

BIOEFFICACY OF *TRICHODERMA* ISOLATES AGAINST *ASPERGILLUS NIGER* VAN TIEGHEM INCITING COLLAR ROT IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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Abstract: Antagonistic effect of 12 isolates of 3 *Trichoderma* strains (*T. virens*, *T. viride*, *T. harzianum*) against the collar rot disease-causing fungus *A. niger*, was studied *in vitro*. It was observed that *T. viride* 60 inhibited maximum (86.2%) growth of test fungus, followed by *T. harzianum* 2J (80.4%). The five varieties of groundnut grown in normal (T_1), sick – *A. niger* infested soil (T_2) and sick + *Trichoderma viride* 60 (seed treatment) (T_3) in pot culture showed significant differences in the per cent of disease incidence of collar rot, up to 15 days after sowing (DAS). The per cent of collar rot disease incidence was higher in the GG-20 (67.4%) variety, followed by moderate in GAUG-10 and GG-13 (46%), and minimum in J-11 and GG-2 (30%) in *A. niger* infected pot culture, at 15 days after sowing (DAS). Based on collar rot disease incidence, groundnut varieties were screened as: susceptible, moderately susceptible and tolerant. *Trichoderma* seed treatment (T_3) reduced 51.6% of the disease incidence in susceptible varieties and 58.1% in tolerant varieties, at 15 DAS, under *A. niger* infection (T_2) in pot culture study.

Key words: antagonism, biological control, rot pathogen, induced resistance, peanut

INTRODUCTION

Because peanut (*Arachis hypogaea* L.) grows underground, it is commonly known as groundnut. It is one of the world's major legume food crops and originated from Brazil in South America. The major production constraint of this crop has been its confinement mainly to dry or rain dependent areas (~6.0–6.5 million ha) (FAO 2008). Groundnut is a crop which is mainly cultivated under rain-fed conditions, thus, pathogens have more of a chance to attack the crop. Grover (1981) listed more than 55 pathogens in groundnut crop. Only a few, such as early leaf spot (*Phaeoisariopsis arichidicola*) late leaf spot (*Phaeoisariopsis personata*), rust (*Puccinia arichidis*), collar rot (*Aspergillus niger* van Tieghem), stem rot (*Sclerotium rolfsii* Sacc.), root rot (*Macrophomina phaseolina*), and aflaroot (*Aspergillus flavus*), are economically important in India. Nematode diseases like root knot, and viral diseases like peanut bud and stem necrosis, groundnut mottle and clump (Ghewande and Reddy 1986) are major diseases that limit groundnut production and productivity. In addition, the pre- and post-harvest aflatoxin contamination in the kernels and meal also reduces groundnut quality as well as export value.

The *A. niger* causing collar rot disease on groundnut seedlings was first reported by Jochem (1926). However, Jain and Nema (1952) first reported the *Aspergillus* blight of groundnut caused by *A. niger* in India. This disease ap-

pears in two phases viz, pre-emergence and post-emergence phase. In the pre-emergence phase, the seed may rot in the soil or be covered with sooty black masses of spore on germination, the emerging hypocotyls are rapidly killed by these spores. In the post-emergence phase, circular light brown lesions appear initially on the cotyledons and as they advance the hypocotyl tissue or stem lesion becomes water-soaked and shows light brown discoloration. The seedlings then collapse and die due to the rotting of the succulent hypocotyls.

A. niger may cause an average 5 per cent loss in yield but in some areas it may cause as high as a 40 per cent loss. Collar rot is a more serious problem in sandy soil (Gibson 1953; Chohan 1965). In Punjab (India), the mortality losses of plants due to *A. niger* may amount to 40 to 50 per cent (Aulakh and Sandhu 1970). Joshi (1969) surveyed groundnut growing areas in the state of Gujarat (India) and found as high as 50 per cent seedling blight in some fields. Similarly, Ghewande *et al.* (2002) reported that losses in terms of mortality of plants due to collar rot range from 28 to 50 per cent.

Thus, among the diseases associated with groundnut, collar rot (*A. niger* Van Tieghem) is one of the most important. Collar rot causes heavy losses in pod and fodder yield of groundnut. Most of the varieties of groundnut are susceptible to this disease. Many seed dressing fungicides are reported to be effective against collar rot of

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groundnut (Gangopadhyay *et al.* 1996; Karthikeyan 1996). But limited work has been done on successful exploitation of bio-control agents, for the management of collar rot disease through induced resistance. The above method is very needed to keep the disease below the economic threshold level without damaging the agro-ecosystem in soil (Papavizas and Lewis 1988). *Trichoderma* have been used as biological control agents against soil-borne plant pathological fungi (Kucuk and Kivank 2003). The main objective of the present study was to find an, *in vitro* *Trichoderma* strain that will act as the best bio-control agent for effectively inhibiting the growth of *A. niger* (as all *Trichoderma* strains do not work equally against a specific disease). The second aim was to determine the overall efficacy of the best *Trichoderma* strain, to control collar rot disease in various groundnut varieties in pot culture study.

MATERIALS AND METHODS

Isolation and maintenance of microbes

Groundnut seedlings which showed typical symptoms of collar rot, were cut into small bits using a sterilized blade. The pure pathogen culture (*A. niger*) was made by the hyphal tip isolation method (Sinclair and Dhingra 1985) on the solidified PDA medium in petri plates. A typical black mycelium (conidia) growth of *A. niger* was observed after 72 h of incubation, at 28±2°C, in an incubator. This was maintained throughout the study by periodical transfers on (PDA) medium under aseptic conditions, to keep the culture fresh and viable.

Various isolates of *Trichoderma* were isolated from different rhizosphere from the Saurashtra region (Gujarat, India) by the Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh. Slants of all 12 isolates, of 3 *Trichoderma* strains (*T. viren* BAN, *T. viride* BAN, *T. viride* JND, *T. harzanium* BAN, *T. viride* 54, *T. viride* 60, *T. viride* 62, *T. harzanium* 2J, *T. harzanium* 4J, *T. harzanium* 5J, *T. harzanium* 6J, *T. harzanium* JND) were collected and maintained throughout the study by periodical transfers on PDA media under aseptic conditions, to keep the culture fresh and viable.

In vitro antagonism between bio-agent *Trichoderma* and pathogen – *A. niger*

The dual culture technique was used to test the antagonistic effect of 12 isolates of 3 *Trichoderma* strains (*T. viren*, *T. viride* and *T. harzanium*) against *A. niger* on PDA media. A 4 mm in diameter mycelial disc, from each *Trichoderma* isolate and test fungus (*A. niger*) were placed on PDA medium in the same petri plate, approximately 4 cm away from each other. The experiment was conducted in four replications for each antagonist. All the inoculated plates were incubated at a temperature of 30±1°C. After six days, the plates were observed for growth of antagonist and test fungus. Index of antagonism as per cent growth inhibition of *A. niger*, was determined by following the method of Watanabe (1984).

Preparation of mass inoculums for pot culture study

The fungal pathogen *A. niger*, which was isolated and maintained as described earlier on PDA media, was mul-

tiplied on potato dextrose broth culture. A 100 ml potato broth culture was then poured in 250 ml conical flasks, and allowed to cool at room temperature in a laminar flow hood (Sinclair and Dhingra 1985). These flasks were then inoculated with a mycelial disc (4 mm diameter) of *A. niger* and incubated at 28±2°C, for 15 days in a BOD incubator. The inoculums thus obtained, were harvested and used for preparation of talc based formulations (Singh *et al.* 2001), for pathogen infection in pot culture study.

The bio-agent *T. viride* 60 which was isolated and maintained earlier on PDA media, was multiplied on sand maize meal medium (SMMM). These flasks were then inoculated with the mycelial disc (4 mm diameter) of *T. viride* 60 and incubated at 28±2°C, for 15 days in a BOD incubator (Sinclair and Dhingra 1985). The inoculums thus obtained, were harvested and used for preparation of talc based formulations (Singh *et al.* 2001) for bio-control agent.

Determination of microbes load (cfu) from talc formulations

The serial dilution plate method was used to determine the microbe load of pathogen *A. niger* or bio-control agent *T. viride* 60 in their respective talc based powder mass formulations (Muhammad and Amusa 2003). Before being used in the experiment, microbe load as a colony forming unit (cfu) was measured by serial dilution, from both the microbe mass formulations and individually.

Preparation of sick soils

Field soil and farm yard manure (FYM) was mixed in a 1:1 proportion, and sterilized in an autoclave in 1:036 kg/cm² for one hour, for three consecutive days. A talc based formulation of pathogen *A. niger* which had the microbial load 1.5x10⁷ cfu/g talc powder, was then added to the soil in a 1:10 proportion (Talc based Inoculums + Sterilized mixture of soil). The pots were filled with these mixtures – 10kg/pot as a sick soil/pot.

Seed sowing and seed treatment

Earthen pots with a 35 cm diameter, were washed thoroughly with tap water. The tap water wash was followed by a 5 per cent formaldehyde solution wash. The pots were allowed to dry before use. Pots were filled with either normal black soil or with inoculated soil (5 kg soil/pot). Pots were watered 48 h before sowing. Following treatment, the five varieties of groundnut seeds were arranged for sowing. All seeds were treated either with talcum powder containing CMC only, or with talc based formulations of *T. viride* 60 bio-control agents – 4 g/kg seeds. Prior to treatment, all groundnut seeds were moistened with water, so that the talc formulations adhered to the seeds.

T1 – Groundnut seeds of all five varieties were treated with talc based powder containing CMC only, and sown in normal soil pots as a control.

T2 – Groundnut seeds of all five varieties were treated with talc based powder containing CMC only, and sown in sick (*A. niger* infected) soil pots.

T₃ – Groundnut seeds of all five varieties were treated with talc powder based formulation of bio-control agent – *T. viride* 60 (microbial load 1.83x10⁶ cfu/g talc powder), and sown in sick (*A. niger* infected) soil pots.

In each pot, 25 groundnut seeds were sown. Three replications were sown for each variety. These five varieties of groundnut were also sown in a similar way: J-11 (V₁), GG-2 (V₂), GAUG-10 (V₃), GG-13 (V₄) and GG-20 (V₅). Observations and recordings were made of the per cent of disease incidence at 3 days intervals, up to 15 days after sowing (DAS) [0 (S₀), 3 (S₁), 6 (S₂), 9 (S₃), 12 (S₄), 15 (S₅) DAS].

Per cent of disease incidence

The incidence of collar rot in each treatment was recorded as pre-emergence rotting, based on germination up to 15 days after sowing (DAS) (Rao and Sitaramaih 2000) using the following formula. The observations were based on 25 seeds sown in each pot.

Statistical analysis

The data obtained by *in vitro* per cent growth inhibition of test fungus were subjected to simple Completely Randomized Design (CRD). However, per cent incidence of collar rot disease in groundnut seedlings in the pot culture study was first subjected to arc sin transformation. Then subjected to analysis by 3FCRD (varieties-5, treatments-3, stages-6) as the statistical tools for interpretation of data (Snedecor and Cochran 1967).

RESULTS

In vitro antagonism between bio-agent *Trichoderma* and pathogen – *A. niger*

Growth inhibition of *A. niger* during *in vitro* interaction with bio-control agents *Trichoderma*, at 6 days after inoculation (DAI), was depicted in figures 1 and 2. Per cent growth inhibition of pathogen (*A. niger*) was significantly higher in T₆ (86.2%) antagonist, followed by T₈ (80.4%), T₃ (74.3%), T₂ (71.9%), T₁ (60.9%) and T₁₂ (50.6%) at 6 DAI. Non significant differences were observed between antagonists T₅ (42.4%) and T₁₀ (40.2%). However, other antagonists were recorded with a below 30% growth inhibition of fungal pathogen. Thus, it was observed that T₆ antagonist (*i.e.* interaction between *Trichoderma viride* 60 and pathogen (*A. niger*)) have a better growth inhibition of test fungus *A. niger*, compared to the other bio-control agents.

Per cent of collar rot disease incidence in groundnut seedlings

The values presented on the per cent of collar rot disease incidence were arc sin transformed values, as zero values obtained in normal treatment (T₁). Mean varietal differences on the per cent of disease incidence were significant (Fig. 3A). The disease incidence in varieties J-11 (V₁) and GG-2 (V₂) was 10.8 and 10.5%, respectively (non significant differences), and it significantly increased to 14.2% in GAUG – 10 (V₃), followed by 18.5% in GG-13 (V₄) and 23.6% in GG-20 (V₅).

Treatments differences - normal (T₁), Sick with pathogen *A. niger* (T₂) and Sick + *T. viride* 60 seed treatment

(T₃) were found to be highly significant in the per cent of collar rot disease incidence (Fig. 3B). Among the treatments, T₂ showed the highest disease incidence (29.8%) followed by T₃ (15.1%) and T₁ showed normal incidence (1.65%). The disease incidence per cent was significantly increased from 1.65 to 23.8% (Fig. 3C) with the advancement of disease, germination and seedling growth at different disease developmental stages *i.e.* 0 DAS (S₀), 3 DAS (S₁), 6 DAS (S₂), 9 DAS (S₃), 12 DAS (S₄) and 15 DAS (S₅). A drastic rise (46%) in seed rotting was recorded during the S₀ to the S₁ stage as a pre-emergence rotting of the seeds, followed by 16.5 % increases in disease incidence from the S₂ to the S₃ stage.

The interaction effect of VxT were significantly different for the per cent of disease symptoms (Fig. 4A). Seeds grown in normal soil (T₁) had no disease incidence, however, seeds sown in sick (T₂) soil had maximum disease incidence. Sick + *Trichoderma* treated seeds (T₃) significantly reduced the disease incidence, compared to the T₂. Minimum disease occurrence (22.2%) was recorded in tolerant varieties (J-11, GG-2) which were significantly reduced to around 8% when the seeds were treated with bio-control agent *Trichoderma* (T₃). However, the susceptible variety (GG-20) had a 42.6 % disease incidence in T₂ and it was also significantly reduced to 26.4 % in T₃ treatment. Moderately susceptible varieties (GAUG-10, GG-13) revealed higher values of disease incidence than tolerant varieties but lower values than susceptible varieties.

The combined effect of VxS was found to be significant (Fig. 4B). During disease developmental stages (S₀ to S₅), collar rot disease per cent incidence increased significantly from 1.65 (S₀) to 17% (S₅) in tolerant varieties (J-11 and GG-2), followed by 1.65 (S₀) to 23.8% (S₅) in GAUG-10, 1.65 (S₀) to 26.5 (S₅) in GG-13 (Moderately), and 1.65 (S₀) to 34.6% (S₅) in GG-20 (Susceptible). Overall, tolerant varieties had lower disease frequency, followed by moderately susceptible, and then susceptible varieties.

The interaction effect of TxS for per cent of disease incidence, was found to be significant (Fig. 4C). Groundnut varieties sown in sick soil (T₂) had the highest percent of disease incidence, followed by T₃ and T₁. Per cent of disease occurrence significantly increased as the disease developmental stages (S₀ to S₅) progressed, in T₂ and T₃ treatments. Seeds sown in sick soil (T₂) had a recorded 44.5% disease incidence at the S₅ stage while sick soil + seeds treated with *Trichoderma* (T₃) had a recorded 25.5% disease incidence at the S₅ stage.

Interaction effect of VxTxS for per cent of collar rot disease incidence revealed significant changes in germination and seedling growth of groundnut varieties (Table 1). At Normal (T₁) and 0 DAS (S₀) stages no disease incidence was recorded. Disease occurrence was maximum (67.4%) in GG-20 (susceptible variety) in sick soil (T₂) at 15 DAS (S₅ stage), however, disease occurrence was significantly reduced to 34.8% under T₃ treatment at S₅ stage. Disease incidence was recorded to be minimum (31%) in J-11 and GG-2 (Tolerant) in sick soil at S₅ stage, and disease incidence also declined to 18% under T₃ treatment at S₅ stage. So, seed treatment (T₃) with *Trichoderma* reduced 51.6% of disease incidence in the susceptible variety (GG-20) under *A. niger* infested conditions at 15 DAS (S₅). Disease

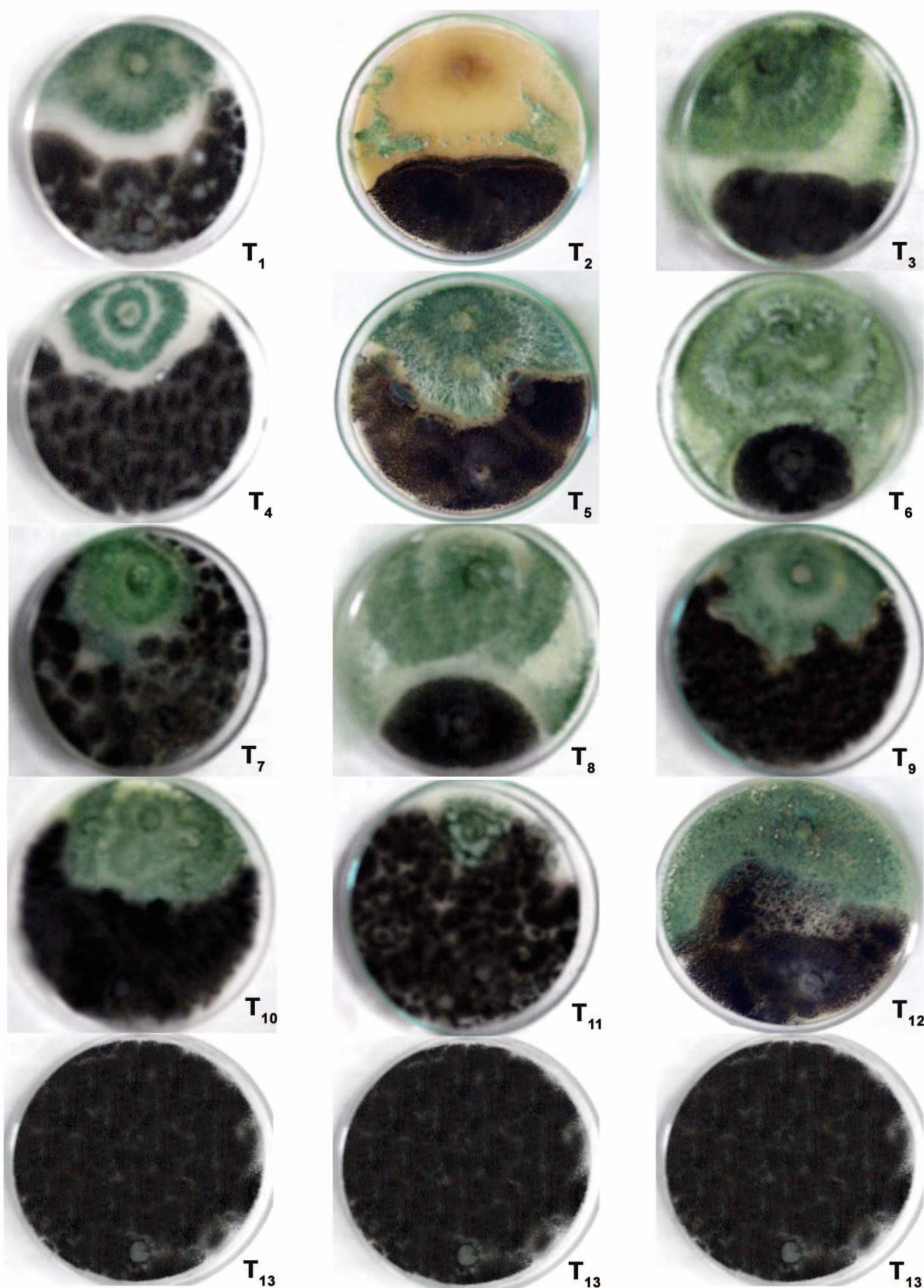


Fig. 1. Antagonism between *Trichoderma* isolates and *A. niger* at 6 DAI (Antagonists petri dish (T1 to T12) have *Trichoderma* isolates at the top and pathogen *A. niger* at the bottom)

T – *T. viren* BAÑ x AÑ; T₂ – *T. wr/de* BAÑ x AÑ; T₃ – *T. wr/de* JND x AÑ; T₄ – *T. harzianum* BAÑ x AÑ; T₅ – *T. wr/de* 54 x AÑ; T₆ – 7: *wr/de* 60 x AÑ; T₇ – *T. wr/de* 62 x AÑ; T₈ – *T. harzianum* 2J x AÑ; T₉ – *T. harzianum* 4J x AÑ; T₁₀ – *T. harzianum* 5J x AÑ; T₁₁ – *T. harzianum* 6J x AÑ; T₁₂ – *T. harzianum* JND x AÑ; T₁₃ – Control – *A. niger* (AÑ)

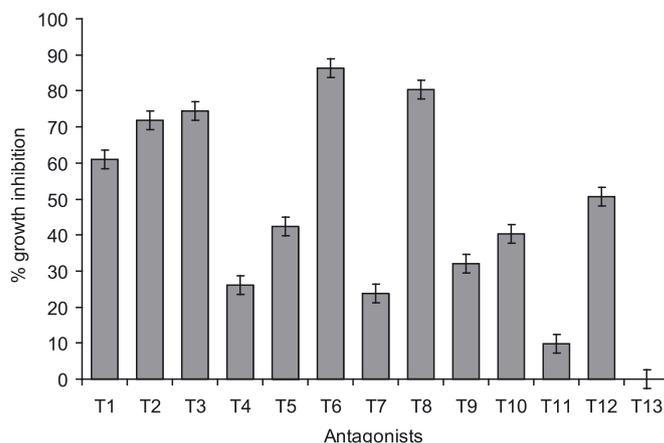
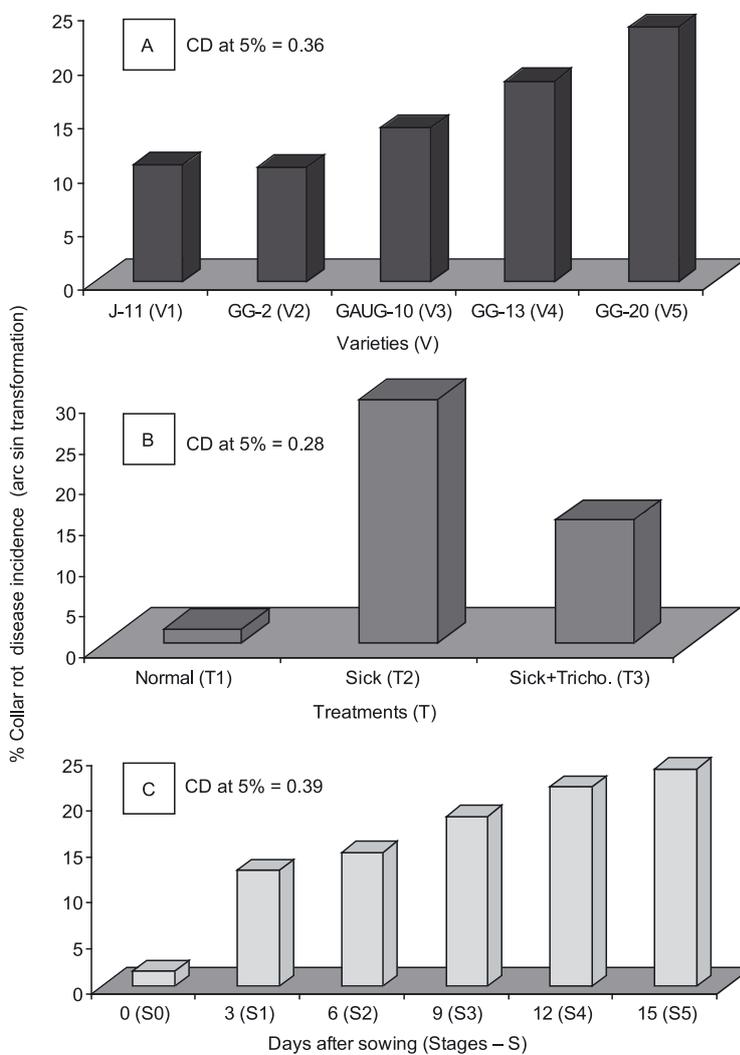
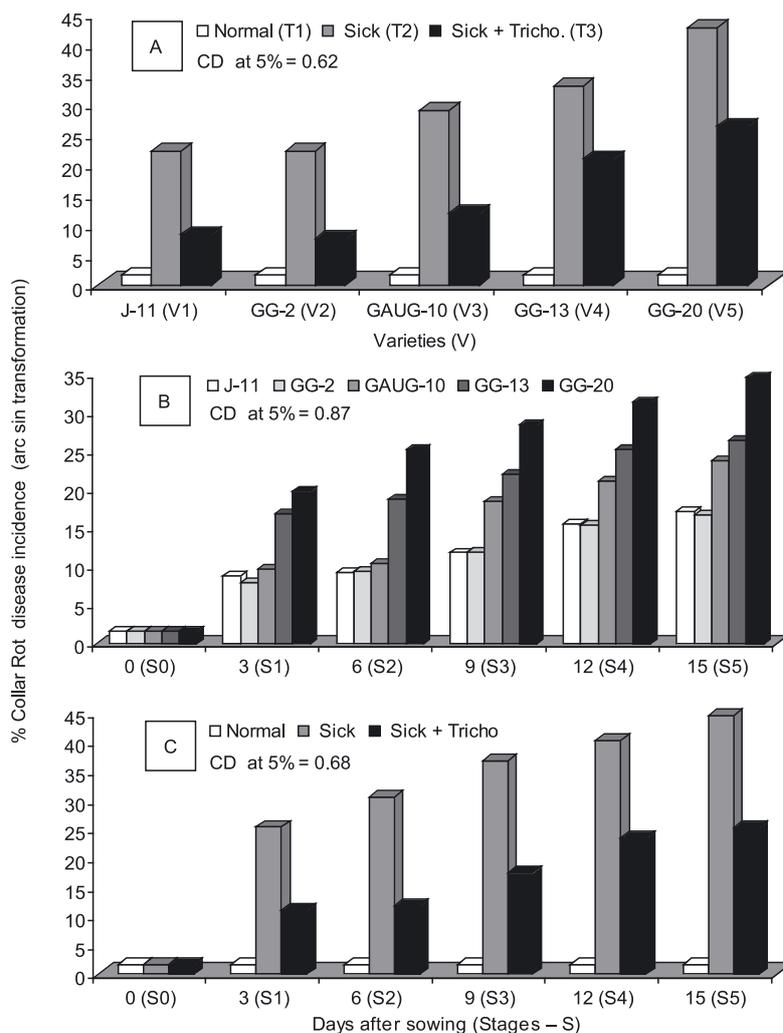


Fig. 2. Per cent of growth inhibition of *Aspergillus niger* during *in-vitro* antagonism with *Trichoderma* at 6 days after inoculation (DAI) T₁ – *T. viren* BAN X *A. niger* (AN); T₂ – *T. viride* BAN X AN; T₃ – *T. viride* JND X AN; T₄ – *T. harzanium* BAN X AN; T₅ – *T. viride* 54 X AN; T₆ – *T. viride* 60 X AN; T₇ – *T. viride* 62 X AN; T₈ – *T. harzanium* 2J X AN; T₉ – *T. harzanium* 4J X AN; T₁₀ – *T. harzanium* 5J X AN; T₁₁ – *T. harzanium* 6J X AN; T₁₂ – *T. harzanium* JND X AN; T₁₃ – control – *A. niger* (AN); Error bar indicates CD value at 5%



CD – critical differences

Fig. 3. Mean effect of varieties (A), treatments (B) and stages (C) on per cent of disease incidence in groundnut seedlings



CD – critical differences

Fig. 4. Combined effect of VxT (A), VxS (B) and TxS (C) on per cent of disease incidence in groundnut seedlings

incidence was reduced up to 58.1% in tolerant varieties (J-11 and GG-2) by T₃ treatment. The data recorded for the moderately susceptible varieties (GAUG-10 and GG-13) were in between the tolerant and susceptible varieties at all disease developmental stages, and for T₂ and T₃. Based on disease incidence, groundnut varieties were categorized into: tolerant (J-11, GG-2), moderately susceptible (GAUG-10, GG-13) and susceptible (GG-20).

DISCUSSION

Groundnut is an economically important crop but the collar rot disease was affecting its growth. The present experiment was initiated to study the comparative efficacy of the bio-control agents *Trichoderma* on different susceptibilities of groundnut varieties against *A. niger* causing collar rot at the pre emergence phase. An antagonistic effect of fungal bio-control agents against the test pathogen fungus (*A. niger*) was observed. *T. viride* 60 (T₆) showed maximum reduction in growth of test fungus followed by *T. harzianum* 2J (T₈), *T. viride* JND (T₃), *T. viride* BAN (T₂), *T. viren* BAN (T₁), *T. harzianum* JND (T₁₂), *T. viride* 54 (T₅), *T. harzianum* (T₁₀), *T. harzianum* (T₉), *T. harzianum* (T₄), *T. viride* 62 (T₇) and *T. harzianum* (T₁₁). These results are in confirmation with the finding of Kishore *et al.* (2001), who

reported that the *T. viride* and *T. harzianum* were found to be effective in reducing the radial growth of *A. niger* *in vitro*. Rao and Sitaramaih (2000) and Prabhu and Urs (1998) also documented that *Trichoderma* isolates significantly inhibited the growth of *A. niger*. The bio-control agent *T. viride* had a greater inhibition on *A. niger* than *T. harzianum* (Raju and Murthy 2000).

Prameala *et al.* (2005) studied the antagonistic effect of *Trichoderma* sp. and *Pseudomonas fluorescense* against isolates of *Fusarium oxysporum* f. sp. *carthami* that cause wilt disease in sunflower. Among three antagonists tested, *T. viride* was found to be more effective than *T. harzianum* and *P. fluorescens*, which confirms the present experimental results that *T. viride* was more effective than *T. harzianum* and *T. virens*. Seventy *Trichoderma* isolates collected from different regions of Morocco were tested for their capacity to inhibit *in vitro* mycelial growth of *Sclerotium rolfsii* (Khattabi *et al.* 2004). Four of these isolates (Nz, Kb2, Kb3 and Kf1) showed good antagonistic activity against *S. rolfsii*, and were also highly competitive in natural soil. These isolates would therefore, be candidates for development in biological control. *Trichoderma* is known to act through several mechanisms such as hyperparasitism, inhibition and antibiosis. Shalini and Kotasthane (2007) screened seventeen *Trichoderma* strains against *R. solani*

Table 1. Interaction effect of varieties, treatments and stages on per cent incidence of collar rot disease in groundnut seedlings

Sr. No.	Treatments/Stages	Disease development stages					
		0 DAS* (S ₀)	3 DAS (S ₁)	6 DAS (S ₂)	9 DAS (S ₃)	12 DAS (S ₄)	15 DAS (S ₅)
1.	J-11+Normal (V ₁ T ₁)	1.65** (0.00)***	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)
2.	J-11+Sick (<i>A. niger</i>) (V ₁ T ₂)	1.65 (0.00)	19.9 (11.8)	24.6 (17.4)	26.6 (20.2)	29.2 (23.8)	31.4 (27.2)
3.	J-11+Sick+Tricho. (V ₁ T ₃)	1.65 (0.00)	5.12 (0.93)	1.65 (0.00)	7.24 (1.59)	16.11 (7.71)	18.6 (10.3)
4.	GG-2+Normal (V ₂ T ₁)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)
5.	GG-2+Sick (<i>A. niger</i>) (V ₂ T ₂)	1.65 (0.00)	20.7 (12.6)	24.8 (17.7)	26.2 (19.5)	29.0 (23.5)	30.9 (26.4)
6.	GG-2+Sick+Tricho. (V ₂ T ₃)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	7.98 (1.98)	15.7 (7.31)	17.9 (9.44)
7.	GAUG-10+Normal (V ₃ T ₁)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)
8.	GAUG-10+Sick (<i>A.niger</i>) (V ₃ T ₂)	1.65 (0.00)	25.66 (18.8)	28.0 (22.1)	37.2 (36.6)	38.2 (38.3)	43.2 (46.9)
9.	GAUG-10+Sick+Tricho. (V ₃ T ₃)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	16.8 (8.4)	23.8 (16.3)	26.5 (19.9)
10.	GG-13+Normal V ₄ T ₁	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)
11.	GG-13+Sick (<i>A. niger</i>) (V ₄ T ₂)	1.65 (0.00)	28.5 (22.7)	31.1 (26.7)	40.8 (42.8)	46.4 (52.5)	49.6 (57.9)
12.	GG-13+Sick+Tricho. (V ₄ T ₃)	1.65 (0.00)	20.4 (12.2)	23.7 (16.2)	23.8 (16.3)	27.9 (22.1)	28.4 (22.4)
13.	GG-20+Normal (V ₅ T ₁)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)	1.65 (0.00)
14.	GG-20+Sick (<i>A. niger</i>) (V ₅ T ₂)	1.65 (0.00)	31.8 (27.7)	41.1 (48.4)	52.4 (62.8)	58.7 (73.0)	67.4 (85.2)
15.	GG-20+Sick+Tricho. (V ₅ T ₃)	1.65 (0.00)	26.3 (16.6)	30.1 (25.2)	31.3 (27.1)	34.0 (31.3)	34.8 (32.8)
VxTxS		±SEm	0.541	CD at 5%	1.51	CV %	6.04

*DAS – days after sowing; **mean of three replications; ***figures in parentheses are retransformed (original) value of arc sin transformation; CD – critical differences; CV – Coefficient of variation

in vitro. All strains, including *T. harzianum*, *T. viride* and *T. aureoviride*, inhibited the growth of *R. solani*.

In pot culture study, bio-control agent *T. viride* 60 reduced the collar rot disease incidence effectively in different groundnut varieties. These results are in agreement with Prabhu and Urs (1998), Raju and Murthy (2000), Kishore *et al.* (2001) who reported that collar rot was reduced more efficiently by *T. viride* compared to other strains of *Trichoderma*, in pot culture. However, Kishore *et al.* (2006) also found, that bio-control agent – *Pseudomonas aeruginosa* GSE 18 reduced the preemergence of groundnut rotting by 60% in *A. niger* infested potting mixture. Their results are in agreement with the present results where the bio-agent *T. viride* 60 reduced the collar rot incidence of groundnut by 51.6% (GG-20) to 58.1% (J-11, GG-2), under sick soil conditions. This indicates that bio-control agent *T. viride* 60 might have a significant role in the control of collar rot disease, by reducing the virulence of *A. niger* in the groundnut rhizosphere.

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POLISH SUMMARY

BIOLOGICZNA SKUTECZNOŚĆ IZOLATÓW GRZYBA *TRICHODERMA* PRZECIWKO *ASPERGILLUS NIGER* VAN TIEGHEM – SPRAWCY ZGNILIZNY ORZESZKÓW ARACHIDOWYCH

Badano *in vitro* antagonistyczne działanie 12 izolatów, trzech gatunków grzyba *Trichoderma* (*T. virens*, *T. viride*, *T. harzianum*) przeciwko zgniliznie powodowanej przez grzyb *Aspergillus niger*. Stwierdzono, że izolat 60 *T. viride* maksymalnie hamował wzrost testowanego patogena w 86,2%, a w dalszej kolejności sklasyfikowano izolat 2J *T. harzianum* (80,4%). W doświadczeniu wazonowym badano reakcję pięciu odmian orzeszka arachidowego na porażenie grzybem *A. niger*, w następujących kombinacjach doświadczalnych: T1 – kontrola nietraktowana; T2 – gleba w wazonach zasiedlona grzybem *A. niger*; T3 – gleba zasiedlona grzybem *A. niger* + izolat 60 grzyba *Trichoderma viride* (potraktowane nasiona). Badane odmiany wykazały zróżnicowaną reakcję po upływie 15 dni od wysiewu. Najsilniej porażona była odmiana GG-20 (67,4%), średnio porażane były odmiany GAUG-10 i GG-13 (46%) oraz najsłabiej odmiany J-11 i GG-2 (30%). Biorąc pod uwagę stopień porażenia, odmiany pogrupowano na: podatne, umiarkowanie podatne i tolerancyjne. Potraktowanie nasion orzeszka arachidowego grzybem *Trichoderma* (T3) ograniczyło nasilenie choroby o 51,6% w przypadku odmian podatnych, a o 58,1% u odmian tolerancyjnych po upływie 15 dni od wysiewu nasion w doświadczeniu wazonowym z infekcją grzybem *A. niger*.