

Biocontrol of *Sclerotium rolfsii* Sacc. in peanuts (*Arachis hypogaea* L.) by *Trichoderma harzianum* Rifai in Malawi

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Abstract

The effect of *Trichoderma harzianum* as a biocontrol agent against *Sclerotium rolfsii* was tested in vitro, in the screen house and under field conditions. In vitro, *T. harzianum* applied as agar blocks or as a spore suspension inhibited mycelial growth and germination of sclerotia of *S. rolfsii* on potato dextrose agar. A suspension of 4 x 10⁶ spores/ml incorporated into potato dextrose agar was as effective as 20ppm mancozeb or 100ppm thiram in inhibiting germination of sclerotia. When *T. harzianum* spores were applied as a seed dressing, the incidence of groundnut damping-off under screen house conditions was reduced by 33% compared to the untreated control, whereas thiram and mancozeb were ineffective. Under field conditions, *T. harzianum* applied as a sorghum culture around the seeds at planting, performed inconsistently in that in 1991-2 season the incidence of damping-off was significantly less in treated than in untreated plants while in 1992-3 season, the disease incidence was generally high in all treatments.

Introduction

Sclerotium rolfsii is a serious pathogen of many crops in tropical and sub-tropical regions of the world, with groundnuts (*Arachis hypogaea*) sustaining the greatest annual losses world-wide from it (Diamonde and Beute, 1977; Porter et al., 1984). Control efforts using fungicides, such as PCNB, have often met with limited success and at best

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have only been 50-70% effective (Hammond et al., 1977; Hill and Waller, 1988). The use of soil fungicides, however, is too expensive for resource-poor subsistence farmers in developing countries.

Biological control of soil-borne pathogens has received a lot of attention as an alternative to the use of expensive and environmentally damaging chemicals (Chet et al., 1979; Grinstein et al., 1979; Bell et al., 1980). Wells et al. (1972) achieved 100% control of *S. rolfisii* with *T. harzianum* on groundnuts and tomatoes under greenhouse conditions. Similar results were obtained by Grinstein et al. (1979) who reported 95% control of *S. rolfisii* with *T. harzianum* in groundnuts under greenhouse conditions. Under field conditions, one to three applications of *T. harzianum* inoculum were reportedly effective in reducing the disease incidence (Wells et al., 1972; Elad et al., 1982; Csinos et al., 1983).

In Southern Malawi, *S. rolfisii* is a serious pathogen on beans, groundnuts and maize in small holder farmer's fields where it causes damping-off, root rot and southern blight (Khonga and Hillocks, 1994; Khonga and Hillocks, 1996). However, not much work has been done on how best the pathogen can be managed. The objectives of this study were to assess:

- the efficiency of a local strain of *T. harzianum* in the suppression of *S. rolfisii* in vitro
- the effect of *T. harzianum* on *S. rolfisii* damping-off and root rot of groundnut under screen house and field conditions

Materials and methods

In vitro test

The antagonistic effects of *T. harzianum* on *S. rolfisii* were tested on potato dextrose agar (PDA) in petri dishes using paired agar blocks and spore suspension methods. The agar block method was used to test hyphal interference and the spore suspension method was used to test inhibition of sclerotial germination. The *T. harzianum* isolate used in the study was isolated from dead coffee leaves which were collected from Mpalanganga estate in Zomba. The isolate had earlier been demonstrated to have strong antagonism against *Colletotrichum coffeanum* Noack (Khonga, 1994).

Paired agar block method

Agar blocks (5mm x 5mm) with mycelia of *T. harzianum* and *S. rolfsii* were placed 2cm apart in each petri dish of PDA. Control petri dishes were inoculated with agar blocks of either *T. harzianum* or *S. rolfsii* alone. Five replicate plates were used per treatment. The plates were incubated at 30°C until the plates were fully covered with the fungi.

Colony diameter was measured every day and when the colony had fully covered the PDA surface, the plates were removed from the incubator and kept under UV light at 25°C for sclerotial production. The number of sclerotia produced in each plate was recorded after two weeks. The experiment was repeated once.

Spore suspension method

A spore suspension of *T. harzianum* was mixed with cool molten PDA and poured into petri dishes giving a final concentration of 4×10^6 spores/ml (Treatment 1). Two broad spectrum fungicides thiram and mancozeb were also incorporated into molten PDA giving final concentrations of 100ppm thiram (Treatment 2) and 20ppm mancozeb (Treatment 3). Untreated PDA plates served as controls. Fifty sclerotia were placed on each of three replicate plates per treatment. The plates were incubated under UV light at 25°C for 72 hours and the numbers of germinated sclerotia were determined and expressed as percentage germination. The experiment was repeated once.

Screen house experiment

In the screen house, *T. harzianum* was tested against *S. rolfsii* as a sorghum inoculum applied to the soil and as a spore seed dressing on groundnut. The sorghum inoculum of *T. harzianum* was prepared by inoculating agar blocks of the fungus into moist sterilised sorghum grain in glass jars and letting the fungus colonise the grain at room temperature (25-30°C) for about 14 days. The sorghum was initially soaked in water for 12 hours, drained and then autoclaved at 121°C at 115kg/cm² for 15 minutes. Inoculum of *S. rolfsii* was also prepared as above. The groundnut variety malimba was planted in pasteurised sandy loam soil in plastic pots (18cm top and 13cm bottom diameters and 16cm deep). The soil was pasteurised in an oven at 100°C for 48 hours in brown paper bags.

Sorghum inoculum experiment

The experiment was carried out from 21 April to 21 July 1992 and repeated from 19 March until 22 June 1993. Pasteurised soil in the pots was infested with 2g of the sorghum culture of *T. harzianum* alone (Treatment 1, control), 2g sorghum culture of *S. rolf sii* alone (Treatment 2, control), Uninoculated soil (Treatment 3, control) a mixture of 2g each of *T. harzianum* and *S. rolf sii* cultures (Treatment 4). The sorghum cultures were evenly sprinkled on the soil surface and mixed with the soil within the top 5cm. Four groundnut seeds were sown 2.5cm deep in 10 replicate pots in 1992 or 12 replicate pots in 1993. The experimental design was completely randomised (CRD). The pots were watered by adding 500mls of water once every three days. Data were collected on the percentage of ungerminated seeds and on the incidence of necrosis on the stems.

Groundnut seed dressing experiment

The effect of *T. harzianum* spore suspension as seed dressing for control *S. rolf sii* was compared with thiram and mancozeb. Seeds of the malimba variety were dipped for 10 minutes in suspensions of 100ppm thiram, 20ppm mancozeb and 4×10^6 spores/ml of *T. harzianum* and then allowed to air dry before planting. Pasteurised soil in the pots was mixed with 2g sorghum culture of *S. rolf sii* within the top 5cm.

The treatments were set up as follows:

1. Untreated seeds planted in uninfested soil (control)
2. Untreated seeds planted in soil infested with *S. rolf sii* (control)
3. Seeds treated with thiram and planted in soil infested with *S. rolf sii*
4. Seeds treated with mancozeb and planted in soil infested with *S. rolf sii*
5. Seeds treated with *T. harzianum* spores and planted in soil infested with *S. rolf sii*

The treatments were arranged in a completely randomised design with six replicate pots. Data were collected only on the percentage of seeds which failed to germinate. A seed was recorded as germinated when the cotyledons emerged through the soil line within seven days of sowing.

Field experiments

The effect of *T. harzianum* on *S. rolf sii* under field conditions was determined using the sorghum culture and the seed dressing methods as above. The sorghum culture exper-

iment was done from 31 December 1991 to 1 May 1992 and repeated from 8 January to 21 April 1993. Groundnut plants were treated as under screen house except that the cultures were infested over each planting station before sowing. In the 1991-2 season, seeds were planted in four replicate plots and each plot had three ridges which were 5m. long and 0.90m apart. In the 1992-3 season, six replicate plots, each with three rows 5m long and 0.90m apart were used. Seeds were sown 10cm apart, one seed per station.

The treatments were arranged in a randomised complete block design (RCBD) with replicate plots 1m apart. All the recommended cultural practices were followed for raising the crop (Ministry of Agriculture, 1990). Data on percentage of ungerminated seeds, incidence of pod rot and final yield were recorded.

The seed dressing experiment was done once from 5 February 1993 at Chancellor College research field. The part of the research field used was previously under fallow. The seeds were dressed with thiram (100ppm), mancozeb (20ppm) and *T. harzianum* (4×10^6 spores/ml) prior to sowing as described above. Six replicate plots were used but the plot size and experimental design were as above. The 2g sorghum culture of *S. rolfsii* was placed together with the treated seeds in each planting station at planting.

Data were collected on percentage of ungerminated seeds one to two weeks after sowing. A lot of wilting was observed due to termite attack and was such that most of the plants did not develop any pods. This problem was also exacerbated by late planting. As a result, the yield was not assessed on this experiment.

Data analysis

All data were subjected to analysis of variance (ANOVA) and if F-values were significant ($p = 0.05$) means were compared using Duncan's multiple range test (DMRT) (Steele and Torrie, 1980).

Results

In vitro test

T. harzianum significantly reduced germination of sclerotia, growth of mycelia and production of sclerotia compared with the untreated controls. In the agar block method, *T. harzianum* alone and *S. rolfsii* alone covered the 9cm diameter agar surface within two and three days, respectively (Table 1). When *T. harzianum* and *S. rolfsii* were

paired, the colony of *S. rolfsii* would grow until it met with the leading edge of *T. harzianum* colony, after which the growth of *S. rolfsii* would cease and that of *T. harzianum* continued. *S. rolfsii* produced only 16 sclerotia when paired with *T. harzianum* compared to 224 sclerotia in the control plates. When the 16 sclerotia were plated on fresh PDA, they failed to germinate and only *T. harzianum* was recovered. Observations under the microscope showed that the hyphae of *T. harzianum* coiled around the hyphae of *S. rolfsii* and the coiling was sparse.

The spores of *T. harzianum* were as effective as the two fungicides in completely suppressing germination of sclerotia on PDA plates (Table 2).

Table 1. The effect of *T. harzianum* on mycelial growth of *S. rolfsii* in dual cultures on PDA

TAS (hrs)*	Treatment	Colony diameter (cm)
48	<i>S. rolfsii</i> alone	1.5 a **
	<i>S. rolfsii</i> + <i>T. harzianum</i>	1.0 a
72	<i>S. rolfsii</i> alone	5.0 a
	<i>S. rolfsii</i> + <i>T. harzianum</i>	1.7 b
96	<i>S. rolfsii</i> alone	9.0 a
	<i>S. rolfsii</i> + <i>T. harzianum</i>	2.0 b

* TAS = Time after setting the experiment
 ** For each TAS means followed by the same letter are not significantly different at p = 0.05 (Duncan's Multiple Range test).

Table 2. The effect of *T. harzianum*, thiram and mancozeb on germination of *S. rolfsii* sclerotia on PDA

Treatment	Concentration	Germination (%)*
Control	0	75
Thiram	100ppm	0
Mancozeb	20ppm	0
<i>T. harzianum</i>	4x10 ⁸ spores/ml	0

* Determination of germination per cent was done after 48 hours of incubation on five replicate plates.

Screen house experiments

When applied as a sorghum culture, *T. harzianum* significantly reduced the incidence of damping-off and the number of stem lesions of groundnuts in pots infested with *S. rolfsii* (Table 3). In pots infested with *S. rolfsii* alone, 65% of the seeds did not germinate as compared with 48% in treated pots where *T. harzianum* was applied. Similarly, the total number of stem lesions were 16 and seven respectively. Low levels of damping-off and stem lesions were observed in untreated control (11%) and where *T. harzianum* alone (10%) was applied. *Aspergillus flavus* was associated with damping-off in the uninfested controls. The number of stem lesions was significantly lower in the control pots where *T. harzianum* alone was applied (two lesions) as compared with those with no *T. harzianum* (five lesions).

Table 3. The effect *T. harzianum* applied as a sorghum culture on *S. rolfsii* damping-off and root rot of groundnuts grown under screen house conditions

Treatment	Pre-emergence damping-off (%) [*]	No. of stem lesions ^{**}
<i>T. harzianum</i>	10 c ^{***}	2 c
<i>S. rolfsii</i>	65 a	16 a
<i>S. rolfsii</i> + <i>T. harzianum</i>	48 b	7 b
Untreated control	11 c	5 bc

^{*} Data were collected on two experiments which were carried out in 1992 and 1993.
^{**} Data were collected on one experiment which was carried out in 1992.
^{***} Means within each column followed by the same letter are not significantly different at P = 0.05 (Duncan's Multiple range test).

Table 4. The effect of *T. harzianum* mancozeb and thiram applied as a seed dressing on groundnut *S. rolfsii* damping-off in the screen house.

Seed treatment	Pre-emergence damping-off (%)
Thiram	87.5 a [*]
Mancozeb	95.8 a
<i>T. harzianum</i> + <i>S. rolfsii</i>	58.3 b
<i>S. rolfsii</i> alone ^{**}	91.7 a
Control	4.2c

^{*} Means within each column followed by the same letter are not significantly different at P = 0.05 (Duncan's Multiple range test)

T. harzianum spores applied as a seed dressing significantly reduced the incidence of *S. rolfisii* damping-off in groundnuts while the fungicides were not effective (Table 4). *T. harzianum* reduced by 33% the percentage of ungerminated seeds over the control while mancozeb seemed to enhance the disease incidence.

Field experiments

The performance of *T. harzianum* as a single application control agent under field conditions was relatively poor and variable. During the 1991-2 season, the incidence of damping-off was significantly lower (16%) in plants treated with *T. harzianum* than untreated (29%) while in the 1992-3 season, the biocontrol agent had no effect (Table 5). The least percentage of ungerminated seeds was obtained where *T. harzianum* was applied alone and in the uninfested controls for both seasons. Similarly, a significantly higher percentage of pod rot and pops (40%) was obtained where *S. rolfisii* alone was applied in the first season while in the second season there was no significant difference. The cause of ungerminated seeds and rotten pods where *T. harzianum* was applied alone and in untreated control was not determined.

The yield of groundnuts in plots treated with *T. harzianum* was significantly higher than in untreated ones during the first season, but no differences were observed in the second season (Table 5). In the second season, the yields in all the treatments were overall much lower than those of the first season corresponding to the overall high disease incidence.

Table 5. The effect of *T. harzianum* applied as a sorghum culture on the incidence of groundnut pre-emergence damping-off and pod rots caused by *S. rolfisii* in the field.

Treatment	Pre-emergence damping-off (%)		Pod rot and pops (%)		Yield (kg/ha)	
	1992	1993	1992	1993	1992	1993
<i>T. harzianum</i>	8.1c*	20.2b	15.0c	28.7a	1207.0a	348.8b
<i>S. rolfisii</i>	29.1a	58.0a	40.0a	35.9a	712.5b	306.2b
<i>S. rolfisii</i> + <i>T. harzianum</i>	16.1b	62.9a	25.0b	31.5a	1053.1a	247.9b
Contr.	10.8bc	10.6b	18.0c	22.8a	1083.8a	623.3a

* Means within a column followed by the same letter are not significantly different at P = 0.05 (Duncan's multiple range test)

Table 6. Effect *T. harzianum*, thiram and mancozeb applied as a seed dressing on the incidence of damping-off due to *S. rolfsii* in groundnuts in the field.

Seed treatment	Pre-emergence damping-off (%)
Thiram (100ppm)	47.6 a*
Mancozeb (20ppm)	58.3 a
<i>T. harzianum</i> (4x10 ⁶ spores/ml)	35.9 ab
<i>S. rolfsii</i> alone	50.2 a
Control	17.4 b

Means within each column followed by the same letter are not significantly different at P= 0.05 (Duncan's Multiple range test).

T. harzianum applied as a seed dressing slightly reduced the incidence of damping-off compared with the treatment where *S. rolfsii* was applied alone or where the seeds were treated with the two fungicides (Table 6). As in the screen house experiment, mancozeb seemed to enhance the disease.

Discussion

T. harzianum showed high antagonism in the form of hyper parasitism against *S. rolfsii* in vitro. This was shown by the failure of *S. rolfsii* mycelia to continue growth once the edges of the two fungi met and the failure of sclerotia to germinate once colonised by the *T. harzianum*. These results confirm earlier reports (Punja, 1985; Upadhyay and Mukhopadhyay, 1986) that hyphae of *S. rolfsii* tend to be parasitised directly by *Trichoderma* spp. in dual cultures. When spores of *T. harzianum* were incorporated into the PDA, the germinating spores quickly parasitised sclerotia of *S. rolfsii* and prevented their germination. The effectiveness of the biocontrol agent was as good as that of mancozeb or thiram.

When applied as a seed dressing or a sorghum culture, *T. harzianum* significantly reduced the incidence of groundnut damping-off by *S. rolfsii* under screen house conditions (Table 4). However, its performance was not as high as in vitro, even though it performed better than the fungicides. The presence of ungerminated seeds in untreated control was probably due to the use of untreated seeds which were infected by seed borne *Aspergillus* spp.

The number of stem lesions was greater where *S. rolfsii* alone was applied (16 lesions) as compared to where *T. harzianum* and *S. rolfsii* were applied together (seven lesions)

(Table 3). This suggests that *T. harzianum* was at least partially effective in reducing the disease due to *S. rolfsii*. The presence of stem lesions in the pots where *T. harzianum* was applied alone and in untreated control was probably due to the presence of *Aspergillus* sp. which was observed on some of the seeds. The lower number of lesions in the plants treated with *T. harzianum* alone suggests that the *T. harzianum* was also able to partially suppress some the seed borne pathogens. The ineffectiveness of the chemicals was probably due to the low concentrations used. Hammond et al. (1977) and Upadhyay and Mukhopadhyay (1986) recommended relatively higher concentrations than those used in this study.

The performance of *T. harzianum* under field conditions was relatively poor and varied from season to season. When the season was very favourable to disease development as seen in the 1992-3 season, *T. harzianum* was unable to reduce disease incidence in the groundnuts (Table 5). The poor performance was probably due to single application of the biocontrol agent. *S. rolfsii* in groundnuts is known to be active throughout the growing period (Thompson, 1984) while *T. harzianum* was probably active for a short period which was not enough for adequate control of the disease. Csinos et al. (1983) reported that *T. harzianum* was active only over a three-to-eight day period and Bell et al. (1980) suggested applications at early bloom, mid-pegging and late pegging for best control of southern blight. *T. harzianum* probably became less active soon after the food base supplied in the inoculum was exhausted.

Although thiram and mancozeb tended to inhibit *S. rolfsii* under laboratory conditions, they did not reduce disease incidence in the screen house and field. These results agree with earlier reports (Diammonde and Beute, 1977; Dalvi and Raut, 1987) that thiram and mancozeb are either moderately or not effective against *S. rolfsii* under field conditions. Factors such as high soil temperatures, ineffective concentrations and application methods have are known to attribute to the poor performance of these chemicals (Diammonde and Buete, 1977; Hammond et al., 1977)

In conclusion, this study has shown that *T. harzianum* has great potential as a means of controlling *S. rolfsii* in groundnuts but more work needs to be done on the use of *T. harzianum* as part of an integrated disease control strategy under Malawian conditions.

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