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BIOLOGICAL CONTROL OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID ROOT ROT IN *VIGNA MUNGO* (BLACK GRAM) WITH *TRICHODERMA SPP*

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ABSTRACT
The charcoal rot of *Vigna mungo* is caused by *Macrophomina phaseolina*. It affects many other crops also causing devastating effect in many regions worldwide. No single control measures are ineffective or not feasible under farmers' conditions. In order to promote biological control as a component of an integrated management approach under arid ecological conditions, various experiments were carried out on dry root rot of black gram and their management by using *Trichoderma spp*. The results are summarized below. Among the *Trichoderma spp* tested, *Trichoderma viride* exhibited strong inhibition of the growth (77.77%) against *M phaseolina*. Their culture filtrates were also found to be effective in inhibiting the *in vitro* growth. Seed treatment of *Trichoderma viride* recorded the maximum root rot incidences (21.4%) followed by *Trichoderma harzianum* (26.6%). The maximum seed germination (75%), shoot length (43.2cm) and root length (16.0cm) was recorded in the same seed treatment of *Trichoderma viride* followed by *Trichoderma harzianum.* Soil application of *Trichoderma spp, Trichoderma viride* recorded the minimum root rot incidence of black gram. Influence of certain treatment combinations in sterilized and unsterilized soils was also studied.

KEY WORDS: *Vigna mungo, Macrophomina phaseolina, Trichoderma* spp, *Trichoderma viride, Trichoderma harzianum.*

INTRODUCTION

Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. It is an economically important disease of a broad range of crops (Srivastava *et al*., 2001**),** particularly in regions with warm and dry weather conditions during the growing season as they are prevalent in the dry zone of Vellore, Tamilnadu. Agronomically important hosts in Vellore include cowpea (*Vigna unguiculata* (L.) Walp), Sorghum (*Sorghum vulgare*), Sesame (*Sesamum indica*), Okra (*Hybiscus esculentus*) and ground nut (*Arachis hypogaea*) (Adam, 1986). Chemical control of the disease is difficult and economically not affordable for low income small scale farmers. Much of the effort to control *M. phaseolina* has focused on soil fumigation (Pearson *et al*., 1984**)**, and applying irrigation water to reduce the disease promoting effects of drought (Kendig *et al*., 2000). But in Vellore, like many Indian states is characterized by farming at small scale level and incomes are extremely low making chemical control unsuitable. Additionally, water supply in this region is difficult. Although a few resistant cultivars have been reported, they often exhibited only partial levels of resistance (Demooy *et al*., 1989) and are not available to the farmers. Other recommended control practices include solarization (Lodha *et al*., 1997), planting later-maturing cultivars (Pearson *et al*., 1984) and crop rotation **(**Singh *et al*., 1990**).**These practices are applicable and affordable in Vellore, and could be implemented in combination with biological control to manage micro sclerotial populations of *M. phaseolina* in the soil. For the biological control of *M. phaseolina*, antagonistic bacteria and fungi have been investigated (Pal *et al*., 2001; Singh *et al*., 2002; Baird *et al*., 2003). Combined application of two bacilli isolates reduced the *Macrophomina* induced charcoal rot of maize by 54% (Pal *et al*., 2001). Among the fungal antagonists, *Trichoderma spp* are generally the most frequently reported. Application of *T. harzianum* as seed treatment, suspension for soil drenching or wheat husk bran culture reduced infection of *Rhizoctonia bataticola* (former name of *M. phaseolina*) to 18%, 28% and 14%, respectively, as compared to 70% in the control variant (Parakhia & Vaishnav, 1986**).** However, most of these potent antagonists are either patented, not available, or would not withstand the dry climatic conditions prevailing in Niger. Therefore, this study was under taken to isolate and evaluate ecologically adapted microbial organisms to be used as a component of an integrated management of charcoal rot in Vellore region. Biological control involves the use of one or more biological organisms to control the pathogens or diseases. Biological control is more specialized and use specific microorganisms that attack or interfere with the pathogens. The members of the genus *Trichoderma* are very promising against soil born plant parasitic fungi. These filamentous fungi are very wide spread in nature, with high population densities in soils and plant liters. They are saprophytic, quickly growing, easy to culture and they can produce large amounts of conidia with long life time. (Manczinger, Antal, & Kredics, 2002**)**. In the US

pesticide residues on foods and another 50-100 induce alone 3000-6000 cancer causes annually by exposure to pesticides during application. Although the third world uses only one sixth of the total pesticides produced globally, at least 37,500 people are poisoned yearly and 10,000 of them fatally. (Whipps & Lumsden, 2001). Chemical control offered a short time effect but not an ultimate approach in the long run. Chemical control measures, with broad-spectrum fungicides, create imbalances in the microbial community, which may be unfavorable for the activity of the beneficial organisms and also lead to the development of resistant strains. The chemical seed treatments are recommended for the management of seed borne fungal pathogens. However they have the following deficiencies. Fungicides have only short term effect. They created environmental pollution. They have harmful side effects on human beings and animals. During the past several years some notable successes in disease control were achieved through introduction of bio control agents in the laboratory, glass house and field. Among the biocontrol agents the most frequently studied genus *Trichoderma*, owing to its elite biocontrol capabilities (Mukherjee, Upadhyay, & Mukhopadhyay, 1989). The following soil borne pathogens is successfully controlled by *Trichoderma spp*, like *Fusarium oxysporum, Pythium spp, Pythium aphanidermatum, Pythium ultimum, Pythium debaryanum, Rhizoctonia solani, Rhizoctonia bataticola, Sclerotium rolfsii and Macrophomina phaseolina.* In the present study an attempt has made to screen effective antagonist against *Macrophomina phaseolina, Macrophomina phaseolina – Trichoderma spp,* interaction in vitro and in vivo, Influence of culture filtrate of *Trichoderma spp.*, on the in vitro growth of *Macrophomina phaseolin,* Pot culture studies, Effect of seed treatment with *Trichoderma spp,* Effect of soil application of *Trichoderma spp and* Fungicide used for comparison with *Trichoderma spp.*

MATERIALS & METHODS

Fungal strains

The isolates of *M. phaseolina* were obtained from plants' samples, from soils, and from seeds collected in vellore, Optimum temperature for the growth of *M. phaseolina* Isolates of *M. phaseolina* from different origins were grown on potato dextrose agar (PDA) in Petri dishes (Ø 85mm, 4 replications per isolate) at different temperatures (32±1^{o}C), and radial growth of the colonies was measured after 48h.

Isolation of antagonists

Isolations were performed using the agar layer method (Herr, 1959). 10 grams of soil were suspended in 50 ml sterile tap water in a beaker and stirred for 10 min. One ml of the suspension was six fold diluted by 1:10 in 9 ml sterile water established at 42-45°C (seeding layer). After solidifying, 5 ml water agar (20 g Agar in 1000 ml H_2O) cooled to 42- 45°C was spread as a second layer over the seeding layer, in order to prevent overgrowth of the seeding layer by fast growing microorganisms. The plates were incubated at 32°C for 48 h to allow growth of antagonists prior to pouring a third layer (test layer) composed of propagules of *M. phaseolina.*

For preparation of the test layer, mycelium/microsclerotia of *M. phaseolina* from 48h old cultures grown in 100 ml potato dextrose broth (PDB; 24 g potato dextrose broth in 1,000 ml H2O, pH 5.5) were blended in 200 ml sterile water after filtrating off the culture medium. Two hundred microliters of mycelial/ sclerotial suspension of *M. phaseolina* were added on top of the seeding layer and evenly mixed with 7 ml PDA stabilized at 42 – 45°C. The plates were incubated at 32°C for another 3-4 days. Zones of inhibition appeared around colonies of antagonistic microorganisms contained in the seeding layer. These colonies were harvested, and used in the following trials.

Antagonist tests *invitro*

Trichoderma antagonists were streaked in the middle of petri dishes containing 15 ml PDA. After 48h incubation at 32°C, mycelial discs of *M. phaseolina* (Ø 8mm) cut from an actively growing 4 days-old culture were laid 3 cm apart from the streak. The plates were incubated at 32°C for one week, or until the colonies of *M. phaseolina* in the control plates had covered the plate. Inhibition zones of growth of *M. phaseolina* due to antagonistic activity were measured. Similarly, the effect of the antagonists on five bacterial species was tested. Inhibition zones of 10 mm or more were considered as important, and at 30 mm, the inhibition of *M. phaseolina* was total.

Identification of antagonists Identification of selected antagonists was carried out by microscopic observation of 24 h old cultures under a phase-contrast microscope, by heat treating 3 week old bacterial cultures at 80^{o}C for 10 min, and by performing amino peptidase (Merck), KOH tests and using API 50 CH strips (bioMérieux) according to the manufacturers' instructions

Culture Media

Czapek,s (DOX) Agar medium was prepared based on Ainsworth, (1961). One hundred ml of the sterilized molasses yeast broth taken in a 250ml Erlenmeyer flask were inoculated with 1ml of spore suspension (1x10^{8}spores ml) of *Trichoderma spp.* prepared from one week old culture and incubate at room temperature for a week in a rotary shaker. The biomass consisting of the mycelium and chlamydospores was blended in a blender and the population of propagules adjusted (Lewis & Papavizas, 1987).

Culture

The different *Trichodrma spp viz., Trrichoderma harzianum, Trichoderma hamatum koningii, Trichoderma pseudokoningii, Trichoderma reesii* and *Trichoderma viride* were collection from Tamilnadu Agricultural University Coimbatore.

IN VITRO GROWTH

Biomass basis

Czapek's broth was prepared based on Ainsworth (1961). 50 ml volume was dispensed in 250 ml Erlenmeyer flasks and sterilized. The flasks were inoculated with 8mm mycelia disc prepared from the periphery of the fungal culture. The flasks were incubated for 10 days at 27^{0}C under illumination in

incubator. After incubation the fungal biomass was separated through filtration in a previously dried and weighted filter paper (whatman No. 41). The dry weight of fungal biomass was determined after drying in the hot air oven at 105^0C unit constant weight was obtained.

Screening of effective antagonist

All the six *Trichoderma spp* were screened against *Macrophomina phaseolina invitro* through dual culture method.

The antagonist fungus was determined by dual culture method. Half portion of each petri dish containing Czapek's medium was inoculated with 8mm disc of the antagonist and other half was inoculated with 8mm disc of the pathogen simultaneously and incubated at 27^0C. Four replications with suitable control were maintained. The linear growth of the pathogen isolated was measured.

Influence of culture filtrate of *Trichoderma spp*. on the in vitro growth of pathogen

Czapek's broth was dispensed in 50 ml quantities in 250ml Erlenmeyer flasks and sterilized. The flasks were inoculated with 8mm disc of *Trichoderma spp*. and incubated for 10 days. After incubation the whole content was filtered aseptically through bacteriological filter. The culture filtrate thus obtained was used for testing their antagonistic action by antibiosis on the pathogen. The antifungal substance of each treatment was mixed with sterilized Czapek's medium at the rate of 20ml/plate. After solidification, a 8mm disc of the pathogen was placed at the center of the plate and incubated. The linear growth of the pathogen was measured.

Pot culture studies

The garden land soil was collected sterilized intermittently and used for the studies. The experiments were done in earthen pots of size 30cm diameter filled with 5kg of soil. The soil was inoculated with pathogen (5%w/w) and made into a sick soil. The effect of *Trichoderma spp,* on the germination percentage, plant growth parameters and disease incidence were studied.

Treatment details for pot culture experiments

T1-*Trichoderma viride* (Seed treatment – ST at $x10^8$ CFU ml)
T2-*Trichoderma viride* (Soil applicaiton – SA at 20kg hal)
T3-*Trichoderma viride* (ST + SA)
T4-Carbendazim at 2g kg^{-1} of seed
T5 -Control

The following observation were made Percentage germination

$$\text{Germination \%} = \frac{No\ of\ seed\ germinated}{Total\ No.of\ seed\ shown} x\ 100$$

Disease incidence

$$\text{Percent Disease incidence} = \frac{\text{No of diseased plants}}{\text{Total No of plants}} x\ 100$$

Effect of seed treatment with *Trichoderma spp*.

The pathogen inoculum was mixed with soil at 5 percent W/W before six days of sowing. Black gram was treated with spore suspension of antagonists ($x10^8$ CFU/ml) prepared from one week old cultures. The treated seeds were sown and the pots were irrigated regularly. The observations were made as mentioned earlier.

Soil application of *Trichoderma spp.*

Trichodrma spp. was multiplied in wheat bran soil medium and applied to the pathogen infested soil at 0.5 percent and mixed thoroughly. Seeds of black gram were sown and observations were made as mentioned earlier.

Fungicide used for comparison with *Trichoderma spp.*

Carbenazim ($2Kg^{-1}$ of seed was used in the present study for comparison its effect with *Trichoderma spp.*

RESULTS & DISCUSSION

Effect of different *Trichoderma spp*. on the in vitro growth of *Macrophominaphaseolina* by Dual culture method

The *Trichodermasppviz, T. harzianum, T. hamatum, T. koningii, T.pseudokoningii, T.reesii*and *T.viride*were screened against *Macrophomina phaseolina* for their biocontrol efficiency in vitro through dual culture growth inhibition method (Fig. 1). The growth inhibition by *Trichoderma spp*was assessed by measuring the linear growth and percent reduction of the linear growth of *Macrophomina phaseolina*.

Among the *Trichoderma spp., Trichoderma viride* exhibited strong inhibition (77.77%) followed by *Trichoderma harzianum* (75.55%) against *Macrophomona phaseolina. Trichoderma reesii* was reported (72.22%) inhibition followed by *Trichoderma hamatum* (68.88%). The lesser inhibition was observed in *Trichoderma pseudokoningii* (48.88%).

Effect of culture filtrate of *Trichoderma spp*. against *Macrophomina phaseolina*

The culture filtrate of individual *Trichoderma spp*. were tested for the assay of antifungal substances (AFS) broth in soil and liquid media against *Macrophominaphaseolina* (Fig. 2)

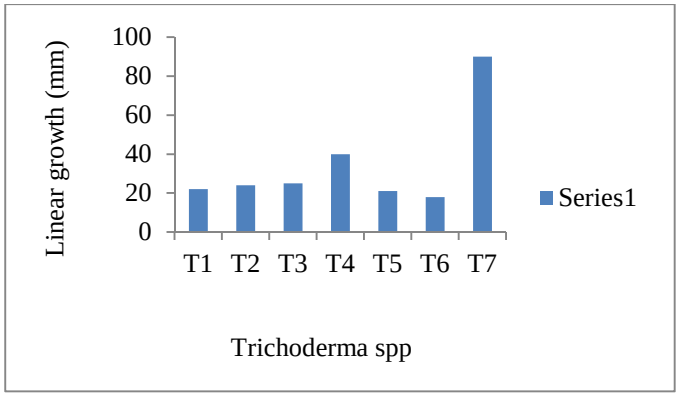


FIGURE1. Effect of different Trichoderma spp on invitro growth of *M. phaseolina*

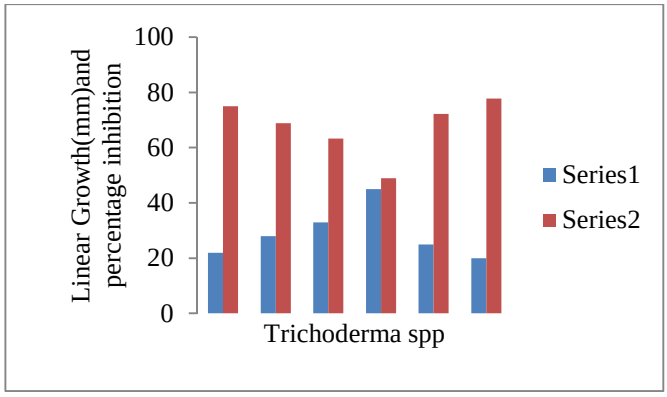


FIGURE 2. Effect of different *Trichoderam spp.* on invitro growth of *Macrophomina phaseolina*

T1 – *T. harzianum*, T2 – *T. hamatum*, T3 – *T. koningii*, T4–*T. pseudokoningii*, T5 – *T. reesii*, T6 – *T. viride*, T7 – Control
Values given are the mean value (X) of 4 datas, d.f. = degrees of freedom = n-1, Significance ++ = p < 0.001, + = p < 0.05, NS = Not significant

The growth inhibition was the maximum in the case of *Trichoderma viride* followed by *Trichoderma harzianum* and *Trichoderma hamatum. In vitro* antagonism by various antagonistic fungi on pathogenic organisms is a field of study in which reports are constantly thronging. In the light of recent emphasis on biological control, this area is gaining special attention. The pre requisite for any attempt to suggest a suitable biocontrol agent against a particular is to screen with all the available antagonists. The possible mechanisms proposed to explain the antagonism are competition. Antibiosis, lysis and hyperparasitism (Park, 1960). Antagonistic action of *Trichoderma spp*, against *Macrophomina phaseolina* was studied by several workers ((Dhingra & Sinclair, 1977; Wyllie, 1988) and (Olaya & Abawi, 1996)).The inhibitory action of culture filtrates of *Trichoderma spp.* might be due to their production of inhibitory volatile metabolite (Dennis & Webster, 1971). (Ghisalberti & Sivasithamparam, 1991) gave a detailed account about the antifungal antibiotics produced by *Trichoderma spp.* The lysis and growth inhibition of the pathogen. *Trichoderma spp.* excreted; lytic extra cellular β-1,3glucanases and chitinases in the growth medium and soil. They caused lysis of the cell walls fo the pathogen, which might also be a reason for the growth inhibition in the presence of antagonists (Chet & Baker, 1981).

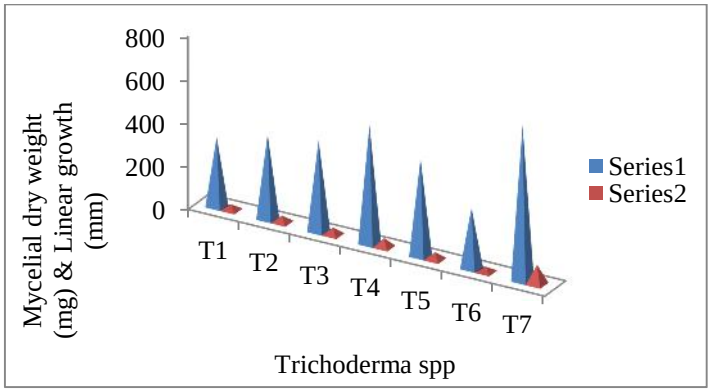


FIGURE 3. Effect of culture filtrates of *Trichoderam spp.* against *Macrophomina phaseolina*
Values given are the mean value (X) of 4 data's, d.f. = degrees of freedom = n-1, Significance ++ = p < 0.001, + = p < 0.05, NS = Not significant

The suppressive effect of *Trichoderma spp*. against *Macrophomina phaseolina* observed in the present study lends support to the phenomenon of antibiosis, mycoparasitism and lysis as reported by the above mentioned workers.

MANAGEMENT OF THE DISEASES

Effect of seed treatment with *Trichoderma spp* on the dry root rot incidence, seed germination and growth parameters in black gram.

The efficacy of various *Trichoderma spp*. on the control of root rot incidence, seed germination and growth parameters in black gram was studied under pot culture conditions. The *Trichoderma spp*, were applied through seed treatment.

Root rot incidence

Among the *Trichoderma spp,* the maximum reduction of the root rot incidence (59.54%) was recorded in *Trichoderma viride.* It was on par with Carbendazim (58.97%) treatment. It was followed by *Trichoderma harzianum* (49.71%) and *Trichoderma hamatum* (32.13%) percent reduction over control. The least percent reduction over control was observed in *Trichoderma reesii* (30.81%). Generally in all the treatments the root rot incidence was considerably reduced.

Seed germination and growth parameters

The maximum seed germination (75.0%) was recorded in the case of *Trichoderma viride*. It was on par with Carbendazim (75.3%) treatment. The maximum length of root (16.0cm), shoot (43.2cm) and Vigour index (4440.0) was observed in *Trichoderma viride* treatments. It was followed by *Trichoderma harzianum, Trichoderma hamatum* treatments. The least length of root (13.8). Shoot (29.0) and Vigour index (2406.6). Generally seed treatment by *Trichoderma spp*. significantly increased the germination pefcentage and growth parameters of black gram.

Effect of soil application with *Trichoderma spp.* on the growth on the dry root rot incidence, seed germination and growth parameters of black gram

Root rot incidences

The maximum disease incidence (25.10%) was observed in *Trichoderma viride* which is 55.16 percent reduction over control (Table) this was followed by *Trichoderma harzianum* (28.60%) and *Trichoderma reesii* (38.00%). In all the treatments, significant reductions of disease incidences were noticed when compared to control. Effect of *Trichoderma viride* on the disease incidences was on par with Carbendazim treatment.

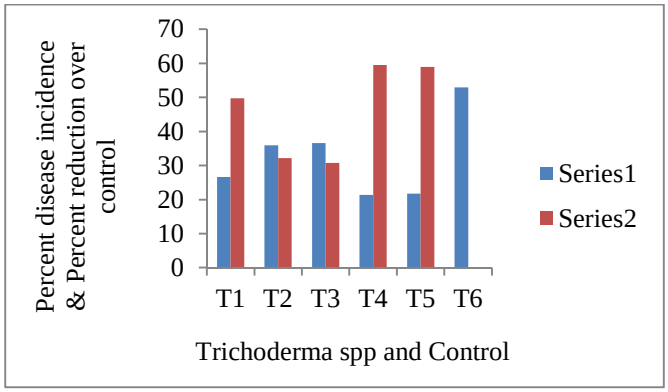


FIGURE 4. Effect of seed treatment with *Trichoderam spp.* On the dry root rot incidence in black gram

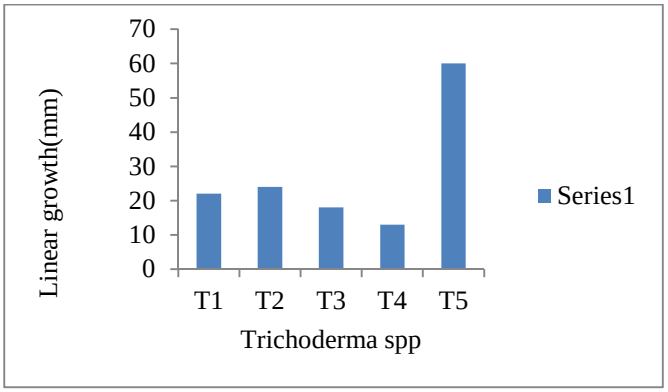


FIGURE 5. Effect of seed treatment with *Trichoderma spp*. on the dry root rot incidence in black gram

T1 – *T. harzianum*, T2 – *T. hamatum*, T3 –*T. reesii*, T4 – *T. viride,* T5 – Control

Values given are the mean value (X) of 4 data's, d.f. = degrees of freedom = n-1, Significance ++ = $p < 0.001$, + = $p < 0.05$, NS = Not significant

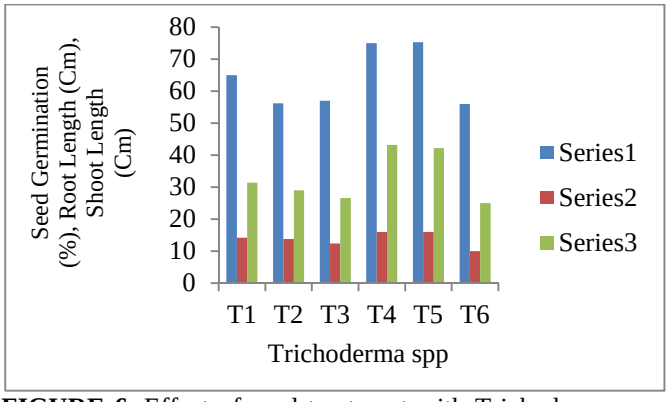


FIGURE 6. Effect of seed treatment with Trichoderma spp. on the seed germination and growth parameters in black gram

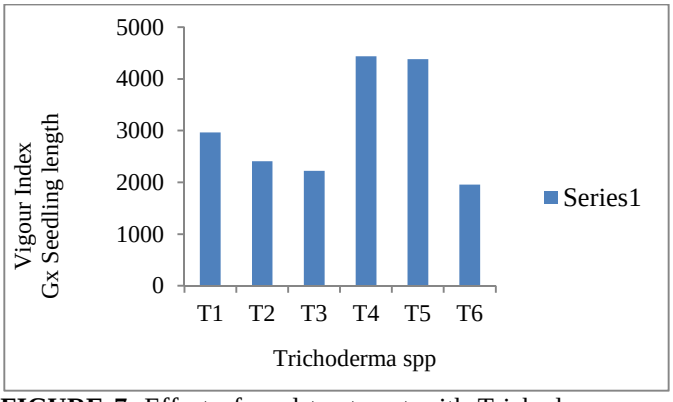


FIGURE 7. Effect of seed treatment with Trichoderma spp. on the seed germination (Vigour Index Gx Seedling length)

Values given are the mean value (X) of 4 data's, d.f. = degrees of freedom = n-1, Significance ++ = $p < 0.001$, + = $p < 0.05$, NS = Not significant

Seed germination and growth parameter

The highest seed germination (72.0%) was observed in Carbendazim treatment, which was on par with *Trichoderma viride* (71.2%) application. It was followed by *Trichoderma harzianum* (67.1%) *Trichoderma hamatum* (59.0%) and *Trichoderma reesii* (55.3%). The maximum length of root (17.6cm) shoot (39.0cm) and vigour index (4029.9) was observed in *Trichoderma viride* treatment. It was

followed by *Trichoderma harzianum, Trichoderma hamatum* treatments. The least length of root (11.4cm), shoot (22.6cm) and Vigour index (1880.2) was recorded in *Trichoderma reesii.* Generally soil application of *Trichoderma spp* significantly increased the germination percentage and growth parameters of Black gram.

Effect of certain treatment combinations on the dry root rot incidences of black gram in unsterilized and sterilized soil

Experiment was conducted to find out the effect of certain treatment combinations against root rot incidences. In unsterilized soil, the maximum reduction of root rot disease incidence (81.72%) was observed in treatment T4 *Trichoderma viride* applied as seed and soil also reduced the root rot incidence 76.89 percent over control. All other treatment considerably reduced the disease incidence. The same trend was observed in sterilized soil also. Where in TY3 recorded 71.14 percent reduction over control. It was on par with Carbendazim treatment. It was followed by treatment T1 where in *Trichoderma viride* applied as soil. It was recorded as 62.52 percent reduction over control.

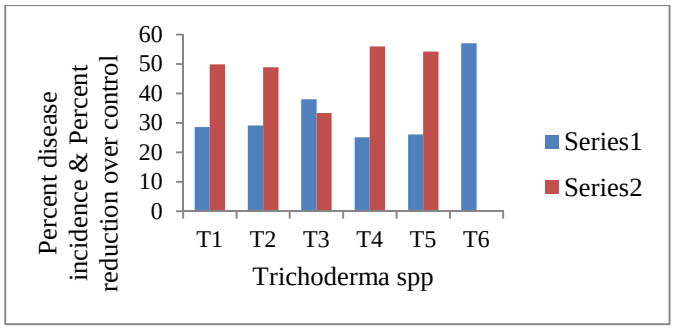


FIGURE 8. Effect of soil application with *Trichoderam spp.* On the dry root rot incidence in black gram

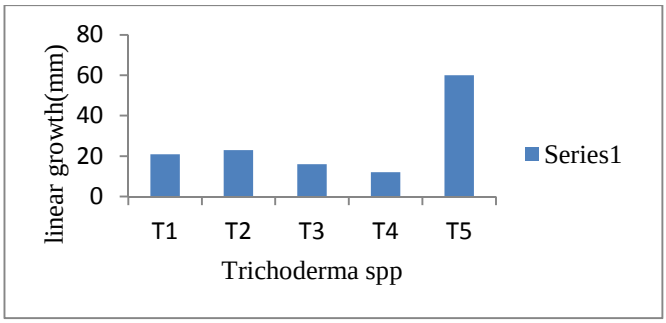


FIGURE 9. Effect of soil application with *Trichoderam spp.* On the dry root rot incidence in black gram

T1 – *T. harzianum*, T2 – *T. hamatum*, T3 –*T. reesii*, T4 – *T. viride*, T5 – Control

Values given are the mean value (X) of 4 data's, d.f. = degrees of freedom = n-1, Significance ++ = $p < 0.001$, + = $p < 0.05$, NS = Not significant

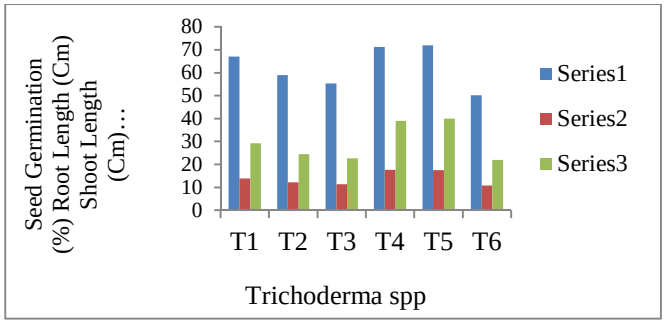


FIGURE10: Effect of soil application of Trichoderma spp. on the seed germination and growth parameters in black gram

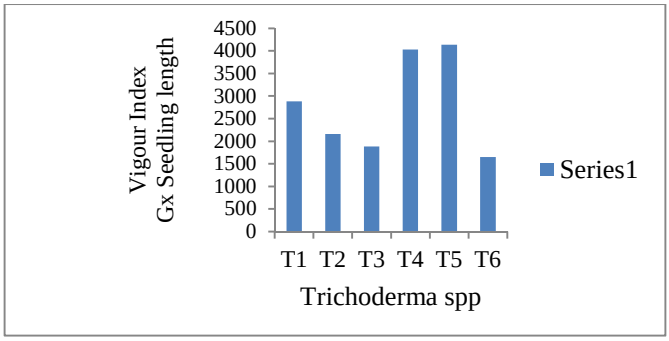


FIGURE 11. Vigour Index Gx Seedling length

T1 – *T. harzianum*, T2 – *T. hamatum*, T3 –*T. reesii*, T4 – *T. viride*, T5 – Control

Values given are the mean value (X) of 4 data's, d.f. = degrees of freedom = n-1, Significance ++ = $p < 0.001$, + = $p < 0.05$, NS = Not significant

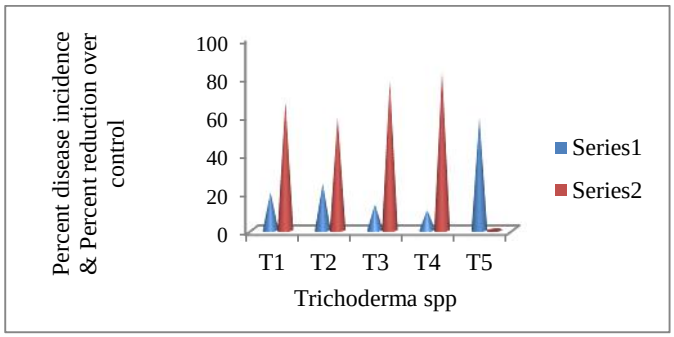


FIGURE 12. Effect of certain treatment with combination on the dry root rot incidence of black gram in unsterilized soil

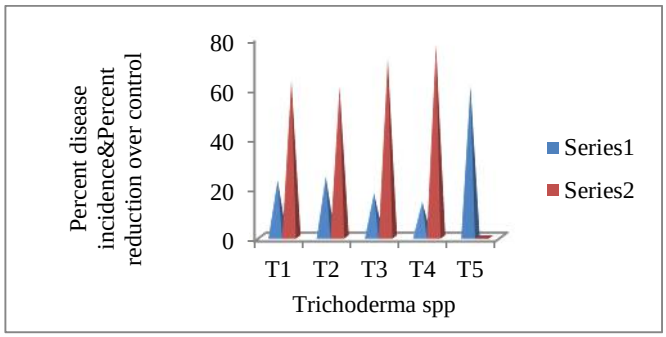


FIGURE 13. Effect of certain treatment with combination on the dry root rot incidence of black gram in sterilized soil

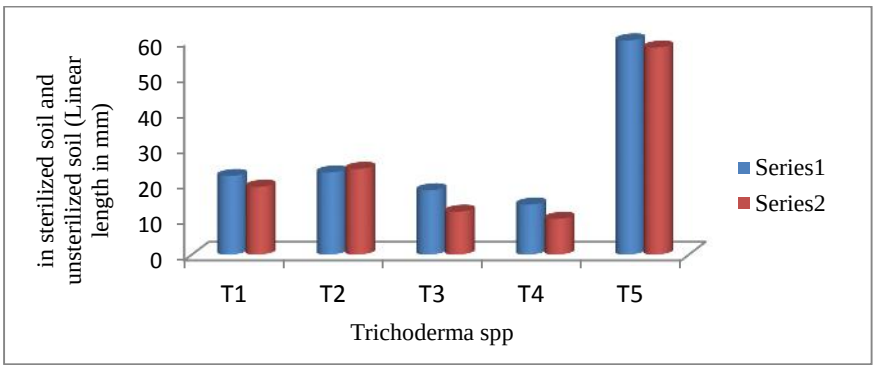
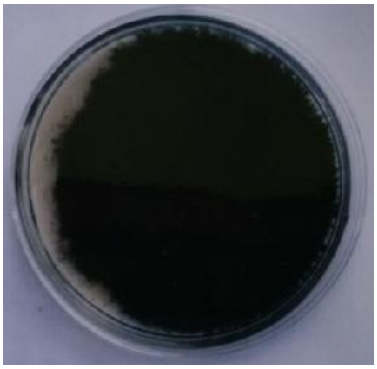
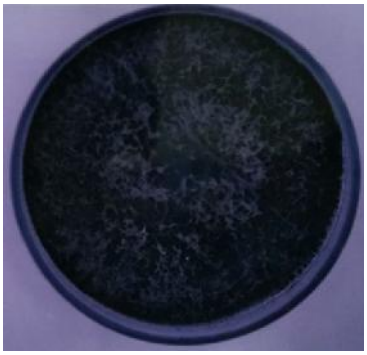


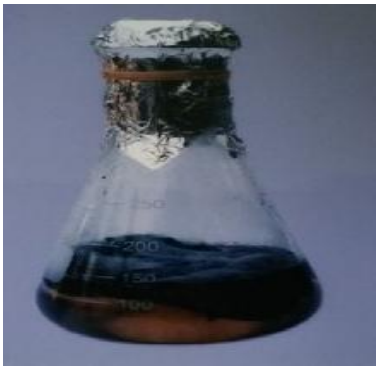
FIGURE 14. Effect of certain treatment combination on the dry root rot incidence of black gram in sterilized soil and unsterilized soil



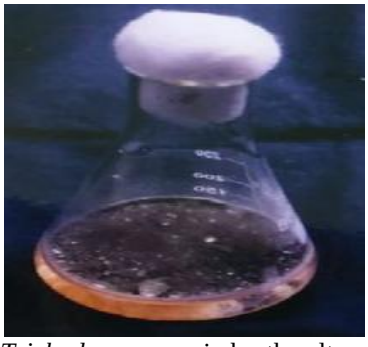
Macrophominaphaseolina in petri plate



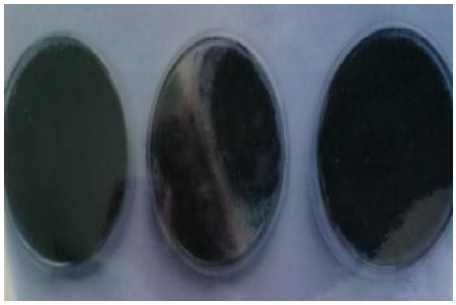
Trichoderma spp., in petri plate



Macrophominaphaseolina in broth culture



Trichoderma spp., in broth culture



Trichodermaspp – Macrophomina phaseolina Healthy plant

Diseased plant Interaction by Dual culture method

Pot culture study

In the present study promising antagonists were isolated from the rhizosphere of healthy as well as diseased cowpea, groundnut and sorghum plants in Niger. Most of the isolated bacterial antagonists irrespective of the site of their isolation inhibited the growth of *M. phaseolina*. However, the antagonist *B. subtilis* A11, most effective was isolated from the rhizosphere of plants in a cowpea field not infected by *M. phaseolina*. Similarly, antagonist isolates from diseased and healthy gram plants, or from potato fields heavily infected with *M. phaseolina* were reported to be effective

against *M. phaseolina* (Broadabent *et al*., 1977; Parakhia and Vaishnav, 1986). As demonstrated in various studies (Pusey and Wilson, 1984; Tilcher, 1995; Montesinos *et al*., 1996), strains of pseudomonads, bacilli and actinomycetes, isolated from the soil were successfully used to control pathogens causing damages in aerial parts of the plants. Differences in inhibition zone diameter observed between different isolates of Bacillus on the same medium suggest that antibiotic production is strain dependent, as also reported by (Becker and Cook (1988) and Landa *et al*. (2003). Thus, the inhibition zone produced in vitro indicates antibiotic production and is regarded as an important criterion for the selection of potent antagonists. The isolation of numerous antibiotic-producers from naturally suppressive soils indicates that antibiotics are involved in the naturally occurring biological control of pathogens (Raaijmakers, 1998). Various authors (Utkhede and Rahe, 1980; Loeffler *et al*., 1986; Pal *et al*., 2001) reported the production of antibiotics with antifungal effect by *B. subtilis* strains. Besides bacterial strains, inhibition of *M. phaseolina* in agar culture by fungal species was also reported (Parakhia and Vaishanav, 1986; Pal *et al*., 2001). In the greenhouse experiments, the Bacillus antagonists A11, B6, C20, which were to a different extent efficient in vitro, revealed effective strain A11 inhibited totally the growth of *M. phaseolina*

In vitro on TSA and was also the most effective antagonist to control the fungus in pot experiments. It is known that good correlation may exist between the efficiency of antagonists in vitro and their effect ad planta (Xu and Gross, 1986; Montesisnos *et al*., 1996; Kempf, 1988) as there may also be lack of correlation between in vitro and ad planta results (Kempe and Sequeira, 1983; Green *et al*., 1995). The performance of antagonists can be influenced by various factors such as the humidity and structure of the soil and other uncontrolled, adverse environmental conditions (Broadabent *et al*., 1977). The high efficiency of *B. subtilis* A11 in the control of *M. phaseolina*, with an overall low disease incidence of 6% and low inoculum level detectable in the plants by ELISA (E405 = 0.24) may be associated not only to antibiotic production, but also probably to plant-growth promotion by the antagonist. Indeed, *B. subtilis* isolates are implicated in the production of organic acids, gibberellin and auxin, in the solubilization of phosphate or inhibition of deleterious root colonizing microorganisms or their toxins (Broadabent and Baker, 1977; Pal *et al*., 2001) and have been successfully used for the management of many plant pathogens (Pal *et al*., 2001; El-Hassan and Gowen, 2006; Schisler *et al*., 2002; Landa *et al*., 2004). Mechanisms of pathogen control by *Bacillus spp*. may be by competition in root colonisation and production of antifungal compounds (Pal *et al*., 2001) by promotion of plant growth, and/or induction of systemic resistance (Kloepper *et al*., 2004). Various communications also report the successful use of microbial antagonists to control *M. phaseolina* (Pal *et al*., 2001; Singh *et al*., 2002), which supports our results. A good rhizosphere competence and colonization of the hypocotyls was observed for *B. subtilis* A11 three weeks after treatment under the dry and hot growth conditions, making this antagonist suitable for use in the field under the ecological conditions of arid ecozones. Also Sasse (1997) and Milus and Rothrock (1993) detected high population densities of *B. subtilis* on the roots, and to a smaller extent on the stem of rapeseed 30 days after planting, and on the roots of wheat. Several studies have demonstrated that rhizobacteria must establish and maintain a threshold population density in the rhizosphere to prevent or limit pathogen infection (Raaijmakers *et al*., 1998). But, reports of rhizosphere colonisation by bacteria of the genus Bacillus are few (Pal *et al*., 2001), as compared to *Pseudomonas spp*., which were extensively investigated for their ability to colonise the rhizosphere (Pal *et al*., 2001; Singh *et al*., 2002; Landa *et al*., 2003). The results of the present study are, however, conflicting with those of Scher *et al*. (1984), who reported a general lack of root colonisation by Gram-positive bacteria. Numerous studies (Lemanceau et al., 1995; Smith and Goodman, 1999; Mazzola and Gu, 2002) have clearly shown that various factors, among them also the plant genotype, can influence the composition and activity of microorganisms in the rhizosphere, and that the effect of antagonists could therefore vary under field conditions. Thus, the combinations of the ability of *B. subtilis* to adapt to extreme environmental conditions such as high temperatures and drought, which favour the occurrence of *M. phaseolina*, with its capability to colonise the plant organs first attacked by the fungus, make this bacterium most suitable as a means to contribute significantly to the control of charcoal rot. In the specific case of Niger, coating seeds with *Bacillus subtilis* A11 in combination with cultural practices is feasible and is suggested to considerably ease the problem of *M. phaseolina*. Therefore, biological control should be one element in an integrated approach for the control of charcoal rot, further comprising the use of clean planting material, crop rotation, timing of the growth period, use of resistant varieties and solarisation.

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