PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(3): 844-852
https://doi.org/10.14719/pst.4472

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RESEARCH ARTICLE

# Management of *Rhizoctonia* blight of groundnut using antagonistic effects of bioagents and organic amendments

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OPEN ACCESS

**ARTICLE HISTORY**

Received: 27 July 2024
Accepted: 08 August 2024

Available online
Version 1.0 : 16 September 2024
Version 2.0 : 20 September 2024

Check for updates

**Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Pranaballava S, Sweta SM, Debashis D, Gayatri B, Mihira KM, Shyama SM, Abhiram D, Jayanta KM, Kartik CP. Management of *Rhizoctonia* blight of groundnut using antagonistic effects of bio agents and organic amendments. Plant Science Today. 2024; 11(3): 844-852. https://doi.org/10.14719/pst.4472

## Abstract

*Rhizoctonia* blight disease caused by *Rhizoctonia solani* in groundnut crop is one of the most devastating diseases occurring worldwide. The disease affects the morphological and physiological parameters of the crop leading to reduction in pod yield as well as oil yield. The pathogen was isolated locally and identified as *Rhizoctonia solani* based on molecular characterization. The efficacy of different bioagents in reducing the radial growth of pathogen was tested *in vitro* and highest mycelia growth inhibition was recorded by *Trichoderma asperellum* (89.07 %). Among the fungicides tested, 100 % mycelial growth inhibition was observed by use of Carbendazim 50 WP, Tebuconazole 25.9 % EC, Hexaconazole 5 % suspension concentrate (SC) and Tebuconazole 50 % + Trifloxystrobin 25 % water-dispersible granule (WG). Among different organic substances tested *in vitro*, neem seed cake achieved maximum mycelial growth inhibition of 50.74 % and 54.08 % at 10 % and 20 % concentrations respectively. In the field experiment, treatment with application of neem seed cake to the soil at 500 kg/ha + application of mustard seed cake to the soil at 500 kg/ha + treatment of seeds with Tebuconazole at 1.5 g/kg of seed + treatment of seeds with *T. asperellum* at 10 g/kg of seed was found to be the best in enhancing plant health, growth promotion and oil yield. The combined treatment of bioagent, fungicide and organic amendment recorded maximum number of branches (14.00), number of leaves (668.33), plant dry weight (64.17 g), 100 pod weight (65.00 g) and oil yield (47.33 %) compared to the control and other treatments along with reduction of the disease (59.61 %). In the physiological parameters study, the same treatment also recorded maximum pigment contents *viz*. Chlorophyll a (1.843 mg/g), Chlorophyll b (0.555 mg/g), total chlorophyll (2.397 mg/g) and carotenoid content (0.084 mg/g) but with minimum phenol content (1.693 mg/g). Thus, it can be concluded that integration of selective inputs in the combined treatment of Neem seed cake, Mustard seed cake, Tebuconazole and *T. asperellum could* enhance the plant health, morphological growth and physiological parameters and increased the oil yield in groundnut along with reduction of the disease.

## Keywords

*Rhizoctonia solani*; *Trichoderma asperellum*; neem seed cake; chlorophyll; phenol

## Introduction

Groundnut (*Arachis hypogaea* L.) is a major oilseed crop in tropical and subtropical region of the world belonging to the Leguminaceae family. Its kernel contains 40-50 % high quality edible oil and 20-50 % protein and other vitamins (1). The crop is affected by several diseases caused by fungi, bacteria and viruses. Among the fungal diseases, *Rhizoctonia* blight disease caused by *Rhizoctonia solani* Kuhn is an important and destructive disease. The drastical reduction in yield and quality of groundnut occur due to this disease (2). *Rhizoctonia solani* causes nearly 31 -60 % yield loss in soybean crop (3). It causes seedling rot which is associated with pre-emergence and post-emergence damping off. It also causes leaf blight, stem blight/rot, root rot and pod rot (4). The fungus develops non differentiated sclerotia, which survive on plant debris, saprophytically. *Rhizoctonia solani* may live on a broad range of hosts, including weed species and rotated crops. Germination of Sclerotia or hyphae on plant debris or in the soil can infect host tissue (5). Foliar pathogens are controlled by the judicious application of fungicides alone. Since *R. solani* is a soil borne pathogen being randomly distributed in the soil and survive through resting structure, it is difficult to manage through conventional methods. The application of fungicides through the groundnut canopy cannot affect the pathogen present in soil. To reduce the yield losses and to manage the *R. Solani*, ecofriendly and sustainable management practices are adopted with the safe and judicious use of fungicide along with application of organic amendments and the biocontrol agents (6). Seed treatment with tebuconazole at 1.5 g/kg is found to be effective in controlling the soil borne diseases of groundnut and thereby increase the yield (7). The combination treatments of biocontrol agents along with organic amendments manages dry root rot disease of mungbean caused by *Rhizoctonia bataticola* and enhances morphological, physiological and yield parameters (8). The present study aimed to evaluate efficacy of bioagents, organic amendments and fungicide *in vitro* as well as *in vivo* against *Rhizoctonia solani* along with promotion of growth and different physiological parameters.

## Materials and Methods

### Isolation and identification of the pathogen

Groundnut plants showing the symptoms of *Rhizoctonia* blight disease in leaves, stems, roots/pegs and pods were collected from the fields of groundnut. The pathogen *Rhizoctonia solani* (9) was isolated from the infected leaves and root pieces grown on potato dextrose agar medium (PDA). The pure culture of the pathogen was obtained through single hyphal tip transfer method and incubated at 28±2 °C in BOD incubator (10). The isolated pathogen is identified as *Rhizoctonia solani* based on morphological studies as well as molecular techniques like sequencing internal transcribed spacer (ITS). The universal primer ITS 1 and ITS 4 were used for the ITS amplification.

### Isolation of bioagents

The bioagent *Trichoderma asperellum* was procured from the laboratory of *Trichoderma* production unit, Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar. Other bioagents like *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma hamatum* were collected from the PG laboratory, Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar. *Pseudomonas fluorescens* and *Bacillus subtilis* were isolated from the talc-based formulations purchased from local market of Bhubaneswar, Odisha state.

### Chemicals and organic amendments

The chemicals like Carbendazim, Tebuconazole, Thifluzamide, Validamycin, Hexaconazole and Tebuconazole + Trifloxystrobin were purchased from local market and the trade name with recommended doses are mentioned in Table 1.The organic amendments like neem seed cake, mustard seed cake, soybean cake, vermicompost and farmyard manure (FYM) were procured from local market.

### *In vitro* evaluation of different bioagents for their antagonistic effect on the radial growth of *Rhizoctonia solani*

Four fungal bioagents *viz*, *Trichoderma viride, Trichoderma harzianum*, *Trichoderma hamatum, Trichoderma asperellum* and 2 bacterial bioagents *viz*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *Rhizoctonia solani* using the dual culture technique (11). Mycelial disc (5 mm diameter) of bioagent and test pathogen were cut and placed on PDA plates at one end opposite to each other. The plates were then incubated at 28 ± 2 °C. Each treatment was replicated 5 times. Percent inhibition (I) of the test pathogen by the bioagent over untreated control was calculated by applying the following formula (12).

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

Where,

I = Growth inhibition of pathogen in %

T = Radial growth (mm) of pathogen in treatment plate

C = Radial growth (mm) of pathogen in control plate

### *In vitro* evaluation of different fungicides on the radial growth of *Rhizoctonia solani*

The efficacy of different fungicides (Table 1) was tested *in vitro* against *Rhizoctonia solani*, at recommended doses using the poisoned food technique in PDA medium. Fungicides at their recommended doses of concentration were added in PDA medium following poisoned food technique (13). Mycelial disc (5 mm diameter) of the test pathogen was cut from a 7-day-old actively growing pure culture and placed in the center of poisoned PDA plate with different concentrations of fungicides. The plates were then incubated at 28 ± 2 °C temperature for 3-5 days or till the untreated control plates were fully covered with the mycelial growth of the test fungus. The percent inhibition (I) of the test fungus over the control was calculated using the formula as mentioned earlier (12). Each treatment was replicated 5 times.

**Table 1.** Fungicides and their doses.

| Treatments | Fungicides | Trade names | Doses |
|---|---|---|---|
| $T_1$ | Carbendazim 50 % WP | Dhanustin | 1.0 g/L |
| $T_2$ | Tebuconazole 25.9 % EC | Folicur | 1.5 mL/L |
| $T_3$ | Thifluzamide 24 % SC | Pulsor | 0.7 mL/L |
| $T_4$ | Validamycin 3 % L | Sheathmar | 2.0 mL/L |
| $T_5$ | Hexaconazole 5 % SC | Hexstar | 1.0 mL/L |
| $T_6$ | Tebuconazole 50 % + Trifloxystrobin 25 % WG | Nativo | 0.6 g/L |
| $T_7$ | Control | | |

## *In vitro* evaluation of different organic substances on the radial growth of *Rhizoctonia solani*

Except for Vermicompost, the rest of the substances were crushed to a fine powder with a pestle and mortar and dispensed at 50 g in 150 mL sterile distilled water (w/v) and allowed to decompose for 7 days. Later, these extracts were filtered through double- layered muslin cloth and the filtrate obtained was further passed through Whatmanno.1 filter paper and autoclaved for 10 min. The final clear extracts/filtrates obtained formed the standard extract of 100 % concentration. In case of vermicompost, it was mixed in sterile water without crushing and rest of the procedures were followed as mentioned earlier. These aqueous extracts were evaluated (each at 10 % and 20 % concentrations) *in vitro* against *Rhizoctonia solani*, using the Poisoned food technique (14) in Potato dextrose agar (PDA) culture medium. Mycelial disc (5 mm diameter) of the test pathogen was cut from a 7-day-old actively growing pure culture and placed in the center of poisoned PDA plate containing different concentrations of organic substances. The plates were then incubated at 28 ± 2 °C temperature for 3-5 days or till the untreated control plates were fully covered with the mycelial growth of the test fungus. The percent inhibition (I) of the test fungus over the control was calculated using the earlier mentioned formula (12). Each treatment was replicated 5 times.

## *In vivo* study

### Mass multiplication of the bioagent and pathogen

The pathogen and bioagents were grown and mass multiplied in the sorghum grain media. The sorghum grains were overnight soaked in water and then filled into polypropylene bags (8 × 10'') up to 1/3 of the capacity and were plugged with non-absorbent cotton with the support of one inch diameter PVC pipe (length 1.5''). The sorghum grain filled bags were sterilized at 121 ºC (15 lbs) for 20 min in autoclave twice at 24 h interval. The sorghum grain media were inoculated with mycelial discs of the biocontrol agents and pathogen from their respective 4 days old actively growing pure cultures. The inoculated grain filled bags were incubated at 28 ± 2 °C temperature for 15 days and shaken occasionally for the uniform growth of mycelia. The grain filled with pathogen inoculums were crushed and artificially inoculated in the experimental field.

### Field experiment

The experimental plots were artificially inoculated using the crushed sorghum grains with pathogen inoculums before sowing of the groundnut seeds. For this field experiment the best fungicide, best bioagent and best organic substance, as found out in the *in vitro* experiments were chosen. Thus, among fungicides tebuconazole, among organic amendments neem cake and mustard cake, among bioagents *Trichoderma asperellum* were selected for evaluation of their effect on *R. solani* in groundnut crop both as sole treatment as well as combination treatments. The field experiment was conducted at AICRP, Groundnut, Central farm, OUAT, Bhubaneswar, Odisha during *kharif* seasons of 2022-23 and 2023-24. The plot size was 2.1 × 4.0 m with plant to plant spacing of 10 cm and row to row spacing of 30 cm. For the treatments the seeds treated with fungicide tebuconazole at 1.5 g/kg, soil application of neem cake and mustard cake at 500 kg/ha and seed treatment with *Trichoderma asperellum* at 10 g/kg were used individually and also in combination. The treatment with inoculation of the pathogen was the control treatment. A total of 10 treatments were taken as mentioned in Table 2 and each treatment was replicated thrice. In the individual treatment of *T. asperellum,* FYM were applied to the soil at 250 kg/ha fortified with *T. asperellum* at 10 kg/ha along with seed treatment.

### Parameters analyzed

The morphological growth parameters of groundnut like number of branches, number of leaves, plant dry weight and 100 pod weight were analysed under field condition. The oil content of the groundnut seeds was determined using the Soxhlet method (15). The percentage of oil yield was calculated using the following formula.

$$\text{Percentage of oil (w/w)} = \frac{W_{oil}}{W_{cs}} \times 100$$

Where,

$W_{Oil}$ is weight of oil obtained (g) using an extraction method

$W_{CS}$ is the weight of crushed seed (g) just before oil extraction

### Physiological parameters

The physiological parameters of the groundnut plants were analysed by using spectrophotometer at Central Instrumentation Facility, OUAT, Bhubaneswar. The photosynthetic pigments like the chlorophyll a, b and total chlorophyll were estimated by using the 80 % v/v acetone solution in falcon tube containing small pieces of fresh leaves. The absorbance (OD) was recorded at 480 nm, 645 nm and 663 nm. The respective pigment content of leaf was calculated using the following formula and expressed as mg/g FW leaf (16).

Chlorophyll a = $(12.7 \times OD_{663} - 2.69 \times OD_{645}) \times [V/ (100 \times W)]$

Chlorophyll b = $(22.9 \times OD_{645} - 4.68 \times OD_{663}) \times [V/ (1000 \times W)]$

Total Chlorophyll = $(20.2 \times OD_{645} + 8.02 \times OD_{663}) \times [V/ (1000 \times W)]$

**Table 2.** Treatment details.

| Treatment | Details |
|---|---|
| $T_1$ | Application of neem seed cake to the soil at 500 kg/ha |
| $T_2$ | Application of mustard seed cake to the soil at 500 kg/ha |
| $T_3$ | Treatment of seeds with *Trichoderma asperellum* at 10 g/kg of seed and application of FYM to the soil at 250 kg/ ha fortified with *T. asperellum* at 10 kg/ ha |
| $T_4$ | Application of neem seed cake to the soil at 500 kg/ha + treatment of seeds with *T. asperellum* at10g/kg of seed |
| $T_5$ | Application of mustard seed cake to the soil at 500 kg/ha + treatment of seeds with *T. asperellum* at 10g/kg of seed |
| $T_6$ | Treatment of seeds with Tebuconazole at 1.5g/kg of seed |
| $T_7$ | Treatment of seeds with Tebuconazole at 1.5g/kg of seed + treatment of seeds with *T. asperellum* at 10g/kg of seed |
| $T_8$ | Application of neem seed cake to the soil at 500 kg/ha + application of mustard seed cake to the soil at 500 kg/ha + treatment of seeds with Tebuconazole at 1.5g/kg of seed + treatment of seeds with *T. asperellum* at 10g/kg of seed |
| $T_9$ | Application of neem seed cake to the soil at 500 kg/ha + application of mustard seed cake to the soil at 500 kg/ha + treatment of seeds with *T. asperellum* at 10g/kg of seed |
| $T_{10}$ | Control |

The carotenoid content was calculated by the following formula and expressed as mg/g FW leaf (17).

Carotenoid content = $[\{A_{480} + (0.114 \times A_{663})\} - (0.638 \times A_{645})] \times [V/ (1000 \times W)]$

Where,

$OD_{645}$ = OD value at 645 nm

$OD_{663}$ = OD value at 663 nm

$A_{480}$ = Absorbance at 480 nm

$A_{645}$ = Absorbance at 645 nm

V = Total volume of extract (mL)

W = Fresh weight of leaf (g)

The total phenol content was calculated with preparation of standard curve (Fig. 1) at different concentrations of catechol equivalents as mg/g of leaf tissue on fresh weight basis (18).

### Experimental design and statistical analysis

*In vitro* experimental studies were conducted using completely randomized design (CRD) and the field experiment was conducted using randomized block design (RBD) (19). The data were analysed by ANOVA using OPSTAT software. Inferences were made based on critical difference (CD) between the means at 5 % level of significance. In case of the field experiments during both years were subjected to pooled analysis.

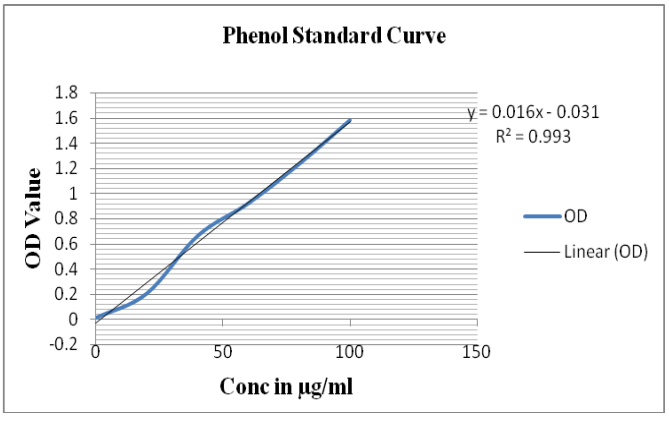


**Fig. 1.** Phenol standard curve.

## Results and Discussion

### *In vitro* evaluation of different bioagents for the antagonistic effect on the radial growth of *R. solani*

Among the fungal bioagents *T. asperellum* was the best showing highest growth inhibition of 89.07 % followed by bacterial biocontrol agent *B. subtilis* with 29.26 % growth inhibition. The other bioagents like *T. viride, T. harzianum* and *T. hamatum* showed their efficiency with percent growth inhibition of 31.48, 29.63 and 36.29 respectively and all the three treatments were statistically at par with each other. The bioagent *P. fluorescens* resulted in the least growth inhibition (22.96 %) of the test fungus (Table 3 and Plate 1). However, all the biocontrol agents tested were found to be efficient in checking the radial growth of the test pathogen. These findings are also in support of earlier reports that *T. asperellum* and compost had reduced the damping-off disease in cucumber plants caused by *R. solani* (20, 21). *Trichoderma* spp. (*T. harzianum* and *T. viride*) and *P. fluorescens* were evaluated (alone and in combination) under pot condition for

**Table 3.** *In vitro* evaluation of different bioagents for their antagonistic effect on the radial growth of *Rhizoctonia solani.*

| Treatments | Bio-agents | Radial growth of pathogen (mm) | % Inhibition |
|---|---|---|---|
| $T_1$ | *Bacillus subtilis* | 63.67 | 29.26 |
| $T_2$ | *Pseudomonas fluorescens* | 69.33 | 22.96 |
| $T_3$ | *Trichoderma asperellum* | 9.83 | 89.07 |
| $T_4$ | *Trichoderma viride* | 61.67 | 31.48 |
| $T_5$ | *Trichoderma harzianum* | 63.33 | 29.63 |
| $T_6$ | *Trichoderma hamatum* | 57.33 | 36.29 |
| $T_7$ | Control | 90.00 | 0 |
| | **CD (0.05)** | **5.60** | |
| | **SEm±** | **1.848** | |

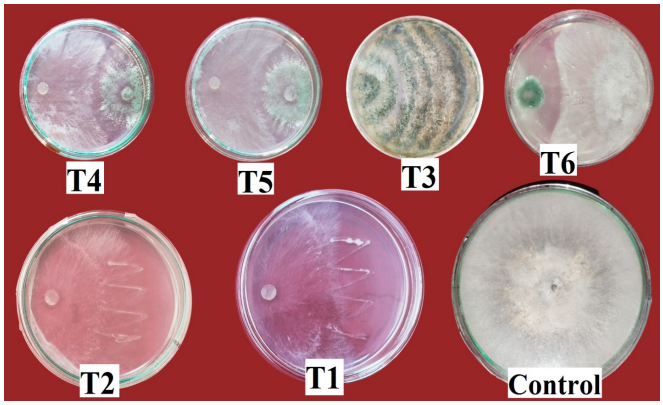


**Plate 1.** *In vitro* evaluation of different bioagents for their antagonistic effect on the radial growth of *Rhizoctonia solani.*

efficacy in suppressing *Rhizoctonia* root rot and promoting plant growth in chili (22). Due to the synthesis of fungitoxic metabolites, the isolates *T. harzianum* (Jn14) and *T. hamatum* (T36) were found to be the most effective at 25 ° C, inhibiting *R. solani* mycelial growth by 42 % and 78 % respectively (23).

## *In vitro* evaluation of different fungicides on the radial growth of *Rhizoctonia solani*

Cent percent inhibition of the radial growth of the test pathogen was observed by use of fungicides like Carbendazim 50 WP, Tebuconazole 25.9 % EC, Hexaconazole 5SC and Tebuconazole 50 % + Trifloxystrobin 25 % WG. But the chemical Validamycin 3 % L showed the least growth inhibition of the test fungus (58.15 %) (Table 4 and Plate 2). However, all the chemicals tested were found to be effective against the test pathogen at their respective doses of concentration. Fungicides have been found to be an integral part of the preventive control of the *R. solani* in different crops. The results in the present studies are in agreement with the earlier reports of other workers (24-27).

## *In vitro* evaluation of different organic substances on the radial growth of *Rhizoctonia solani*

The inhibition of the radial growth of the test fungus was also observed with the use of different organic substances. The results showed that neem seed cake showed highest growth inhibition (50.74 %) at 10 % concentration followed by mustard seed cake (35.92 %) at the same concentration. The least inhibition of the radial growth of the test pathogen

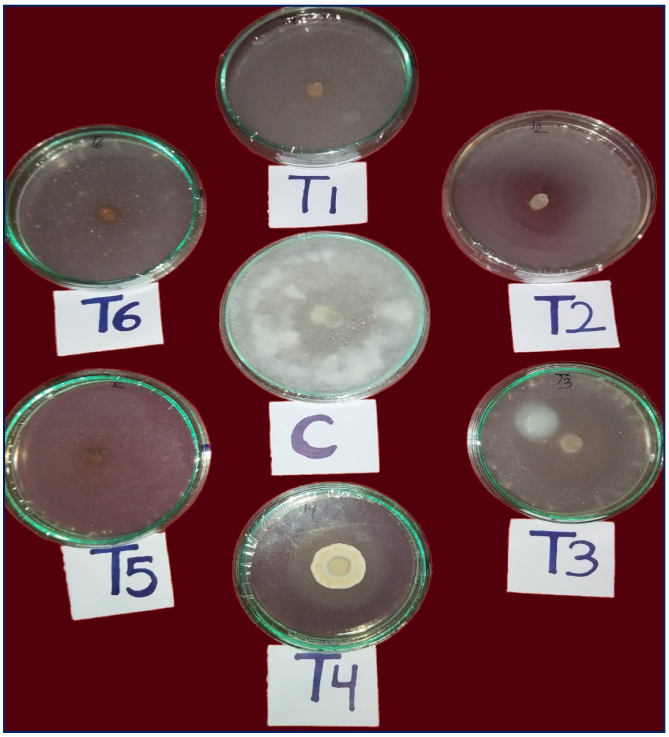


**Plate 2.** *In vitro* evaluation of different fungicides on the radial growth of *Rhizoctonia solani.*

was observed in case of soybean cake (5.19 %) at 10 % concentration. In the similar way, the neem seed cake showed the highest inhibition (54.08 %) on the radial growth of the test pathogen followed by mustard seed cake (50.92 %) at 20 % concentration, both being statistically at par with each other. The least inhibition was observed in case of farmyard manure (1.48 %) at 20 % concentration (Table 5 and Plate 3). Similar findings were observed by several earlier researchers (28-30).

## *Efficacy of bioagents, fungicides and organic amendments in field condition*

Considering the maximum growth inhibition of the test pathogen *in vitro* the best bioagent *T. asperellum,* best fungicide tebuconazole and best organic amendments like neem seed cake and mustard seed cake were selected for use either alone or in combination under field condition for management of the disease as well as to observe the growth promotion.

**Table 4.** *In vitro* evaluation of different fungicides on the radial growth of *Rhizoctonia solani.*

| Treatments | Fungicides | Doses | Radial growth (mm) | % inhibition |
|---|---|---|---|---|
| T1 | Carbendazim 50 WP | 1.0 g/L | 0.00 (0.71)* | 100 |
| T2 | Tebuconazole 25.9 % EC | 1.5 mL/L | 0.00 (0.71) | 100 |
| T3 | Thifluzamide 24 % SC | 0.7 mL/L | 12.00 (3.54) | 86.67 |
| T4 | Validamycin 3 % L | 2.0 mL/L | 37.67 (6.17) | 58.15 |
| T5 | Hexaconazole 5SC | 1.0 mL/L | 0.00 (0.71) | 100 |
| T6 | Tebuconazole 50 % + Trifloxystrobin 25 % WG | 0.6 g/L | 0.00 (0.71) | 100 |
| T7 | Control | | 90.00 (9.51) | 0 |
| | **CD (0.05)** | | **0.189** | |
| | **SEm±** | | **0.062** | |

Figures in parentheses indicate corresponding √(X+0.5) values

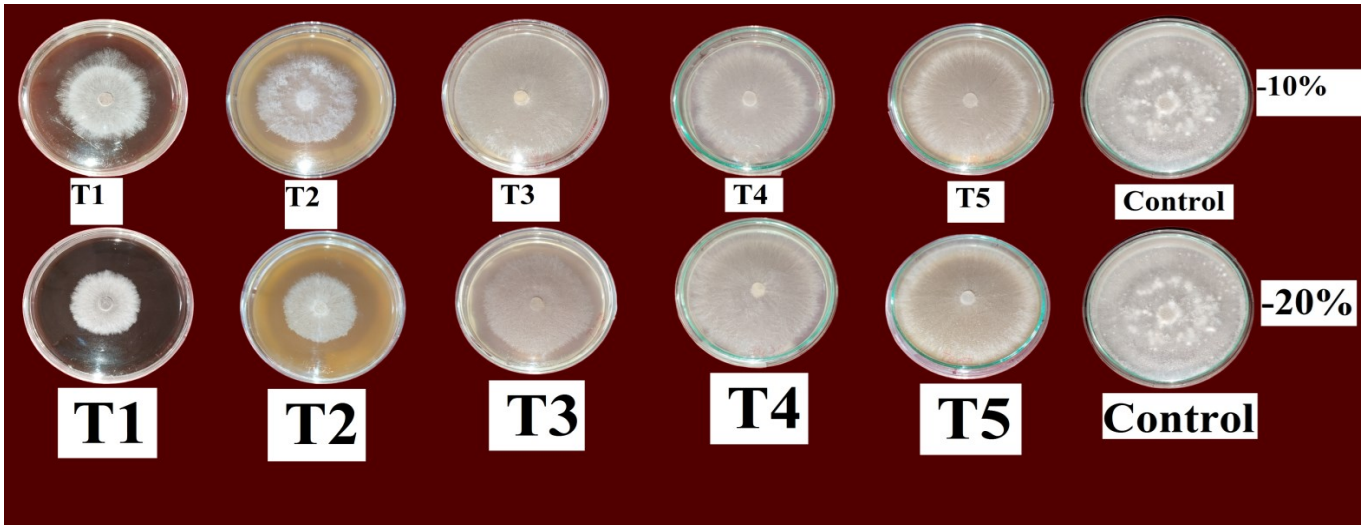


**Plate 3.** *In vitro* evaluation of different organic substances on the radial growth of *Rhizoctonia solani.*

**Table 5.** *In vitro* evaluation of different organic substances on the radial growth of *Rhizoctonia solani.*

| Treatments | Organic substances | Radial growth (mm) | | % Inhibition | |
|---|---|---|---|---|---|
| | | 10 % | 20 % | 10 % | 20 % |
| $T_1$ | Neem seed cake | 44.33 | 41.33 | 50.74 | 54.08 |
| $T_2$ | Mustard seed cake | 57.67 | 44.17 | 35.92 | 50.92 |
| $T_3$ | Soybean cake | 85.33 | 66.67 | 5.19 | 25.92 |
| $T_4$ | Vermicompost | 77.67 | 86.33 | 13.7 | 4.08 |
| $T_5$ | Farmyard manure (FYM) | 74.67 | 88.67 | 17.03 | 1.48 |
| $T_6$ | Control | 90.00 | 90.00 | 0 | 0 |
| | **CD (0.05)** | **6.44** | **7.24** | | |
| | **SEm±** | **2.091** | **2.350** | | |

## Effect of different treatments on morphological parameters and oil yield of groundnut under field condition with *Rhizoctonia* blight infestation

Among various treatments tested, maximum number of branches (14.00), number of leaves (668.33), plant dry weight (64.17 g) and 100 pod weight (65.00 g) were observed in the treatment $T_8$ (Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum*) followed by $T_9$ (Neem seed cake + Mustard seed cake + *T. asperellum*) with number of branches (12.84), number of leaves (622.50), plant dry weight (62.83 g) and 100 pod weight (63.67 g). The least number of branches (7.0), number of leaves (274.50), plant dry weight (36.50 g) and 100 pod weight (35.83 g) were recorded in the control treatment (T10). The maximum oil yield (47.33 %) was recorded in the treatment $T_8$ (Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum*) followed by $T_9$ (Neem seed cake + Mustard seed cake + *T. asperellum*) with oil yield of 40.00 % and both were statistically at par with each other. The lowest oil yield (27.93 %) was recorded in the control treatment ($T_{10}$). The highest percentage increase in oil yield (40.99) over control was observed in the treatment $T_8$ (Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum*) but the lowest percentage increase in oil yield (6.37) over control was observed in the treatment $T_2$ (Mustard seed cake) (Table 6). The morphological and growth parameters were found to be highest in the combined treatment of bioagent, fungicide and organic amendments. The present result is in accordance with the earlier reports (31). *Trichoderma* spp. when interact with plants, it promotes the nutritional availability and thereby promotes growth parameters and yield (32). The organic amendments resulted in the decrease of root rot incidence (pre-emergence and post-emergence) and there by increased the yield of pods in french bean (33). Neem seed cake which contains azadirachtin was found highly effective in the promotion of the plant growth by reducing the disease incidence caused by *R. solani* as reported earlier (29). Mustard seed cake which is having antifungal compounds which are released to the soil on hydrolysis as isothiocyanates (34). These isothiocyanate compounds suppress the germination of *R. solani* inoculum (35). The fungicide when applied for the soil borne diseases also reduced the foliar blight disease (36). Application of bioagent, fungicide and organic amendments reduced the soil borne diseases caused by *R. solani* (37). The combination treatment enhanced the oil yield by improving plant health and growth compared to the individual treatments (38, 39). In the present study, the treatment $T_8$ (Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum*) was found to be the best treatment promoting morphological characters and there by increased oil yield in addition to reduction in the disease incidence.

## Effect of different treatments on physiological parameters of groundnut under field condition with *Rhizoctonia* blight infestation

The data of physiological parameters indicated that all the treatments had significant effect on the host physiology as mentioned in Table 7. The leaf pigments like chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were significantly reduced in the pathogen infected plants due to blockage of xylem vessels by the toxins released by the pathogen affecting chlorophyll synthesis. The highest chlorophyll a (1.843 mg/g), chlorophyll b (0.555 mg/g), total chlorophyll (2.397 mg/g) and carotenoid (0.084 mg/g) contents were recorded in the combined treatment $T_8$ (Neem seed cake + Mustard seed cake + Tebuconazole + *T.*

**Table 6.** Pooled data on effect of different treatments on morphological parameters and oil yield of groundnut under field condition with *Rhizoctonia* blight infestation.

| Treatments | % disease reduction over control | Number of branches | Number of leaves | Plant dry weight (g) | 100 pod weight (g) | Oil yield (%) | % increase in oil yield over control |
|---|---|---|---|---|---|---|---|
| $T_1$- Neem seed cake | 25.41 | 9.50 | 310.50 | 39.17 | 40.00 | 33.83 | 17.44 |
| $T_2$- Mustard seed cake | 18.28 | 8.33 | 402.00 | 41.83 | 40.50 | 29.83 | 6.37 |
| $T_3$- *Trichoderma asperellum* | 34.44 | 10.33 | 437.50 | 45.00 | 39.67 | 34.00 | 17.85 |
| $T_4$- Neem seed cake + *T. asperellum* | 45.13 | 12.00 | 550.67 | 49.50 | 49.67 | 34.67 | 19.44 |
| $T_5$- Mustard seed cake + *T. asperellum* | 41.80 | 11.50 | 457.00 | 48.00 | 45.50 | 35.00 | 20.20 |
| $T_6$- Tebuconazole | 44.89 | 11.67 | 485.67 | 39.67 | 47.17 | 36.97 | 24.45 |
| $T_7$- Tebuconazole + *T. asperellum* | 52.49 | 12.83 | 605.83 | 60.33 | 61.83 | 37.08 | 24.68 |
| $T_8$- Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum* | 59.61 | 14.00 | 668.33 | 64.17 | 65.00 | 47.33 | 40.99 |
| $T_9$- Neem seed cake + Mustard seed cake + *T. asperellum* | 54.39 | 12.84 | 622.50 | 62.83 | 63.67 | 40.00 | 30.18 |
| $T_{10}$- Control | - | 7.00 | 274.50 | 36.5 | 35.83 | 27.93 | 0 |
| **S.Em ±** | **-** | **0.358** | **23.542** | **2.287** | **1.597** | **1.670** | **-** |
| **CD@0.05** | **-** | **1.065** | **69.940** | **6.794** | **4.745** | **4.963** | **-** |

**Table 7.** Effect of different treatments on physiological parameters of groundnut under field condition with *Rhizoctonia* blight infestation.

| Treatments | Pigment content (mg/g) FW of leaf | | | | Phenol content (mg/g FW) |
|---|---|---|---|---|---|
| | Chlorophyll a | Chlorophyll b | Total Chlorophyll | Carotenoid content | |
| $T_1$- Neem seed cake | 0.785 | 0.27 | 1.034 | 0.038 | 2.232 |
| $T_2$- Mustard seed cake | 0.764 | 0.225 | 1.010 | 0.037 | 2.249 |
| $T_3$- *Trichoderma asperellum* | 0.900 | 0.313 | 1.213 | 0.044 | 2.123 |
| $T_4$- Neem seed cake + *T. asperellum* | 1.270 | 0.421 | 1.714 | 0.059 | 1.948 |
| $T_5$- Mustard seed cake + *T. asperellum* | 1.176 | 0.391 | 1.597 | 0.056 | 2.088 |
| $T_6$- Tebuconazole | 1.331 | 0.444 | 1.721 | 0.062 | 1.945 |
| $T_7$- Tebuconazole + *T. asperellum* | 1.446 | 0.470 | 1.915 | 0.064 | 1.929 |
| $T_8$- Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum* | 1.843 | 0.555 | 2.397 | 0.084 | 1.693 |
| $T_9$- Neem seed cake + Mustard seed cake + *T. asperellum* | 1.569 | 0.547 | 2.115 | 0.072 | 1.913 |
| $T_{10}$- Control | 0.443 | 0.153 | 0.595 | 0.023 | 2.286 |
| **S.Em±** | **0.001** | **0.001** | **0.001** | **0.000** | **0.156** |
| **CD at 0.05** | **0.002** | **0.002** | **0.003** | **0.001** | **0.462** |

*asperellum*) followed by $T_9$ (Neem seed cake + Mustard seed cake + *T. asperellum*) having chlorophyll a (1.569 mg/g), chlorophyll b (0.547 mg/g), total chlorophyll (2.115 mg/g) and carotenoid (0.072 mg/g) contents compared to the control treatment, where these were observed to be reduced. The control treatment ($T_{10}$) recorded the least chlorophyll a (0.443 mg/g), chlorophyll b (0.153 mg/g), total chlorophyll (0.595 mg/g) and carotenoid (0.023 mg/g) contents. Among the individual component treatments, the highest total chlorophyll (1.213 mg/g) and carotenoid (0.044 mg/g) contents were recorded in the treatment $T_3$ (*Trichoderma asperellum)* followed by $T_1$ (Neem seed cake) having total chlorophyll (1.034 mg/g) and carotenoid (0.038 mg/g) contents and $T_2$ (Mustard seed cake) having total chlorophyll (1.010 mg/g) and carotenoid (0.037 mg/g) contents. The maximum total chlorophyll and carotenoid contents were recorded in the combination treatment of bioagent, fungicide and organic amendments due to least incidence of the blight/rotting caused by pathogen *R. solani*. The enhancement of physiological parameters in the combined treatment attributed the same trend in the other growth and morphological parameters. Maximum phenol content 2.286 mg/g FW was recorded in the control treatment ($T_{10}$) followed by $T_2$ (Mustard seed cake) 2.249 mg/g FW. Least phenol content 1.693 mg/g FW was recorded in the treatment $T_8$ (Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum*). Similar findings were earlier reported (8) where total phenol content was enhanced by the dry root rot infection caused by *Rhizoctonia bataticola* in mungbean. Total pigment contents were least recorded in the control treatment where maximum phenol content was recorded. This indicated that due to low pigment contents, the energy is utilised for the synthesis of phenol which provided mechanical strength to the plant in response to the pathogen infection, leading to low yield due to diversion of energy (40). Similar results were also reported by the earlier workers that combined treatment of bioagent, fungicide and organic amendments recorded

maximum chlorophyll, carotenoid pigment contents and minimum phenol content (8, 31, 41-44). Combined treatment of *T. harzianum* with neem based pesticide caused reduction in disease severity caused by *F. oxysporum* f.sp. *capsici* and increased the nutrient uptake in plants and thereby increased physiological parameters like chlorophyll pigment content (45, 46). Similar results were also obtained with increase in chlorophyll by the addition of organic amendments (47). Hence the treatment with Neem seed cake + Mustard seed cake + Tebuconazole + *T. asperellum* was found to be the best treatment which recorded maximum pigment content but lowest phenol content due to reduced pathogenic stress compared to the control treatment showing less pigmentation but higher phenol content.

## Conclusion

The result of the present study concludes that combination of fungicide, bioagent and organic amendments in the treatments promotes plant health and growth parameters as well as physiological parameters with less incidence of the *Rhizoctonia* blight/stem rot/root rot disease caused by *R. solani* compared to the individual input treatment. The combined application of organic amendments like neem cake, mustard cake, bioagent *T. asperellum* and fungicide tebuconazole increased the morphological and physiological parameters along with oil yield in addition to highest disease reduction. Hence the treatment was found superior in managing disease by reducing the *R. solani* infection and promoting the morphological and physiological parameters which ultimately attributed towards enhanced pod and oil yield. This ecofriendly approach with application of bioagent, organic amendments and judicious application of effective fungicide can manage the soil borne and foliar disease-causing pathogen *R. solani* in groundnut crop along with enhanced pod and oil yield and can be an effective strategy towards sustainable agriculture. However, further research may be carried out to find out the mechanism of disease reduction as well as yield enhancement in order to generate a concrete finding for recommendation to the farmers of the state.

## Acknowledgements

Authors are thankful to the Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India for providing the required financial assistance and technical support.

## Authors' contributions

Conceptualization PS and GB, Methodology, PS and GB, Investigation, PS, Resources, GB, MKM, writing original draft, PS, Formal analysis, SSM, writing review and editing, GB, AD, JKM, KCP, SSM, SM and DD.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None.

**Did you use generative AI to write this manuscript?**

Ans: No

**Declaration of generative AI and AI-assisted technologies in the writing process**

Ans: NIL

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