**Effect of different application methods of *Trichoderma* isolates on growth and disease response against stem and root rot disease of ground nut seedlings (*Arachis hypogea L.*) incited by *Sclerotium rolfsii***

**ABSTRACT:**

The pot culture experiment of groundnut seedlings was performed to study the efficacy of UBT 21 & UBT 23 strains of *Trichoderma spp.* and their application methods on seedling growth parameters, rhizosphere colonizing ability, sclerotial wilt disease reduction and induction of defense enzymes (total phenols and polyphenol oxidase). It was found that shoot and root length varied significantly with *Trichoderma* treated seedlings irrespective to method of application but soil application provided better effect on biomass development. The rhizosphere colonization by *Trichoderma spp*. was maximum in combined application with soil and seed treatment. The incidence of stem and root rot disease was reduced by 65-80% in *Trichoderma* treated pots over control. Also, there was an increased total phenols and polyphenol oxidase activity in treated plots. Comparing the methods of application, the combined application by soil and seed showed better results for growth as well as disease reduction. The UBT 23 strain was found most effective than UBT 21 strain.

Key words: *Trichoderma*, Groundnut, *Sclerotium rolfsii*, Phenols and Polyphenol oxidase.

**INTRODUCTION:**

Groundnut (*Arachis hypogaea L.*) is one of the prominent oilseed crops in India, cultivated during *kharif* and *rabi-summer* seasons. Various diseases have been reported in groundnut crop, in that few were found to cause significant economic losses. The stem and root rot disease caused by *Sclerotium rolfsii* is major threat for groundnut growers that it infects all the stages of crop growth. More than 27% yield losses have been reported in India due to this disease (Chohan, 1974). The pathogen *Sclerotium rolfsii* infects both the living plant cells and plant debris, as well as the sclerotia can survive for several years in soil. The previous crop residue present can significantly influence the sclerotial germination, the growth of mycelium, and there by infection process of *S. rolfsii* in groundnut (Kumar *et al*., 2010). Due to its wide host range and soilborne nature of this pathogen, it becomes a challenge to manage the disease by using conventional and chemical methods. Biological control offers environment friendly and natural alternative to chemical method for management of various soil-borne plant pathogens including *S. rolfsii* (Papavizas, 1985).

Among fungi, *Trichoderma* species have been widely used for management of various plant pathogens. Several species, including *T. hamatum*, *T. viridae, T. harzianum*, *T. virens*, *T. asperellum*, and *T. atroviride* are identified as potential biological control agents against a wide range of phytopathogenic fungi (Bell, 1994). *Trichoderma spp*. promotes the disease reduction by various mechanisms that include antibiotic production (antibiosis), mycoparsitsim and competition for nutrients and space (Harman *et al.,* 2004). Besides that, the root-colonizing beneficial microbes like *Trichoderma* can enhance plant health and stimulate systemic resistance to phyto-pathogens by triggering defense response mechanisms in plants (Hossain *et al.,* 2016). Biswas *et al.,* (2000) reported that two *Trichoderma* isolates significantly reduced the stem rot incidence in ground nut with a reduction of 33-50% when seed treated and 72-83% when applied to the soil in pot trials.

By considering the above stated research facts, a research study was carried out to know the efficacy of *Trichoderma* isolates and their methods of application on seedling growth, disease reduction and defense enzymes induction on infection with *S. rolfsii*.

**MATERIALS AND METHOD:**

Under greenhouse conditions, the pot culture experiment of ground nut seedlings was conducted at Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal during the *rabi* season (2018-19). Soil and Farm yard manure were collected and fumigated with 4% formalin and mixed thoroughly in 9:1 ratio in pots. The pure culture of *S. rolfsii* with mycelia and sclerotium are finely grounded and mixed with the soil to create sick soils. Then it is left for a week by sprinkling water for inoculum development and make the soil sick. Later groundnut seeds of TG-37A variety were sown in the pots. For this, two *Trichoderma* isolates (UBT21 & UBT 23) from culture repository of Department of Plant Pathology were used. The culture filtrate was obtained from the leftover culture media after harvesting of mycelial mat i.e., strained through Whatman no.1 filter papers by taking care that no spore or mycelia present in it. Sodium azide was added to prevent further spore development. The Trichoderma talc formulations was prepared using the harvested mycelial mat and keeping the viable population of 1011 to 1012cfu/g talc formulation which were used for soil and seed treatment in the experiment. The *Trichoderma* isolates along with their method of application were designed into 10 treatments with 10 pots each.

**Treatment details:**

T1: Soil application of UBT 21, T2: Seed treatment of UBT 21, T3: Seed treatment with culture filtrate of UBT 21, T4: Seed treatment + Soil application of UBT 21, T5: Soil application + seed treatment with culture filtrate of UBT 21, T6: Soil application of UBT 23, T7: Seed treatment of UBT 23, T8: Seed treatment with culture filtrate of UBT 23, T9: Seed treatment + Soil application of UBT 23, T10: Soil application+ seed treatment with culture filtrate of UBT 23

**Seedling growth parameters:**

The germination (%) was estimated by the following formula (ISTA, 1999).

Germination (%) = No. of seed germinated/ Total seed kept for germination × 100

Measurement of shoot length, root length and canopy width:

Seedlings were randomly selected from 15 and 21days old seedlings. Nearly 10 seedlings were selected, measured for its canopy width. Later cut the shoot and root portions and length measured by using a scale.

Determination of shoot and root dry weight:

 After recording shoot and root length, they are taken in brown paper bags and placed in hot air oven (700C± 20C) for 48 hours. The dry weights were measured on a weighing balance.

Determination of vigour index

Seed vigour index was calculated according to Abdul-Baki and Anderson (1973) formula.

Vigour index = Germination percentage × Seedling dry weight

**Estimation of rhizosphere *Trichoderma* population:**

Soil samples (10g) were collected from the rhizosphere region of 10 treatments. The estimation of *Trichoderma* population was done by serial dilution followed by Agar plate method (Clark, 1965). They are grown in Trichoderma specific medium (Elad *et al*., 1982) and later the number of colonies formed are multiplied to their dilution factor to get the number of *Trichoderma* population per gram soil collected. Later they are transformed to log scale.

**Disease incidence:**

The disease incidence percentage was calculated using Kokalis-Burelle *et al*. (1992) formula.

Disease incidence (%) = No. of infected plants / Total no. of plants ×100

**Total phenol estimation:**

The leaf samples were collected from 15 and 21days old seedlings. The total phenol estimation was done using Vinson *et al.* (2001) procedure. 100 mg of leaf tissue was crushed with 15 mL of 1.2 N methanolic HCl. Then heated in a water bath at 72–80°C for approximately one hour and allowed to cool. The extract was centrifuged at 10,000 rpm for 30 minutes and supernatant was collected. The final volume was adjusted to 25 mL solution using 1.2 N methanolic HCl. For the reaction mixture, 0.2 mL of the aliquot was combined with 2.8 mL of water and 0.5 mL of 50% Folin-Ciocalteu’s reagent. After 3 minutes, 2 mL of 10% sodium carbonate solution was added to extract and mixed thoroughly. Later the samples were incubated in a water bath at 50–60°C followed by cooling to room temperature. The absorbance of the final extract was measured at 650 nm using a SHIMADZU UV-1800 spectrophotometer. The total phenol content was estimated in mg/g of fresh wt.

**Polyphenol oxidase activity:**

The polyphenol oxidase activity was estimated by following the method of Mayer *et al.* (1965) with slight modifications. The collected leaf samples were immediately placed in liquid nitrogen to prevent enzyme degradation. 200 mg of the leaf tissue was grounded with 2 mL of sodium phosphate buffer (pH 6.6). The obtained extract was then centrifuged at 4°C with 20,000 rpm for 30 minutes. Then, 100 µL of the aliquot was made by adding 2.4 mL of sodium phosphate buffer (pH 6.0) and 0.5 mL of pyrogallol. Absorbance was measured at 495 nm by using a SHIMADZU UV-1800 UV-VIS Spectrophotometer. A blank solution was made with 3 mL of sodium phosphate buffer (pH 6.0), and readings were taken for every 30 seconds interval for 5 times. The enzyme activity was predicted in “ΔA495 min-1 g-1 fresh weight”.

**RESULTS:**

**Germination and growth parameters:**

From the Table 1 & 2, the germination percentage was higher in T9 and T4 treatments i.e., combined application of both soil and seed of UBT 23 (31.43%) and UBT 21 (28.57%) isolates. Root and shoot length varied significantly with *Trichoderma* treated seedlings with irrespective to method of application but soil application provided better effect on development of biomass. There was a significant increase in canopy width and highest canopy width observed in T1 & T6 treatments at 25 DAS of groundnut seedlings. The 25 days old seedlings showed maximum increase in shoot and root dry weight. In 25days old seedling, the vigor index recorded highest at T1 (114.28) followed by T2 (110.62) treatment indicating the better performance of UBT 21 strain when applied independently.

**Table 1:** Germination and growth parameters of Groundnut seedlings (15 DAS) under different application methods of UBT 21 & UBT 23 strains of *Trichoderma spp*.

|  |  |
| --- | --- |
| **Treatment No.** | **Per cent increase over control** |
| **Germination** | **Shoot Length** | **Root Length** | **Canopy width** | **Leaf number** | **Root dry wt.** | **Shoot dry wt.** | **Vigor index** |
| T1 | 14.29 | 68.91 | 56.31 | 0.51 | 89.42 | -3.43 | 1.57 | 17.51 |
| T2 | 25.71 | 71.9 | 61.96 | -8.25 | 57.85 | 0.88 | 4.28 | 44.03 |
| T3 | 17.14 | 27.06 | 18.64 | -0.41 | 47.33 | 20.91 | 0.03 | 33.73 |
| T4 | 28.57 | 58.45 | 46.89 | -18.4 | 57.85 | 48.54 | 5.46 | 93.52 |
| T5 | 24.29 | 56.95 | 45.01 | -29.46 | 47.33 | 1.57 | 4.02 | 41.92 |
| T6 | 15.71 | 17.34 | 11.11 | -14.71 | 57.85 | 14.52 | 14 | 79.95 |
| T7 | 7.14 | 50.97 | 43.13 | -20.7 | 89.42 | 0.33 | 4.47 | 23.01 |
| T8 | 18.57 | 24.07 | 16.76 | -18.86 | 36.81 | 23.65 | 3.09 | 49.43 |
| T9 | 31.43 | 46.49 | 37.48 | -31.77 | 47.33 | -8.94 | 5.06 | 45.24 |
| T10 | 30 | 94.32 | 88.89 | -28.54 | 68.38 | 19.4 | 8.12 | 81.47 |
| SEm± |  | 10.29 | 12.1 | 5.31 | 11.16 | 29.01 | 1.45 | 23.58 |
| CD (P=0.05) |  | 30.07 | 36.22 | 15.89 | 34.17 | NS | 4.34 | NS |

**Table 2:** Germination and growth parameters of Groundnut seedlings (25 DAS) under different application methods of UBT 21 & UBT 23 strains of *Trichoderma spp*.

|  |  |
| --- | --- |
| **Treatment No.** | **Per cent increase over control** |
| **Germination** | **Shoot Length** | **Root Length** | **Canopy width** | **Leaf number** | **Root dry wt.** | **Shoot dry wt.** | **Vigor index** |
| T1 | 14.29 | 62.96 | 17.92 | 52.30 | 9.99 | 31.68 | 95.43 | 114.28 |
| T2 | 25.71 | 55.56 | 12.68 | 31.70 | 40.70 | 27.57 | 71.78 | 110.62 |
| T3 | 17.14 | 39.89 | 7.98 | 41.43 | 41.42 | 35.10 | 32.24 | 61.09 |
| T4 | 28.57 | 63.82 | 16.82 | 43.92 | 7.85 | 58.66 | 28.07 | 80.22 |
| T5 | 24.29 | 71.79 | 21.79 | 42.79 | 48.56 | 49.87 | 30.06 | 73.58 |
| T6 | 15.71 | 43.02 | 3.56 | 57.05 | 39.99 | 123.19 | 40.24 | 91.25 |
| T7 | 7.14 | 51.28 | 9.64 | 37.59 | 85.70 | 99.26 | 76.51 | 99.95 |
| T8 | 18.57 | 37.04 | 5.63 | 25.37 | 5.71 | 61.71 | 11.93 | 52.87 |
| T9 | 31.43 | 49.86 | 8.26 | 36.68 | 34.28 | 40.48 | 57.83 | 108.00 |
| T10 | 30.00 | 47.01 | 5.77 | 28.99 | 37.13 | 60.30 | 52.85 | 105.72 |
| SEm± |  | 5.85 | 2.66 | 4.77 | 3.69 | 7.22 | 6.75 | 5.71 |
| CD (P=0.05) |  | 17.51 | 7.97 | 14.28 | 11.06 | 21.60 | 20.13 | 17.48 |

**Rhizosphere population of *Trichoderma spp.***

 The figure1 shows the colonizing ability of Trichoderma spp. of groundnut seedlings under different application methods of UBT 21 & UBT 23 strains of *Trichoderma spp.* The highest Trichoderma population was recorded at T9 (7.58 log) and T4 (7.56 log cfu/ g soil) treatments i.e., combined application by both soil and seed followed by sole seed treatment application with T7 (7.41 log) and T2 (7.98 log cfu/g soil).

**Figure 1:** Soil *Trichoderma* population of Groundnut seedlings under different application methods of UBT 21 & UBT 23 strains of *Trichoderma spp.*

**Disease incidence of *Sclerotium rolfsii*:**

From the figure 2, the lower disease incidence was found at T9 (14.29) and T4 (190.5) treatments when compared to control (85.71%). Seed treatment showed the disease reduction by 66.66% to 72.77% and same trend followed by soil application. The combined application by soil and seed provided better results against the disease. The disease reduction was also higher in T9 treatment followed by T4. The results showed that all the *Trichoderma* treated pots has reduced disease incidence with significant differences compared to untreated.

**Figure 2:** Percent incidence and reduction of stem and root rot disease by *S. rolfsii* of groundnut seedlingsunder different application methods of UBT 21 & UBT 23 strains of *Trichoderma spp.*

**Activity of defense enzymes (Phenol and Polyphenol oxidase):**

The figure 3 depicts the increase in total phenol content in *Trichoderma* treated pots. The highest phenol content was observed at T4 treatment at both 15 (0.65) and 21(0.49 mg/ g fresh wt.) days old seedling. The phenol content of untreated control was 0.164 at 15 DAS and 0.50 at 21 DAS. The combined application by both soil and seed showed better result with 1.5 to 2 times increase in activity of total phenols. The Polyphenol oxidase enzyme activity was shown in figure 4. The highest polyphenol oxidase activity was recorded at T9 (3.42) followed by T8 (3.33 ∆495nm/min/g fresh wt.) treatments for 21 days old ground nut seedlings. The UBT 21 strain showed the highest phenol concentration whereas the polyphenol oxidase activity recorded highest with application of UBT 23 isolate when applied as both soil and seed treatment.

**Figure 3:** Effect of different application methods of UBT 21 & UBT 23 strains of *Trichoderma spp.* on inducing total phenol in ground nut seedlings on inoculation with *S. rolfsii*

**Figure 4:** Effect of different application methods of UBT 21 & UBT 23 strains of *Trichoderma spp.* on inducing polyphenol oxidase in ground nut seedlings on inoculation with *S. rolfsii*

**DISCUSSION:**

Application of *Trichoderma* isolates by either seed or soil treatment, it multiplies and develops along with developing root system of plant (Harman, 2000; Howell *et al*., 2000). The germination and seedling parameters (height, dry weight, root & shoot length, leaf number) has significantly increased in *Trichoderma* treated seeds (Harman *et al*, 2004). The *Trichoderma* application also aids in enhanced root nodulation and in turn increased photosynthetic activity thereby promotes plant growth (Lugtenberg *et al*., 2013). Partiban *et al*., (2002) found the reduction of sclerotium wilt disease of ground nut by 92.58% with soil application of *T. harzianum*. Similar report by Kartikeeyan *et al*. (2006) stated that the culture filtrate of *T. viride* suppressed the *S. rolfsii* growth by 87.05% as well as sclerotial germination by 62.50%. Mohammadi and Karr (2002) and She-ze*et al.* (2008) reported that following biological method increased activity of peroxidases, phenols and polyphenol oxidase against pathogen attack and aided in resistance to plants.

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