 months. After seeding, regular watering with distilled water was done. After 4 to 5 days sown to seed in the pot, the germination started. When the germination starts the inoculation of *Rhizobium* and *Trichoderma* bioformulation to the plant. Plants treated with numerous treatments such as Control, T7, T8, T9, T10, PR4, and combinations like PR4+T7, PR4+T8, PR4+T9, PR4+T10. In every pot except uninoculated soil, each plant is given 40ml bioformulation, to evaluate the formation of nodules in each plant after 30 days and also checked the biometric parameter of the plant [15].

Results

Soil survivability

The four types of *Trichoderma* species managed to survive in all soil types. According to the results of recovery on diluted plates from the sterile soils (45 days later), *Trichoderma* survived in the soils after being added. Longer survival of *Trichoderma* was linked to soil sterilization by autoclaving. The fungus concentration increased by almost one hundred times in 16 of the sterile soils. In all soil types, the soil was dry for 45 days after inoculation. Soil survivability of *Trichoderma* in soil is illustrated in figure 1.

Root colonization

Although the control was kept as a standard, the *Trichoderma* strain was used to treat seeds in a closely monitored test tube assay to assess its ability for colonization. It was discovered that *Trichoderma* was growing on a medium with various plants in the soil after 7 days of incubation, including *Penicillin s*pp, and *Aspergillus*, No *Trichoderma s*trains were detected in the control plate Figure 2, however, other fungi including *Aspergillus* were visible. *Trichoderma* was found to colonize roots readily according to the root colonization experiment.

Compatibility

The twin culture plating method was performed to check for compatibility between *Trichoderma* and *Rhizobium pusense* PR4. *Trichoderma* was found to be dominant and to have outgrown *Rhizobium pusense* PR4. Additionally, *Rhizobium pusense* PR4 was shown to be sensitive when *Pseudomonas aeruginosa* was employed as the reference. However, *Trichoderma* was unable to extend on Pseudomonas since that species was sensitive to *Trichoderma.* All *Trichoderma* was able to inhibit the growth of *Rhizobium* on agar medium result is demonstrated in Figure 2.

Nodulation and pot study

In the nodulation effect check in chickpea, the plant was observed the plant treated with *Trichoderma* and *Rhizobium pusense* PR4 developed the fewest nodules, as compared to the control no nodules were observed in 35 days. According to nodule estimation by dual culture *Rhizobium + Trichoderma,* the minimum nodules were observed. In result shows that *Trichoderma* works as a biofertilizer. Also, biometric parameters were increased plant growth, no leaves, root length, and shoot length. The nodules were decreased when performing the co-inoculated culture employed in soil. In the pea plants after the seed germination due to less time could not check nodule formation. In the period only after seed germination only 25 days, therefore, checked the biometric parameter like root length, and shoot length, and no leaves were observed. We were not able to conduct this study on all of the leguminous plant species, their produced nodules, and the effects of various types of *Trichoderma* on the early sprouting of roots. Further, we were unable to determine the best way to suppress the growth of developing bacteria as well as helpful microorganisms in the environment. All data is depicted in Figures 10, 11, 12 13, and 14. The plant was treated with different treatments and evaluated the number of nodules formed after 35 days. Hence, we calculated the number of nodules in each pot as we found Control (00), T7 - (03), T8 – (02), T9-(03), T10 - (02), PR4 - (04), and the plant treated with co-inoculated culture produced some nodules like PR4+T7 - (02), PR4+T8 - (02), PR4+T9 - (02), PR4+T10 - (01). The highest number of nodules was found in PR4 Figure 3. The smaller number of nodules produced plants treated with PR4 +T10. As compared to other treatments, however, the control had produced 00 nodules. The Result indicates when they combined the *Rhizobium* and *Trichoderma* they reduced the nodules, furthermore, we analyzed the biometric parameter of the treated chickpea plant. The chickpea plant increased in length T9 - (52cm), T8 – (48cm), T10 –(46cm), T7 – (44cm). T9 has the highest length observed Figure. In the other one also the control plant pot is observed whose treated only with distilled water. In the control plant, the length of the whole plant is only 26. 5 cm and is less compared to other plants.

Discussion

Trichoderma is a bio-fertilizer and biocontrol agent. Hence, nowadays farmers are using these agents in higher amounts for gaining more products and plant health. In this study, we used different sorts of *Trichoderma viride, Trichoderma longibranchium, Trichoderma harmonium,* and *Trichoderma ressie*. Testen., *et al*. 2017 has demonstrated that *Trichoderma* can enhance the growth of peanut and soybean plants [18]. Whereas, we also focus that all *Trichoderma* increasing plant growth in check pea and pea plant as working biofertilizer. Marra., *et al*. 2006 have described that *Trichoderma* has the potential to control plant pathogens and soil pathogens [13]. In this framework, *Trichoderma* lives in the soil for up to 45 days they destroy some harmful pathogens from the soil. In the soil inoculated with *Trichoderma,* they help to soil more fertile, which is the correlated result of they have already checked the soil survivability of *Trichoderma* [20].

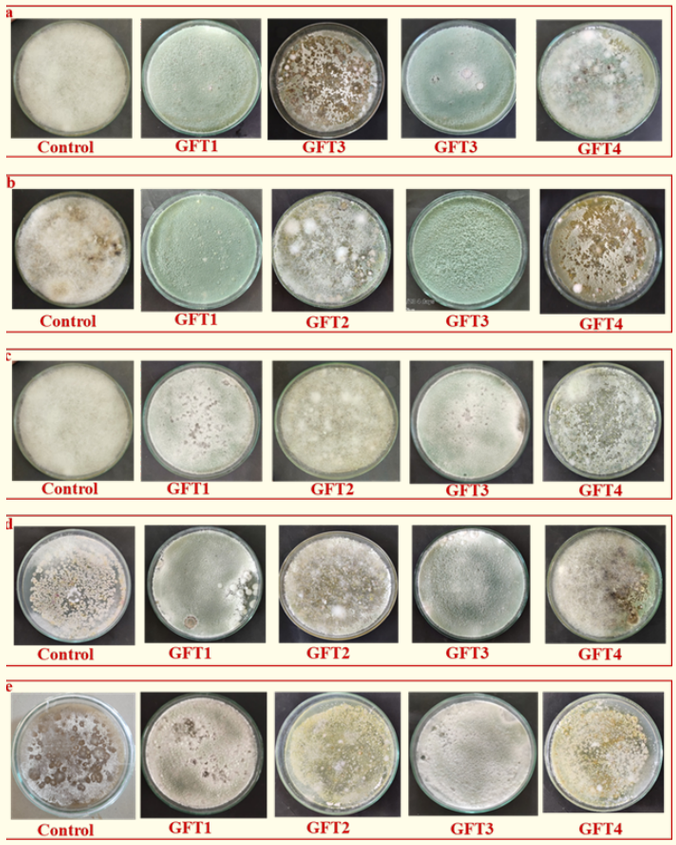

Figure 1: Displays the (a) zero-day (b) 10 days (c) 20 days (d) 3o days (e) 40 days soil survivability of Trichoderma species in soil with 30% moisture.

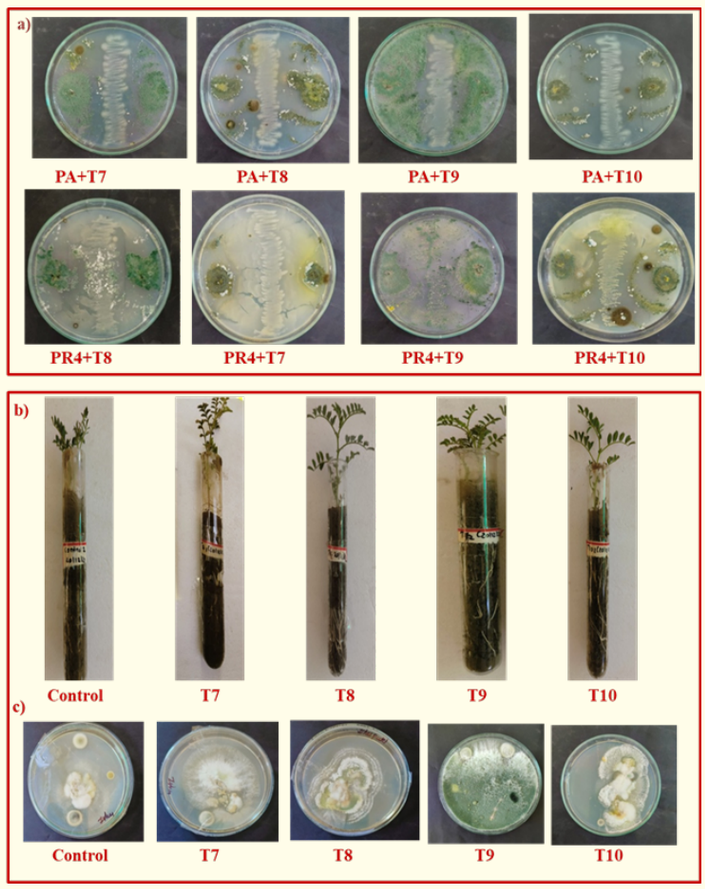

Figure 2: Displays a) compatibility test results b) chickpea plant root colonization results.

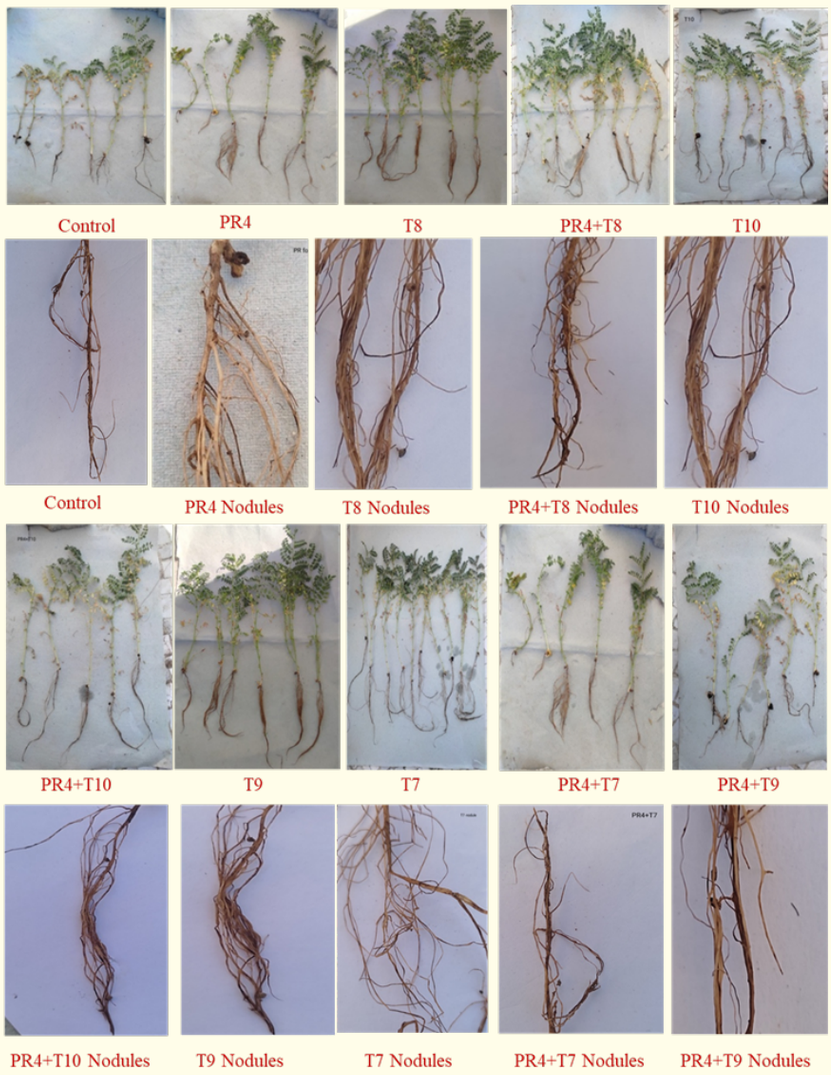

Figure 3: Nodule formation on chickpea plant after 35 days plant treated with different treatments.

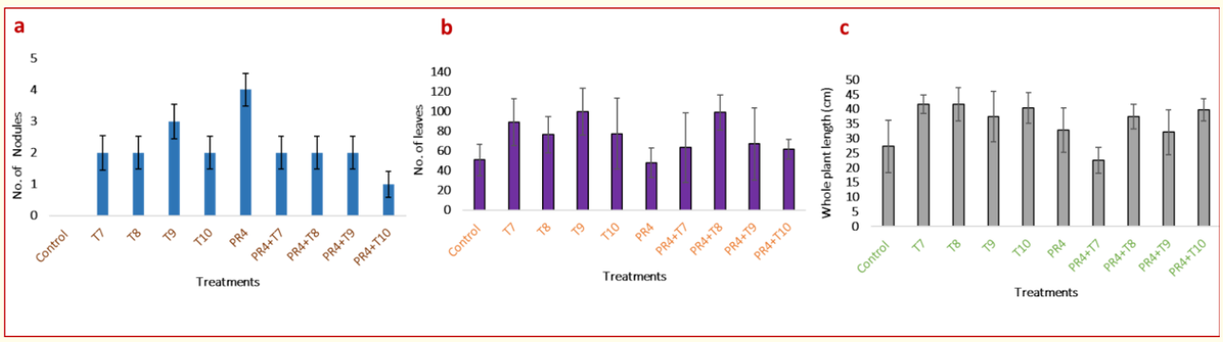

Figure 4: Display of (a) No nodules produced by plants treated with various organisms (b) No of leaves produces plants treated with various organisms (c) Whole plant length plant treated with various organisms.

Whereas we performed the compatibility test the *Trichoderma of* various species inhibit the growth of *Rhizobium* when we grow on the same medium provided. *Trichoderma longibrachiatum* cannot affect the *Rhizobium pusense* and other *Trichoderma species* which have we used they inhibit the growth of *Rhizobium. Pseudomonas aeruginosa* and *Trichoderma* in grow on same medium *Trichoderma* cannot affect the *pseudomonas aeruginosa. Pseudomonas aeruginosa* was resistance the *Trichoderma*. Longa., *et al*. 2017 have demonstrated that *Trichoderma* also inhibited the growth of *Rhizobium.* In this framework, root colonization assay *Trichoderma* has the potential to colonize chickpea plants' roots [21]. *Trichoderma* works as a biocontrol agent they also killed harmful pathogens from the soil, so it the around the root surface.

In this research work when we provided the formulation of *Trichoderma* + *Rhizobium*, *Rhizobium pusense* number nodules were produced 04. In the chickpea, plant nodulation decreased, as compared to but only *Trichoderma* has provided to increase the plant's root length, shoot length, and no leaves, as compared to the control plant no nodules were found and the length of the control plant decreased. Subsequently, Mulani., *et al*. 2023 demonstrated that peanut plant nodulation also decreases when the *Trichoderma longibranchium* + *Rhizobium* are provided [22]. No. of leaves also have increased. Khan., *et al*. 2023 under the nodulation decreased [27]. Since we were unable to identify the right method to suppress the growth of modulating bacteria as well as beneficial microorganisms in the soil, we were unable to carry out this study on all of the leguminous plants, their produced nodules, and the impact of different types of *Trichoderma* on early root development. Therefore, our future perspectives are employed *Trichoderma* and *Rhizobium* spp on various leguminous plants for nodule formation and study the metagenomics to get more idea about soil microflora that has been deprived by *Trichoderma*.

Conclusion

The present work said that *Trichoderma* species lived in soil for up to 2 months. *Trichoderma* species as would be assumed of a typical soil naturally saprophytic fungus, *Trichoderma* was able to use a variety of substances as its only sources of carbon and nitrogen. *Trichoderma* was used to create formulations for the research we conducted because it has been extensively studied and is currently marketed as biological pesticides, bio-fertilizers, and amendments for the soil because of its ability to protect plants, enhance vegetative growth, and reduce pathogen populations under various agricultural conditions. *Trichoderma* species also as a biocontrol agent and as a biofertilizer work. In the non leguminous plant, *Trichoderma* has the most effective proven.It enhances the plant growth-promoting factor as well as yield production. *Trichoderma* species encouraged plant growth, but they also quickly established the root system surface and helped the plant develop resistance to biotic and abiotic stress. Around the root surface of the plant, the *Trichoderma* quickly grew so the disease-causing pathogen cannot attack pathogen the plant, and for this reason, too they planted to lengthen the root and shoot length of the plant. The number of root nodules reduced when *Trichoderma* and *Rhizobium* were present together because *Trichoderma* prevents Rhizobium from growing. The *Trichoderma* inhibit the growth of *Rhizobium*, and *pseudomonas aeruginosa* resistance to the *Trichoderma* Furthermore, *Trichoderma* and species the inhibitor declined the growth of the root nodules. Leguminous plants the decreasing nodules so yield production decreases. In *Trichoderma,* it inhibits all leguminous plant nodules decreased or not they not studied it. In the future aspects of work, the more plant effect checked so, whether *Trichoderma* is an inhibitor or not proven.

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Conflict of Interest

The authors declare no competing interests.

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