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Use of bio-agent and organics for sustainable management of pea root rot

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Key words: Root rot, *Trichoderma,* Bioagents, Organics, *Fusarium solani*

Abstract

Efficacies of *Trichoderma*, neem and garlic extract were evaluated for managing pea root rot disease sustainably through seed treatment. The experiment employed six treatments alongside a control (T_0= Control, T_1= *Trichoderma viride*, T_2= *Trichoderma harzianum*-BD, T_3= *Trichoderma harzianum*-TH, T_4= Neem extract, T_5= Garlic extract, T_6= Chemical i.e., Carbendazim 50 WP) in a randomized complete block design. Results from *in vitro* experiments revealed substantial inhibition of *Fusarium solani* mycelial growth by *Trichoderma* and plant extracts. *Trichoderma harzianum*-TH exhibited the highest activity in suppressing fungal growth, closely followed by chemical treatment. Additionally, garlic extract showed notable inhibition in fungi. Field trials demonstrated significant reductions in both root rot incidence and severity of diseases in treated plots. Plots treated with *Trichoderma harzianum*-TH showed the most promising outcomes in regards to *in vitro* and field experiment. Moreover, *Trichoderma viride*, *Trichoderma harzianum*-BD, and neem extract also exhibited considerable effectiveness in curbing disease progression. This research contributes valuable insights into sustainable disease management strategies for enhancing pea crop productivity and resilience.

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Introduction

Bangladesh with its predominant agricultural economy relies heavily on a variety of crops for sustenance. Pulses following cereals emerge as the second-most crucial crop category providing vital protein sources for the populace. Among these pulses green pea (*Pisum sativum*) stands out as a significant crop cultivated alongside other varieties like lentil, chickpea, and grass pea. The cultivation of peas in Bangladesh spans the Rabi season, typically as a monoculture but occasionally intercropped with wheat, mustard, and other crops. Peas belonging to the Leguminosae family are annually grown crops with a life cycle of one year. They are extensively cultivated worldwide with nations like India, China, the UK, and the US contributing significantly to their production. In Bangladesh pea remains a staple crop with green pod yields ranging from 2500 to 4500 pounds per acre and dry pea yields ranging from 1200 to 2000 pounds per acre (Sharma *et al.*, 2022). This crop was recently grown on 18501 acres in Bangladesh, yielding 435.22 kg per acre and 8051.97 tons of output per year (BBS, 2021). Peas hold substantial nutritional value addressing protein deficiency and serving as a rich source of fiber, minerals, and antioxidants. Their nutrient profile encompassing essential minerals like phosphorus, manganese, and vitamins such as B6 and K (Dixit *et al.*, 2023) underscores their significance in combating chronic diseases like cancer and heart disease. However, peas are susceptible to root rot; a fungal disease caused by pathogens like *Rhizoctonia solani* and *Fusarium solani* (Kaur *et al.,* 2024). The affected plants exhibit stunted growth, yellowing leaves, weakening roots that darken with black lesions and a reddish discoloration on the lower stem. Additionally, the taproot suffers severe rotting. Consequently, this disease detrimentally impacts plant development, leading to decreased yields. Approximately 46% of pea plants in Bangladesh are afflicted by root rot disease (Anonymous, 1986). This disease commonly infects pea plants at an early stage, hampering their proper growth resulting ultimate yield loss. To mitigate the impact of root rot on pea crops, the application of bioagents such as *Trichoderma* spp. and plant extracts has gained attention. *Trichoderma* spp. have already used in reducing disease incidence and severity, thereby enhancing yield. Imran *et al.* (2023) demonstrated that *Trichoderma* spp. effectively controls common diseases like root rot, reducing both disease incidence and severity while simultaneously increase yield. Similarly, plant extracts as organics derived from sources like neem leaves, garlic, and cloves exhibit fungicidal properties, contributing to sustainable disease management. Plant extracts have been utilized by numerous researchers in agriculture to regulate pathogens, as evidenced by studies conducted by Meena (2020), Khan *et al.* (2020), and Lakshmeesha *et al.* (2019). According to Ncise (2020), these extracts exert a potent fungicidal effect, effectively suppressing phytopathogenic organisms. Integrated approaches combining biocontrol agents and plant extracts present promising avenues for managing root rot in pea crops effectively. This study aims to explore *in vitro* and field effects of bioagents and plant extracts ie. neem (*Azadirachta indica*) and garlic extracts (*Allium sativum)* on the root rot pathogen of pea.

Materials and methods

The study conducted at the Plant Pathology Laboratory and Agronomy Experimental Field, University of Rajshahi, from November 2021 to March 2022, aimed to assess the efficacy of biological agents and plant extracts (neem and garlic) for sustainable management of pea root rot through seed treatment. The experiment utilized one variety of pea and seven treatments comprised T_0 (Control), T_1 (*Trichoderma viride*), T_2 (*Trichoderma harzianum*-BD), T_3 (*Trichoderma harzianum*-TH), T_4 (Neem extract), T_5 (Garlic extract), and T_6 (Chemical). The experiment was laid out in RCBD with 3 replications.

Collection and culturing of Trichoderma spp.

Three *Trichoderma* strains, including *Trichoderma viride* collected from the plant pathology lab. at the Dept. of AAE, R.U., Bangladesh; *Trichoderma harzianum*-BD; obtained from the Disease Resistance Lab. from the Department of Plant Pathology, BAU, Mymensingh, Bangladesh; and *Trichoderma* *harzianum*-TH sourced from Thailand through Jate Sathornkich; Researcher at the Horticulture Innovation Lab., Regional Center of Kasetsart University were cultured in the Plant Pathology Laboratory at the University of Rajshahi. These strains were inoculated onto previously prepared PDA plates and incubated at 25±2°C for 7 days until green conidia appeared. The harvested conidia were preserved at 4°C in a refrigerator for future use.

Preparation of plant extracts

Neem (*Azadirachta indica*) and garlic extracts (*Allium sativum*) were prepared using sterile distilled water at a concentration of 2.5%. Fresh and healthy plant parts, totaling 25 grams, were washed, dried and blended with 100 ml of sterile distilled water for 10 minutes to create a fine paste. The resulting aqueous solutions were separated from the blended neem leaf and garlic bulb extracts which used to make culture media for the growth of respected fungi.

Seed treatment with Trichoderma, plant extracts and chemical

For *Trichoderma* treatment, harvested conidia were mixed with distilled water to achieve a concentration of 1x10^{-7}cfu/ml. The required quantity of seeds was then immersed in this *Trichoderma* solution, thoroughly mixed, and air-dried under laminar airflow. Alternatively, for plant extract treatment, 36 grams of pea seeds were placed in a sterilized beaker and soaked in 500 ml of each plant extract for 2 minutes with continuous stirring. Control seeds were soaked only in distilled water. After treatment, all seeds were dried in sunlight and stored in airtight polythene bags at 15°C. Carbendazim, used for chemical treatment, was prepared at a concentration of 0.25% and applied to seeds in a similar manner.

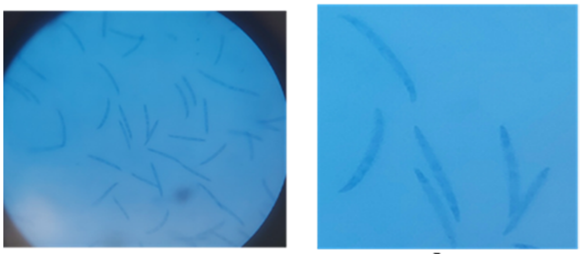
Phytopathological study

Collection of infected roots of pea and isolation of Fusarium solani

Infected plant samples were collected and brought to the Plant Pathology Laboratory. These samples underwent thorough washing and surface sterilization before being dissected into small pieces. These pieces were then plated onto Potato Dextrose Agar (PDA) plates and incubated for 7 days at room temperature. Fungal growth on the plates was observed, and any fungi present were isolated using hyphal tip culture method. For fungi producing spores, a single spore culture method was employed to obtain pure cultures. The resulting pure cultures were preserved as stock cultures in PDA slants at 4°C in a refrigerator.

Identification and growth study of the isolated fungi

The isolated fungi were identified by assessing their morphology, growth characteristics, colony color, conidia, and sclerotia following the guidelines from standard mycological literature such as Booth (1971) and Singh (1982). *Fusarium solani* exhibits three types of microscopic spores: small, one-celled elliptical microconidia, larger, septate, slightly curved macroconidia, and thick-walled rounded chlamydospores (Fig. 1).



a b

Fig. 1. Microscopic view of *Fusarium solani* (a-overall, b-close view)

Inhibition of mycelial growth in root rot fungi by Trichoderma on dual plate

Trichoderma sp. was placed on one side of the PDA plates, while root rot fungi were placed on the opposite side using 6mm blocks. The distance between the blocks was measured. Control plates only had pathogenic fungi at the center. The plates were then put in a growth chamber and checked regularly for fungal growth. When *Trichoderma* came into contact with the pathogenic fungi, their growth was slowed down. The percentage of inhibition of the pathogenic fungus was calculated using the following formula.

Inhibition (%) = $\{(X-Y)/X\} \times 100$

Where,

X = Mycelial growth of the root rot fungus alone without antagonist (control).

Y = Mycelial growth of the root rot fungus dual plate with antagonist.

Determination of growth inhibition by plant extracts

Appropriate proportions of prepared garlic and neem extracts were mixed during PDA preparation. Subsequently, PDA plates were inoculated with cultured pathogen blocks measuring 6mm in diameter and placed for incubation. Following the incubation period, the mycelial growth of the isolated pathogen on treated PDA plates was measured in diameter for the next 7 days. Finally, the growth was compared to that of the control.

Field study

Conducting the field experiment

For the field experiment, the land was thoroughly prepared, clearing weeds and stubbles. Fertilizers were applied according to crop recommendations. Before sowing, seeds underwent treatment with *Trichoderma*, plant extracts and chemical respectively and testing of seed germination was observed by blotter method in the laboratory. Seeds were sown in rows using the line sowing method, with 1200 seeds per plot at a depth of 5 cm. Intercultural operations were performed on schedule. Plots were regularly observed for changes in plant characteristics and pest attacks. Root rot disease incidences and severities were recorded at 15-day intervals during crop growth stages.

Data analysis

The collected data were organized and analyzed statistically using the SPSS program (version 2022). Mean differences were assessed using Duncan's Multiple Range Test.

Results and discussion

Effect of seed treatment on germination of pea

Pea seed showed the significant difference of germination rate. All six treatments are applied on the pea for seed germination. The maximum germination (95.66%) was recorded from T_3 (*Trichoderma harzianum*-TH) and the second highest germination (94%) was found in T_2 (*Trichoderma viride*) while minimum germination (78%) was recorded from T_0 (control). Considering the plant extracts T_5 (Garlic extract) responded the most (Fig. 2).

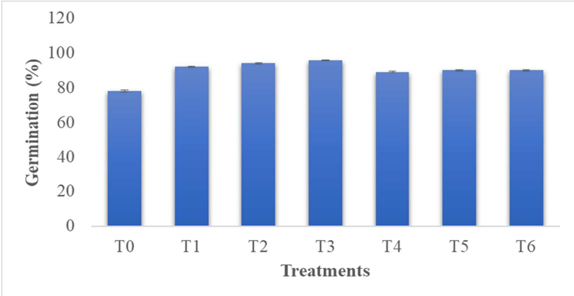


Fig. 2. Effect of seed treatment on germination of pea. Here, T_0= Control, T_1= *Trichoderma viride*, T_2= *Trichoderma harzianum*-BD, T_3= *Trichoderma harzianum*-TH, T_4= Neem extract, T_5= Garlic extract, T_6= Chemical (Carbendazim 50 WP). BD= Bangladesh and TH= Thailand

Inhibited mycelial growth of Fusarium solani by Trichoderma and plant extracts

Growth inhibition of *Fusarium solani* by *Trichoderma* spp. and plant extracts were assessed using dual culture technique on the fifth day of incubation. *Trichoderma harzianum*-TH (T_3) displayed the highest inhibition rate at 69.88%, followed by the chemical treatment (T_6) with 65.03% of inhibition. Among the bioagents, *Trichoderma viride* (T_1) exhibited 62.65% inhibition, while *Trichoderma harzianum*-BD (T_2) showed the lowest inhibition of 57.80%. In terms of organics, Garlic extract (T_5) demonstrated the highest inhibition at 59.03%, while Neem extract (T_4) showed inhibition at 57.80% (Table 1, Fig. 3).

Table 1. Inhibited mycelial growth of *Fusarium solani by Trichoderma and plant extracts*

Treatment	(%) Inhibition
T_1	$62.65^{bc}\pm1.04$
T_2	$57.80^{d}\pm1.52$
T_3	$69.88^{a}\pm1.05$
T_4	$57.80^{d}\pm1.52$
T_5	$59.03^{cd}\pm1.03$
T_6	$65.03^{b}\pm1.46$
Level of significance	***

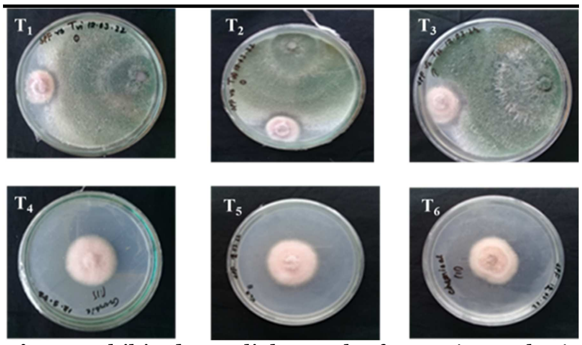


Fig. 3. Inhibited mycelial growth of *Fusarium solani* by *Trichoderma* and plant extracts

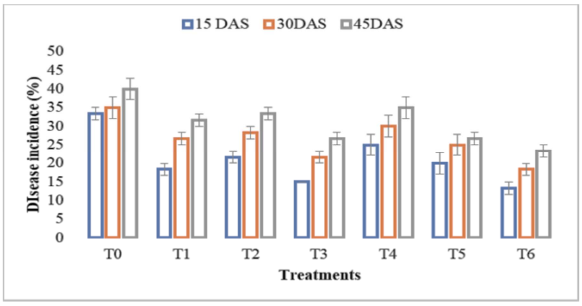


Fig. 4. Effect of seed treatment on root rot incidence (%) of pea in the field. Here, T_0= Control, T_1= *Trichoderma viride*, T_2= *Trichoderma harzianum*-BD, T_3= *Trichoderma harzianum*-TH, T_4= Neem extract, T_5= Garlic extract, T_6= Chemical (Carbendazim 50 WP). BD= Bangladesh and TH= Thailand

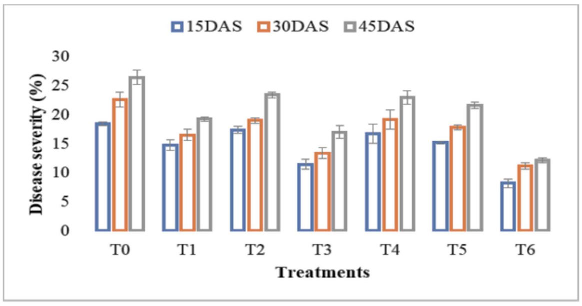


Fig. 5. Effect of seed treatment on root rot severity (%) of pea in the field. Here, T_0= Control, T_1= *Trichoderma viride*, T_2= *Trichoderma harzianum*-BD, T_3= *Trichoderma harzianum*-TH, T_4= Neem extract, T_5= Garlic extract, T_6= Chemical (Carbendazim 50 WP). BD= Bangladesh and TH= Thailand

Effect of seed treatment on a root rot incidence (%) of pea in the field

Treated pea plots exhibited significantly reduced disease incidence compared to untreated controls. Disease incidence was lowest at the early stage (15 DAS), gradually increasing thereafter (30 DAS, 45 DAS).

At 15 DAS, the highest disease incidence was observed in the control group (T_0) at 33.33%, while the lowest was in plots treated with *Trichoderma harzianum*-TH (T_3) at 15% and chemical treatment (T_6) at 13.33%. At 30 DAS, the highest disease incidence was in the control group (T_0) at 35%, whereas the lowest was in plots treated with *Trichoderma harzianum*-TH (T_3) and chemical treatment (T_6) at 21.66% and 18.33%, respectively (Fig. 4). At 45 DAS, the highest disease incidence was in the control group (T_0) at 40%, whereas the lowest was in plots treated with *Trichoderma harzianum*-TH (T_3) and chemical treatment (T_6) at 26.66%. Other treatments, including *Trichoderma viride* (T_1), *Trichoderma harzianum*-BD (T_2), and Neem extract (T_4), also exhibited positive results compared to the control.

Effect of seed treatment on a root rot severity (%) of pea in the field

In this field experiment, treated pea plots exhibited significantly reduced disease severity compared to untreated control. Disease severity was lowest at the early stage (15 DAS) but increased progressively thereafter (30 DAS, 45 DAS). At 15 DAS, the highest disease severity was observed in the control group (T_0) at 18.44%, while the lowest severity was in plots treated with chemical (T_6) at 8.16%, followed by *Trichoderma harzianum*-TH (T_3) at 11.41%. At 30 DAS, the highest disease severity was in the control group (T_0) at 22.58%, while the lowest severity was in plots treated with chemical (T_6) at 11.10%, followed by *Trichoderma harzianum*-TH (T_3) at 13.33% (Fig. 5). At 45 DAS, the highest disease severity was observed in the control group (T_0) at 26.41%, while the lowest severity was in plots treated with chemical (T_6) at 12.08%, followed by *Trichoderma harzianum*-TH (T_3) at 17%. *Trichoderma viride* (T_1) and *Trichoderma harzianum*-BD (T_2) also showed lower disease severity. For plant extracts, garlic extract (T_5) exhibited 21.58% severity and neem extract (T_4) showed 22.91%. Overall, *Trichoderma* spp. and plant extracts demonstrated effective reduction in disease severity compared to the control, presenting a safer alternative to Agrochemicals.

Discussion

Trichoderma spp. have been renowned for their effectiveness in controlling plant pathogens, providing a more environmentally friendly option compared to chemical pesticides. *Trichoderma* spp. control disease by competing with phytopathogens for space and nutrients (Woo *et al.*, 2023). *Trichoderma* spp. is rapidly growing fungi that establish a protective barrier around the roots and aerial parts of plants, colonizing the rhizosphere and phyllosphere (Halifu *et al.*, 2019). Their colonization hinders the growth of harmful organisms by depriving them of necessary nutrients, thus decreasing their ability to infect and cause diseases. *Trichoderma* spp. have the ability to produce a diverse range of secondary metabolites (Lakhdari *et al.*, 2023), such as antibiotics, enzymes, and volatile organic compounds. These antimicrobial compounds have the dual effect of inhibiting pathogen's growth and boosting plant defense mechanisms, thereby strengthening the plant's resistance to infection (Singh *et al.*, 2023). In addition, studies have demonstrated *Trichoderma* spp. can activate systemic resistance in plants (Dutta *et al.*, 2023), preparing them for better defense against a wide range of pathogens. When *Trichoderma* spp. is detected, plants respond by activating different signaling pathways that lead to the production of defense-related proteins, phytohormones, and secondary metabolites (Lakhdari *et al.*, 2023). This systemic defense response not only offers protection against the target pathogen but also grants long-lasting immunity against future infections, a phenomenon referred to as induced systemic resistance (ISR) (Dutta *et al.*, 2023). Neem extract and garlic extract have gained substantial popularity as biocontrol agents for managing plant diseases. The neem tree (*Azadirachta indica*) produces neem extract, which contains bioactive compounds like azadirachtin, nimbin, and nimbidin (Wasim *et al.*, 2023). These compounds have strong antimicrobial properties and can effectively combat a variety of plant pathogens. These compounds disrupt important physiological processes in pathogens, such as cell division, membrane function, and enzyme activity (Su *et al.*, 2023). This ultimately hinders the growth and reproduction of the pathogens. In addition, research has demonstrated that neem extract can stimulate systemic acquired resistance (SAR) in plants, leading to the synthesis of defense-related proteins, phytoalexins, and antioxidants (Yousif *et al.*, 2023). This systemic defense response prepares plants for stronger resistance against pathogens, resulting in a decrease in disease occurrence and intensity. In the same vein, garlic extract derived from Allium sativum bulbs, contains sulfur-containing compounds like allicin, ajoene, and diallyl sulphide (Okoro *et al.*, 2023) which have powerful antimicrobial properties. These compounds interfere with crucial cellular processes in pathogens, such as cell wall synthesis, respiration, and DNA replication (Moiketsi *et al.*, 2023). This ultimately hinders the growth and virulence of the pathogens. In addition, garlic extract has the ability to enhance the production of reactive oxygen species (ROS) in plants (Das *et al.*, 2024) which in turn triggers defense mechanisms like hypersensitive response (HR) and the synthesis of pathogenesis-related (PR) proteins (Nadarajah *et al.*, 2024). This localized defense response strengthens the plant's ability to resist invading pathogens, effectively reducing the spread and severity of diseases.

Conclusion

The study concludes that *Trichoderma* spp. and plant extracts, particularly *Trichoderma harzianum*-TH and garlic extract are effective in managing pea root rot. These bioagents and organics offer a sustainable alternative to chemical treatments, exhibiting significant inhibition of pathogen growth and reducing disease incidence and severity in the field as well. Therefore, integrating *Trichoderma* spp. and plant extracts into pea cultivation practice can enhance disease management and improve pea productivity promoting sustainable Agriculture.

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