RESEARCH | Egyptian Journal of Agricultural Research | PLANT PATHOLOGY

# Integration of *Trichoderma harzianum* with organic amendments for controlling major soil-borne diseases in chickpea

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**Received:** 18-02-2023 **; Accepted:** 11-09-2023 **; Published:** 17-02-2024 **DOI:** 10.21608/ejar.2023.194622.1367

## ABSTRACT

*In vitro* and *planta* field experiments were conducted to check the effect of *Trichoderma harzianum* organic amendments in controlling chickpea's major soil-borne diseases. *T. harzianum*, isolate Tri-9 was selected for its *in vitro* efficacy against *Sclerotium rolfsii* and *Rhizoctonia solani* the causal organisms of important chickpea diseases in the field. Subsequently, spore suspension and wheat grain colonized inoculum of isolate Tri-9 were prepared for further *planta* use. Three different organic amendments such as poultry refuse, cow dung, and mustard oil cake after being fortified with wheat grain colonized *T. harzianum* isolate Tri-9. Both the fortified soil amendments and Tri-9 spore suspensions were checked in the chickpea growing field under natural conditions. Besides disease suppression, the growth-promoting parameters and yield were also recorded. *Trichoderma* fortified poultry refuge was found significantly effective in plummeting pre-and post-emergence seedling mortality, disease incidence, and severity. All the treatments significantly increased the growth-promoting components, yield, and improved seed quality compared to those of untreated control. *Trichoderma* fortified compost mixed with poultry refuge was the best to control soil-borne diseases of chickpea as well increase yield and their quality.

**Keywords:** *T. harzianum*, *S. rolfsii*, *R. solani*, Organic amendments, Chickpea.

## INTRODUCTION

Pulse covered about 877.94 thousand hectares of the cultivated area of Bangladesh, of which 11.89 thousand ha area was covered by chickpea with an average yield of 0.49 t $ha^{-1}$ in 2018-2019 (BBS, 2019). Chickpea is the third most extensively cultivated pulse crop in Bangladesh, comprising approximately 12% of the overall pulse yield (*Salma et al.,* 2016). It has been traditionally cultivated in Bangladesh under rainfed conditions. About 85% of chickpea was grown in Jessore, Faridpur, Rajshahi, Kustia, Pabna, Chapai Nawabgonj, and Dinajpur districts. Most of these areas belong to the Agroecological zone (AEZ) 11 and 12 (Rashid *et al.,* 2014). There are many factors are responsible for the low production of chickpea whereas disease is the most important one (Jambhulkar *et al.,* 2015). There are more than 50 diseases that have been reported in different regions across the world, which affect this particular crop. (Nene, 1980; Fakir, 1983). The estimated yield of chickpea loss due to insects and diseases varies depending on the region, with temperate regions experiencing a loss of 5-10% and tropical regions seeing a much higher loss of 50-100%. (Kukreja *et al.,* 2018). Most of the diseases (> 30) are caused by fungi. So far, 17 chickpea diseases have been identified in Bangladesh, 12 of which are caused by fungus (Bakr *et al.,* 2007). Out of the 12 fungal diseases of chickpea, four are soil-borne, namely wilt, blight, Botrytis grey mould (BGM), root rot and collar rot (Bakr, 1994). It is well known that plant diseases deteriorate the yield quality and quantity, for instance collar rot disease can cause 10-30% chickpea yield loss annually (Maurya *et al.,* 2008). Therefore, it is assumed that these diseases limit the chickpea yield in Bangladesh in contrast to other chickpea cultivating countries of this world.

However, management of soil-borne diseases is challenging because of its long-lasting sclerotia, chlamydospores or fragmented mycelium in soil or crop residues (Mehta *et al.,* 2014; Panth *et al.,* 2020). The asexual resting spores of soil-borne fungi are highly tolerant under adverse environmental conditions and remain as inactive vegetative spore for up to 50 years (Khan and Rao, 2019). In this context, chemical control is generally preferred for soil-borne plant diseases due to its relatively rapid effectivity and easy operation (Panth *et al.,* 2020). Alternatively, continuous and overuse of chemical fungicides often causes water pollution, reduce soil fertility, eliminate non-target beneficial flora, increase greenhouse gas production and arises fungicide resistance pathogen variants (Arias-Estévez *et al.,* 2008; Komárek *et al.,* 2010). Therefore, emphasize should be given to alternative disease management technologies such as biocontrol to achieve sustainability in agriculture. According to Imran *et al.* (2020), the use of *T. harzianum* (a well-established bio control agent) is increasing in the current times as a substitute to chemical pesticides. However, the use of *T. harzianum* is restricted on research and its acceptance at farmers' level is not adequate yet in Bangladesh because of some issues such as; sluggish speed of reproduction and colonization, prone to biotic and abiotic stresses, partial abolition of pathogens. The study examines the use of *T. harzianum* for controlling crop diseases through an analysis of research conducted by experts in the field. It aims to identify strains of *T. harzianum* that can rapidly multiply and colonize, resist environmental stress, and target a diverse range of pathogens. Additionally, the study explores the current significance and mechanisms of *T. harzianum*, methods for its application and multiplication, challenges to its adoption on a large scale, and potential solutions to these challenges.

In addition, natural compost or soil amendment application to agricultural fields is an excellent natural alternative approach against soil-borne pathogens mitigation (Mehta *et al.,* 2014). The microbial communities present in compost or amendment are considered to be one of the major driving forces for plant pathogen suppressiveness of composts (Joshi *et al.,* 2009). Therefore, an attempt was taken to determine the effectiveness of *T. harzianum* integration with organic amendments in controlling soil-borne diseases of chickpea and enhancement of yield.

## MATERIAL AND METHODS

**Collection, isolation and preservation of microorganisms:**

The rhizosphere soil (approximately 500 g) of different vegetable (carrot, radish, tomato, potato, brinjal and chilli) crop fields of BSMRAU (24°02'16.5"N and 90°23'46.9"E) were collected to isolate fungi. Fungi were isolated from individual samples following standard technique (Mian, 1995). The culture was purified on PDA following hyphal tip culture technique (Tuite, 1969) and verified according to Swehla *et al.* (2020). A total of seven *Trichoderma* isolates were identified as *T. harzianum* on the basis of morphological characters following the prior references (Gams and Bissett, 2002; Siddiquee *et al.,* 2009; Sharma and Singh, 2014) and standard key (Barnett and Hunter, 1972). In addition, three pre-identified isolates were collected from the Plant Disease Diagnostic Clinic (PDDC) of the Department of Plant Pathology, BSMRAU. Similarly, two prior identified and tested virulent isolates of *S. rolfsii* and *R. solani* were collected from Department of Plant Pathology, BSMRAU, Bangladesh.

**Screening of *T. harzianum* isolates against virulent isolates of test pathogens:**

All 10 isolates of *T. harzianum* were separately screened *in vitro* against *R. solani* and *S. rolfsii* on 90 mm PDA plates by dual culture technique (Dhingra and Sinclair, 2017). The dual cultured plates inoculated with *T. harzianum* were observed closely during the incubation period. A distinct inhibition zone was observed of which arms and height were measured. Then, antagonistic effect of 10 isolates was recorded on the basis of the inhibiting potentiality after the incubation period. Radial growth of *R. solani* and *S. rolfsii* percentages were calculated according to Sunder *et al.* (1995)

**Efficacy of *T. harzianum* fortified compost in controlling chickpea soil-borne fungi:**

**Preparation of *T. harzianum* Tri 9 isolate fortified compost:**

Three different soil amendments, such as cow dung, poultry refuge and mustard oil cake were used to prepare the *T. harzianum* isolate Tri 9 fortified compost. Three compost pits (1.0 m x 1.0 m x 1.5 m) were prepared separately in the experimental field of BSMRAU. Each compost pit was filled with 40 kg each of soil amendments separately. Water was applied as per necessary to accelerate the decomposition process. *T. harzianum* isolate Tri 9 inoculum was prepared on wheat grain according to previously described method (Akter *et al.,* 2016; Rubayet and Bhuiyan, 2016). Twenty-one days old inoculum was used to fortified the decomposed soil amendments. After forty-five days of decay, wheat grain colonized inoculum of *T. harzianum* isolate Tri 9 inoculum was mixed @2.5 kg in every compost pit. Then it was left for 45 days for further decomposition according to Chang and Hsu (2008).

**Preparation of *T. harzianum* isolate Tri-9 spore suspension:**

The spore suspension was prepared using PDA plates from *T. harzianum* isolate Tri-9 that were six days old. PDA plates were flooded with 20 mL of sterilized distilled water, and the conidia were plucked gently with a sterile inoculation needle. The conidial suspension was agitated for 10 minutes before filtering through a fine mesh screen to remove the hyphal debris. Spore concentration was adjusted to 5 $x10^5$ $ml^{-1}$ using spore counting hemocytometer according to Chung and Hoitink (1990) dilution plate technique.

**Field preparation and application of soil treatment:**
The study was conducted in Gazipur, Bangladesh, which is situated in the Madhupur tract agroecological zone. The experimental site had shallow red-brown terrace soils and silty clay soil with poor nutrient content, a pH of 6.5, moderate rainfall, clear sunshine, and moderate temperature.

A Randomized Complete Block Design (RCBD) field experiment (plot size was 3 m × 2 m) was conducted to evaluate the most effective application method and formulation of *T. harzianum* isolate Tri-9 for controlling major diseases of chickpea. Chemical fertilizers such as Urea, Triple Super Phosphate (TSP), Muriate of Potash (MP) were applied @ of 25, 50, 21 kg per hectare. *Trichoderma* fortified composts @ 5kg plot $^{-1}$ (8.33 t ha $^{-1}$) were applied as treatment during the land preparation. Besides, well decomposed poultry refuge and mustard oil cake also applied as separate soil treatments. Including this soil amendments, there were 8 treatments such as $T_1$ = Fresh seeds (Control), $T_2$ = Seed treatment with *T. harzianum* isolate Tri-9 spore suspension (@5×10$^5$ ml$^{-1}$), $T_3$ = Foliar spray (@5×10$^5$ ml$^{-1}$) of *T. harzianum* isolate Tri-9 spore suspension (three times: 35, 50, and 65 days after sowing), $T_4$ = *T. harzianum* isolate Tri-9 fortified cow dung compost, $T_5$ = *T. harzianum* isolate Tri-9 fortified poultry refuge compost, $T_6$ = *T. harzianum* isolate Tri-9 fortified mustard oil cake compost, $T_7$ = Mustard oil cake, and $T_8$ = Poultry refuge.

**Seed sowing, intercultural operation:**
Chickpea variety BARI Chola-5 was collected as seed sample from Bangladesh Agricultural Research Institute (BARI). Seeds were sown by manually in the field on 26$^{th}$ December 2019. The row-to-row distance 20 cm, plant to plant distance 15 cm and depth 2.5 cm (approximately) was maintained during sowing of 200 seeds/6m$^2$ area. Immediately after sowing, seeds were covered with pulverized soil and gently pressed with hands, followed by light irrigation for better germination. Weeding mulching irrigation were done at an interval of 15 days and as per necessary.

**Disease and yield data recording:**
Mortality of chickpea were observed regularly after the seed sowing up to 4 weeks. In addition, two specific disease symptoms such as wet root rot (*R. solani*) and collar rot (*S. rolfsii*) developed were also recorded on the standing crop continuously at 3 days interval from 30 days of sowing to the final harvest. The severity of wet root rot and collar rot was evaluated using a scale of 0 to 4, with 0 indicating no symptoms and 4 indicating complete coverage of chickpea organs with lesions. The severity rating was based on the percentage of coverage with lesions, with higher numbers indicating more severe symptoms (Morid *et al.,* 2012). Disease incidence and severity were calculated by the following formulae:

$$\text{Disease incidence (\%)} = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plant\ in\ the\ plot} \times 100$$

$$\text{Percent disease index (PDI)} = \frac{Summation\ of\ all\ rating}{Number\ of\ plant\ observed \times Maximum\ ratings} \times 100$$

Five plants were selected randomly (from each plot separately) at 7 days prior to harvest to record the yield contributing characters such as plant height, number of branches per plant, were recorded. Pods were harvested on the last week of March 2020 and sun dried properly. Finally, number of pods/plants, 1000 seed weight, and seed yield per plot were recorded to calculate the yield per hectare of land.

**Data analysis**
The data collected on different diseases and yield components were subjected to statistical analysis using SPSS 24 software. When necessary, suitable transformations were carried out before performing the analysis. The means were compared using DMRT.

## RESULTS

**Screening of *T. harzianum* isolates against selected pathogens:**
All the 10 isolates of *T. harzianum* successfully controlled the growth of *R. solani* and *S. rolfsii* (Table 1 and Fig. 1) *in vitro*. The percentage of radial growth inhibition varied from 62.2 to 83.3% for *R. solani*, and 65.5 to 90.0% for *S. rolfsii*. The clear inhibition zone along with mycelial overgrowth of *T. harzianum* isolates against two specific soil-borne pathogen in the present *in vitro* test supports the mentioned mode of action. However, among the ten isolates, Tri-9 demonstrated highest %inhibition of radial growth, such as 83.3% and 90% for *R. solani* and *S. rolfsii* respectively (Table 1). Therefore, Tri-9 selected for further *in vivo* experiments in the present study.

**Table 1.** Percent inhibition of radial growth of *R. solani* and *S. rolfsii* by selected *T. harzianum* isolates

| **Isolates of *T. harzianum*** | **% inhibition of radial growth** | | | |
|---|---|---|---|---|
| | ***R. solani*** | **Bell's scale*** | ***S. rolfsii*** | **Bell's Scale*** |
| **Tri-1** | 66.6 | R2 | 68.8 | R2 |
| **Tri-2** | 65.5 | R2 | 71.1 | R2 |
| **Tri-3** | 68.8 | R2 | 65.5 | R2 |
| **Tri-4** | 70.0 | R2 | 77.7 | R1 |
| **Tri-5** | 62.2 | R2 | 67.7 | R2 |
| **Tri-6** | 73.3 | R2 | 78.8 | R1 |
| **Tri-7** | 72.2 | R2 | 74.4 | R2 |
| **Tri-8** | 66.6 | R2 | 65.5 | R2 |
| **Tri-9** | 83.3 | R1 | 90.0 | R1 |
| **Tri-10** | 71.1 | R2 | 68.8 | R2 |
| **Control** | 9 cm | - | 9 cm | - |

*$R_1$ =100% Overgrowth of *T. harzianum*, $R_2$= 75% Overgrowth of *T. harzianum*, $R_3$= 55% Overgrowth of *T. harzianum*, $R_4$= Block at point of contact and $R_5$= Pathogen overgrows against antagonist (Bell *et al.* 1982).

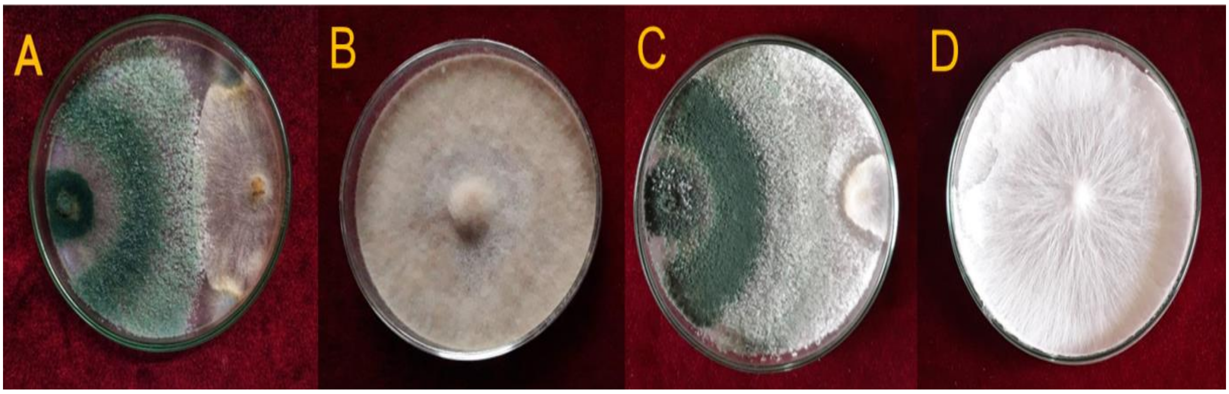


**Fig. 1.** *In vitro* antagonistic effect of *T. harzianum* isolate Tri 9 against *R. solani* and *S. rolfsii*. A) Dual culture plate of isolate Tri 9 against *R. solani*. B) Control plate of *R. solani*. C) Dual culture plate of isolate Tri 9 against *S. rolfsii*. D) Control plate of *S. rolfsii*.

**Efficacy of *T. harzianum* fortified compost in controlling chickpea soil-borne fungi:**

*T. harzianum* isolate Tri-9 fortified compost successfully reduced the natural disease development in the seedling and standing crop of chickpea in field. Significantly lower pre-and post-emergence chickpea seedling mortality were recorded after application of different Tri-9 fortified composts up to five weeks of the field growth (Fig. 2 and 3). Among the three Tri-9 fortified composts, the lowest pre-emergence seedling mortality (4.17%) was observed with the Tri-9 fortified poultry refuse compost. This result was followed by the Tri-9 fortified cow dung compost (6.25%) applied plot (Fig. 2A). Similarly, the lowest post emergence seedling mortality (7.38%) was recorded from Tri-9 fortified poultry refuse applied plot. However, Tri-9 fortified cow dung and mustard oil cake applied plot also provided statistically similar post-emergence seedling mortality (8.65%) with Tri-9 fortified PR applied plot (Fig. 2B). However, seed treatment/ foliar application of *T. harzianum* isolate Tri-9 or soil application of MOC/CD lowered both the pre- and post-emergence seedling mortality compared to control, but the results were significantly higher than the Tri-9 fortified poultry refuse applied plots.

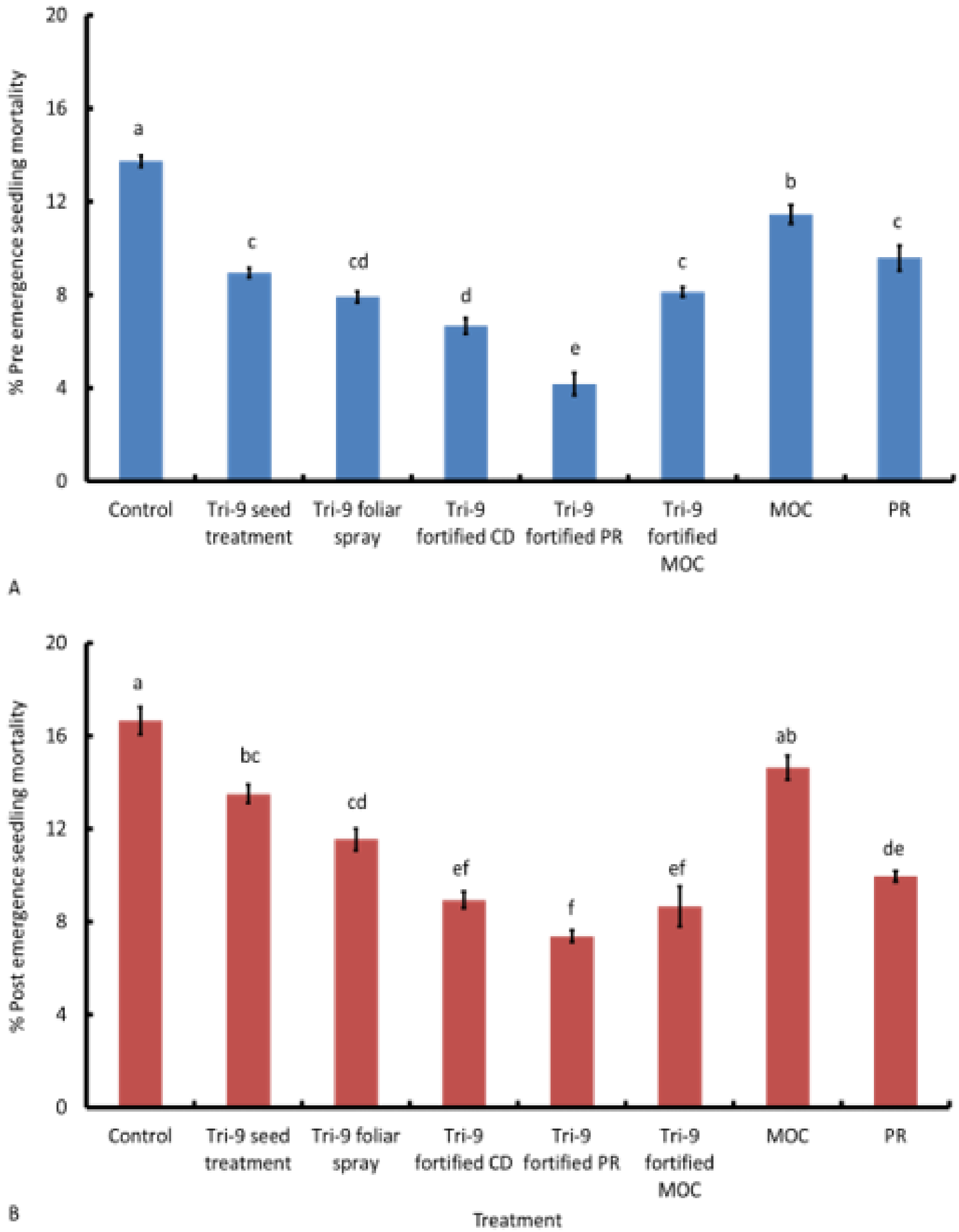


**Fig. 2.** Percent seedling mortality after applying different Tri-9 fortified compost. A) % pre-emergence seedling mortality. B) % post-emergence seedling mortality. Here, CD= Cow dung, PR= Poultry refuge, and MOC= Mustard oil cake.

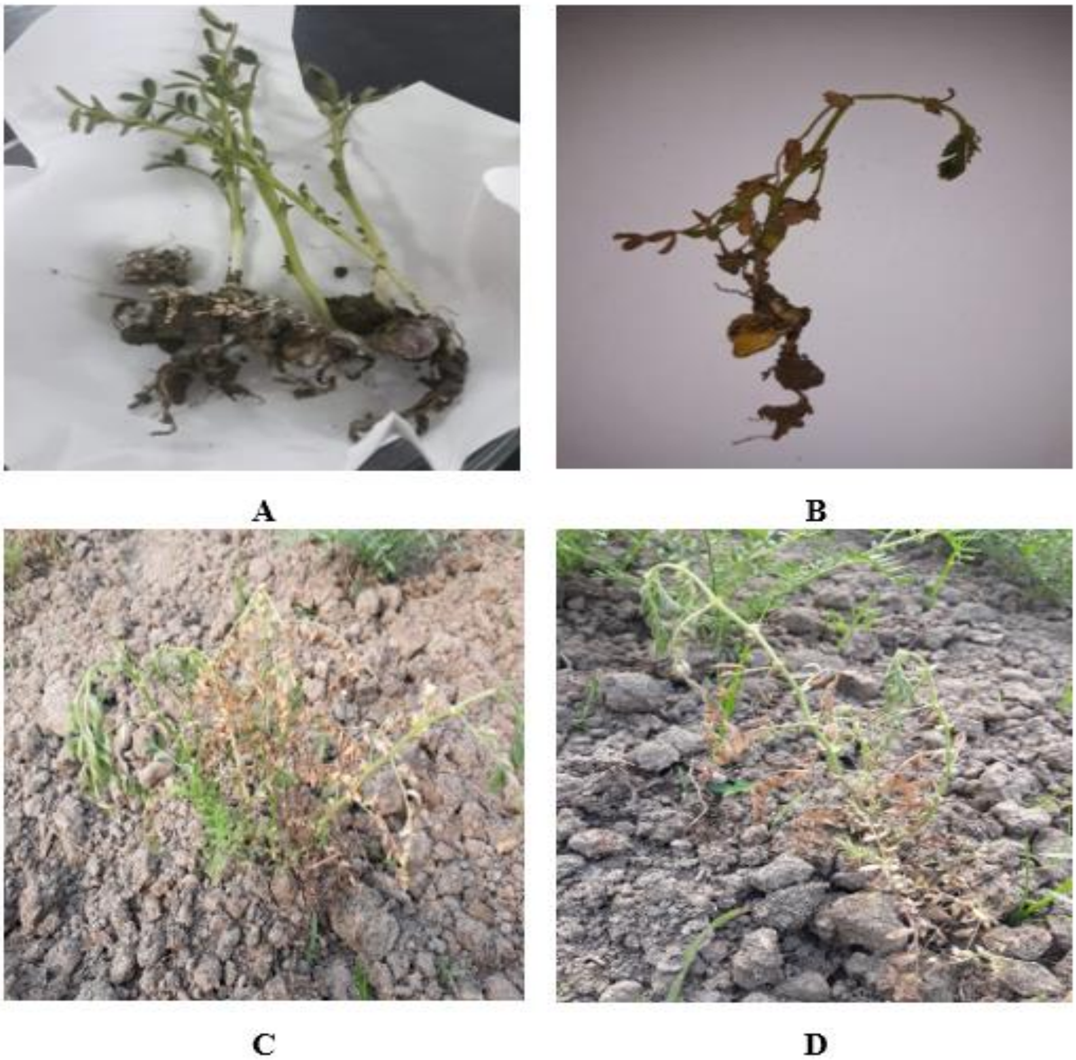

**Fig. 3.** Post-emergence seedling mortality and disease appearance in the experimental field of chickpea. A) Post-emergence seedling mortality by *S. rolfsii*; B) disease appearance in chickpea field caused by *S. rolfsii*; C) Post-emergence seedling mortality by *R. solani*; D) disease appearance in chickpea field caused by *R. solani*.

Tri-9 fortified compost significantly reduced the incidence and severity of two specific disease symptoms such as collar rot (*S. rolfsii*) and wet root rot (*R. solani*), compared to control in the standing chickpea during the entire growing period (Fig. 4). However, both the disease incidences and severity were significantly varied among different treatment applied plots (Fig. 4). The highest disease incidence was recorded in the untreated control plot in case of both diseases (27.08% and 27.71% for collar rot and root rot, respectively). Whereas, the lowest disease incidence (16.67% and 15.21% for collar rot and wet root rot, respectively) were recorded from Tri-9 fortified PR treated plots. In addition, Tri-9 fortified cow dung (17.50%) Tri-9 fortified mustard oil cake (18.54%), and soil amendments with poultry refuge (19.17%) also reduced collar rot incidence compared to control. In the meantime, Tri-9 fortified cow dung applied field provided 17.08% wet root rot incidence, which was statistically similar with Tri-9 fortified poultry refuge applied soil (Fig. 4A).

Similarly, the highest PDI (40.20% and 42.15% for collar rot and wet root rot, respectively), were recorded from the control treatment where no antagonist was applied (Fig. 4B). The disease was significantly lower in all the treatments as compared to the untreated control. The lowest PDI (compared to control) was observed in the treatment Tri-9 fortified poultry refuge (13.20 and 14.38% for collar rot and wet root rot, respectively) applied plot. In addition, Tri-9 fortified cow dung (15.95% and 16.60%) and Tri-9 fortified mustard oil cake (21.10% and 21.55%) also reduced the PDI of both diseases (Fig. 4B).

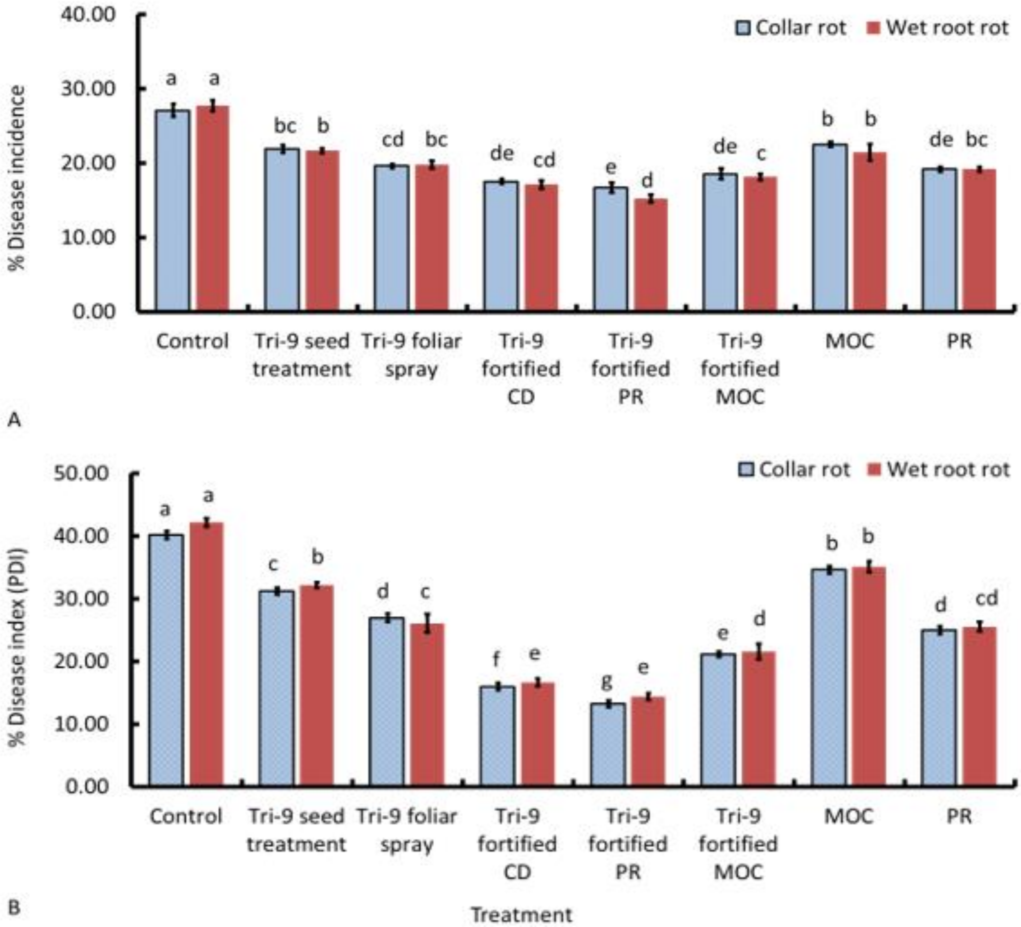


**Fig. 4.** Percent disease incidence and percent disease index (disease severity) of chickpea after applying different Tri-9 fortified compost. A) % disease incidence. B) % post emergence seedling mortality. Here, CD= Cow dung, PR= Poultry refuge, and MOC= Mustard oil cake.

**Performance of different organic amendments along with *T. harzianum* on chickpea**

All the treatments increased the yield contributing characters including the number of branch plant$^{-1}$, plant height, No of pods plant$^{-1}$, and 1000 seed weight/g compared to those of untreated control (Table 2). The yield contributing characters and yield were significantly different among different treatments. The plant height varied from 43.5 to 55.50 cm in different treatment. However, the highest plant height (55.50) and number of branches (4.75) were obtained from Tri-9 fortified PR treated plots. Whereas, other two Tri-9 fortified composts (CD and MOC) provided statistically similar results with the previous one in case of plant height and number of branches. Compared to these results, statistically lower plant height and number of branches were recorded when Tri-9 or soil amendments were applied separately. The highest no of pods per plants (42.25) were recorded from tri-9 fortified PR compost applied plots. All other treatments, including Tri-9 fortified composts (CD and PR), or separate application provided statistically lower result (33.50 to 39.50) compared to the Tri-9 fortified PR applied plot. The 1000 seed weight and seed yield were highest (160.88 g and 1.61 t ha$^{-1}$) in the Tri-9 fortified PR applied plot and was statistically similar with other two Tri-9 fortified compost applied plots.

Finally, the highest yield over control was also recorded from the same treatment, Tri-9 fortified PR compost. It could be concluded that, Tri-9 fortified composts were effective in respect to yield contributing characters and yield. Among the three fortified composts, the performance of Tri-9 fortified PR compost was best. However, seed treatment or foliar spray of Tri-9 spore suspension and soil application of MOC or PR provided better result in compare to control.

**Table 2.** Effect of *T. harzianum* isolate Tri-9 fortified organic compost on yield contributing characters and yield of chickpea in the field

| Treatment | Plant height (cm) | No. of branches plant$^{-1}$ | No of pods plant$^{-1}$ | 1000 seed wt. (g) | Seed yield (t ha$^{-1}$) | % increase of seed yield (Over control) |
|---|---|---|---|---|---|---|
| Control | 41.25 (±0.48) d | 2.50 (±0.29) c | 31.75 (±0.48) e | 138.75 (±1.49) e | 1.07 (±0.02) d | - |
| Tri-9 seed treatment | 43.5 (±0.29) cd | 2.75 (±0.25) bc | 33.50 (±0.29) e | 148.00 (±0.82) cd | 1.34 (±0.06) bc | 25.65 (±3.92) d |
| Tri-9 foliar spray | 45.50 (0.50) c | 3.25 (±0.25) bc | 34.25 (±0.25) de | 149.00 (±0.58) c | 1.44 (±0.04) abc | 34.91 (±1.28) c |
| Tri-9 fortified CD | 52.25 (±1.11) ab | 3.75 (±0.25) ab | 39.50 (±0.65) b | 157.75 (±1.03) ab | 1.57 (±0.04) a | 46.93 (±0.61) ab |
| Tri-9 fortified PR | 55.50 (±0.65) a | 4.75 (±0.25) a | 42.25 (±0.63) a | 160.88 (±1.61) a | 1.61 (±0.04) a | 50.71 (±2.21) a |
| Tri-9 fortified MOC | 51.50 (±1.55) b | 3.75 (±0.25) ab | 37.75 (±0.85) bc | 156.75 (±0.48) ab | 1.53 (±0.03) ab | 43.43 (±0.48) abc |
| MOC | 42.25 (±0.48) cd | 2.75 (±0.25) bc | 33.25 (±0.25) e | 143.25 (±1.38) de | 1.31 (±0.05) c | 22.88 (±2.23) d |
| PR | 49.50 (±0.65) b | 3.50 (±0.29) bc | 36.50 (±0.65) cd | 153.25 (±1.38) bc | 1.49 (±0.04) abc | 40.11 (±0.89) bc |

CD= Cow dung, PR= Poultry refuge, and MOC= Mustard oil cake. Numbers in parenthesis (± 0.00) indicate the SE of each mean. Means within same column followed by bold common letter(s) are significantly different (*p=0.05*) by DMRT

## DISCUSSION

The effective *in vitro* antagonism of *T. harzianum* isolates against different pathogen of different host crop such as *Alternaria porri* (onion), *F. oxysporum* f. sp. *lentis*, *F. oxysporum* f. sp. *ciceri*, (lentil, chick pea), *R. solani* (lentil, chickpea), *S. rolfsii* (potato, carrot, chick pea), *Macrophomina phaseolina* (mungbean, soybean), *Colletotrichum capsici* (Chilli) has been reported recently (Akter *et al.,* 2016; Rubayet and Bhuiyan 2016; Talukdar *et al.,* 2017; Das *et al.,* 2019; Simi et al., 2019; Rubayet *et al.,* 2020; Swehla *et al.,* 2020; Rahman *et al.,* 2021; Roy *et al.,* 2022). All these studies including the present study revealed two important factors of the *in vitro* antagonism efficacy of a bioagent. The antagonistic efficacy of *T. harzianum* isolates can be varied according to the variation of the test pathogen or according the isolate variation of the mentioned antagonist. Al-Saeedi and Al-Ani (2014) reviewed that, this different level of antagonism might be due to either to the presence of different types of chemical constituents in different isolates, or interaction of several genes of both pathogen and antagonist. In addition, Sevugapperumal *et al.* (2018) listed a number of modes of action which involves the biocontrol efficacy of *Trichoderma* species including production of lytic enzyme.

Biological management of chick pea diseases (wilt, collar rot, wet root rot) through *T. harzianum* is an important and continuously studied research issue in different countries of the Subcontinent (Khan *et al.,* 2014; Nirmalkar *et al.,* 2017; Talukdar *et al.,* 2017). According to the present study, the *T. harzianum* fortified compost provided better performance in suppressing natural chickpea diseases, compare to the single application of either *T. harzianum* or compost. It is known that *Trichoderma* spp. possessed a number of mode of actions against plant pathogen, such as mycoparasitism, competition, antibiosis and induced resistance (Harman *et al.,* 2004; Sevugapperumal *et al.,* 2018; Bahadur and Dutta, 2022; Rubayet and Bhuiyan, 2023). *Trichoderma* can compete with plant pathogens for nutrients and space, thereby limiting their growth and proliferation. It can produce secondary metabolites such as antibiotics and enzymes (chitinases and glucanases) that can kill or inhibit the growth of plant pathogens. Additionally, *Trichoderma* can induce a plant's defense response and enhance its ability to resist infection by pathogens. Moreover, *Trichoderma* helps to reduce the population and activity of plant pathogens in the soil, leading to healthier and more productive plants. Therefore the *in vitro* pathogenic growth and *in vivo* disease suppression by *T. harzianum* isolates Tri-9 in the present study could be due to one or more of these modes of action. In addition, the substrate such as poultry refuse served as an ideal food base for the antagonistic *T. harzianum* isolates Tri-9. Therefore, the fortified compost increased the microbial biomass of *Trichoderma* spp. and increased the disease suppressive effect against chickpea collar rot and wet root rot. It also aids introduction and establishment of *Trichoderma* into the soil as soil micro biota for sustained biocontrol activities. The result of current study suggests the superiority poultry refuse as a base substrate of *Trichoderma* fortified compost over other bases substrates in controlling seedling mortality of chickpea and other crops ( Morsy and El-korany, 2007; Uddin *et al.,* 2011; Akter *et al.,* 2016; Talukdar *et al.,* 2017; Bhuiyan and Rubayet, 2023).

*Trichoderma* species have the ability to modify the rhizosphere soil environment of plants, leading to enhanced plant growth (Halifu *et al.,* 2019). When *Trichoderma* species colonize plant roots, it can result in significant alterations to the plant's proteome and metabolic processes. This association can lead to an improvement in nutrient uptake, increased root and shoot growth and development, enhanced crop productivity, and greater resilience to abiotic stresses. (Benítez *et al.,* 2004; Harman *et al.,* 2004; Bahadur and Dutta, 2022). Similar to the present investigation, the improved seed germination, shoot length, root length, vigour index of chickpea due to soil application *T. harzianum* fortified farm yard manure has been reported previously (Jambhulkar *et al.,* 2015; Sharma *et al.,* 2018). Recently, Vasava *et al.* (2019), suggested the integrated application of chemical seed treatment along with soil application of *T. harzianum* fortified compost as a successful technology to improve chickpea yield in the farmer's plot in India. The result of the present study was in consistent with several investigators (Talukdar *et al.,* 2017; Das *et al.,* 2019; Liton *et al.,* 2019; Ahmed *et al.,* 2019; Rahman *et al.,* 2020a; Rahman *et al.,* 2020b) who applied *Trichoderma* fortified compost to reduce disease and increase crop yield of chickpea, bush bean, lentil, carrot, Soybean in Bangladesh. Here *Trichoderma* fortified compost with poultry manure appeared to be the promising amendment along with *T. harzianum* in controlling soil-borne diseases of chickpea. However, no chemical treatment was compared in the present study and in Talukdar *et al.* (2017).

Therefore, it is important to compare the chemical treatment with the present finding to draw a firm conclusion. Finally, a multi-location field trial is needed to evaluate the present finding as a green technology.

## CONCLUSION

*T. harzianum* isolate Tri-9 was found to be the most effective antagonist against *R. solani* and *S. rolfsii* in in vitro screening. Additionally, *Trichoderma* isolate Tri-9 fortified compost mixed with the poultry refuge @833kg/ha was appeared to be promising amendment of *Trichoderma* in controlling soil-borne diseases of chickpea and increasing yield. This method is to be sustainable and ecofriendly for soil-borne diseases management across the globe.

**Acknowledgments:**

The authors are thankful to the Ministry of Science and Technology in Dhaka, Bangladesh, for providing financial assistance for this research.

**Funding:**

Ministry of Science and Technology, Dhaka, Bangladesh.

**Conflict of Interest:** The authors declare no conflict of interest.

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