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Efficacy of *Trichoderma harzianum* Rifai alone or in combination with fungicides against Sclerotium wilt of groundnut

A. K. PATIBANDA, J. P. UPADHYAY [1] AND A.N. MUKHOPADHYAY[2]
Agricultural Research Station
Tandur 501 141, Ranga Reddy District, Andhra Pradesh, India
E-Mail: patibandaanil @ rediffmail.com

ABSTRACT: ***Trichoderma harzianum*** **Rifai application either to soil as wheat bran saw dust (WBSD) preparation or on the groundnut seeds as spore coat proved effective against sclerotium wilt caused by *Sclerotium rolfsii* Sacc. Synergistic and positive effects on disease control were registered when *T. harzianum*-WBSD preparation was applied to soil in integration with Vitavax or Vitavax-200. Integration of Thiram (seed coating) and soil application of antagonist was found compatible and synergistic. However, seed treatment with both antagonist and Thiram was found incompatible and hence may not be practically feasible for disease reduction.**

KEY WORDS: Biocontrol, fungicides, groundnut, integration, *Sclerotium rolfsii*, *Trichoderma harzianum*

Sclerotium rolfsii Sacc. causes pre-emergence rot, collar and stem rot, and wilt in groundnut. The disease infected crops show poor stand in the field. The disease is reported to cause huge yield losses (Kolte, 1984). Several fungicides have been reported to inhibit *S. rolfsii* (Patil and Rane, 1982). However, under field conditions, fungicide application alone is neither feasible nor practicable owing to high cost and environmental concerns. Biological control in integration with fungicidal seed treatment offers a more reliable approach in managing soil-borne plant pathogens (Mukhopadhyay, 1987).

The present investigation was undertaken to explore the feasibility of using *T. harzianum* for the management of sclerotium wilt of groundnut and its efficacy in integration with fungicides.

MATERIALS AND METHODS

The pathogenic fungus, *Sclerotium rolfsii* was isolated from diseased groundnut plants at Crop Research Center, G. B. Pant University of Agriculture and Technology, Pantnagar, India. The antagonist *T. harzianum* (Th-3) was obtained from Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, G. B. P. U. A. & T., Pantnagar. Susceptible groundnut cultivar TMV-2 was included in pot experiments.

1. Department of Plant Pathology, Tirhut College of Agriculture, Dholi, Rajendra Agricultural University, Muzaffarpur 843 121, Bihar, India.

2. 151, Akansha, Udyan-II, Raibareilly Road, Lucknow 226 025, U. P., India.

Mass culturing of *S. rolfsii* was done on autoclaved sorghum grains. Wheat bran-saw dust (WBSD) preparation was used for mass multiplication of the antagonist (Mukhopadhyay *et al.*, 1986). Seed coating of the antagonist was done by antagonist spore suspension (Mukhopadhyay *et al.*, 1992). Pot experiments were carried out in plastic pots (15cm diam) with 2kg soil capacity to find the efficacy of the antagonist when applied through seed or soil.

The pathogen was applied to soil @ 4g/kg soil. Twenty-four hours later, antagonist and/or fungicide coated groundnut seeds were sown @ 6 seeds/pot. For soil application of the antagonist, WBSD preparation was added to soil 24h after pathogen inoculation and sowing was done five days later. Water was added to pots as and when required to maintain optimum soil moisture. Observations on plant mortality were taken 35 days after sowing (DAS). In the integration experiment, ED_{50} and ED_{75} values of WBSD preparation were used.

Five fungicides *viz.*, Thiram-75 SD (Thiram from Rhone Poulenc Agro- Chemical (India) Ltd., West Bombay), Vitavax-75 SP (Carboxin from Rallis India Ltd., Bombay), Vitavax–200-75 SP (Carboxin 37.5%+Thiram 37.5% from Rallis India Ltd., Bombay), Ziride 80WP (Ziram from the Alkali & Chemical Corporation of India Ltd., West Bengal) and Calixin 80EC (Tridemorph from BASF India Ltd., Bombay) were assayed for their efficacy against *S. rolfsii* using poisoned food technique (Grover and Moore, 1961). Fungicide efficacy was also tested in pot experiments @ 0.2 percent as seed coating. Based on the results obtained, three fungicides were selected to test the antagonist sensitivity and their compatibility in integration. ED_{50} and ED_{90} values were calculated for fungicidal efficacy *in vitro* against *S. rolfsii* (Nene and Thapliyal, 1987). Sensitivity of the antagonist at these fungicidal concentrations was assayed using poisoned food technique and dual culture technique (Morton and Straube, 1955). Fungicidal sensitivity of the antagonist was also tested by placing groundnut seeds coated with fungicide (at doses recommended

TABLE 1. Biological control of Sclerotium wilt of groundnut by *T. harzianum in vivo*

Bioagent	Disease control over check (%)
Th-3 @ 1.25g/kg soil SA	25.90 (30.59)
Th-3 @ 2.5g/kg soil SA	48.03 (43.72)
Th-3 @ 5.0g/kg soil SA	77.74 (67.58)
Th-3 @ 10.0g/kg soil SA	92.58 (82.49)
Th-3 @ 2.43×10^{10} spores/ml ST	83.00 (76.30)
Th-3 @ 1.22×10^{10} spores/ml ST	66.66 (53.70)
Th-3 @ 6.10×10^{9}spores/ml ST	41.60 (39.50)
Th-3 @ 3.00×10^{9} spores/ml ST	33.33 (34.52)
Th-3 @ 1.50×10^{9} spores/ml ST	33.33 (34.52)
Th-3 @ 1.90×10^{8} spores/ml ST	33.33 (34.52)
CD (P=0.01)	(3.0)

Figures in parentheses are arcsine transformed values.

Th-3: *T.harzianum* isolate –3

SA: Soil application ST: Seed treatment

for field application) at the center of the potato dextrose agar plate and inoculating with the antagonist at three places around the seed. Observations on inhibition zone corresponding to *T. harzianum* around the seeds were recorded four days after incubation at 28±1°C.

Integration effect of antagonist (applied either to soil or seed) and fungicide (0.1% seed coating) in reducing groundnut plant mortality was assayed in pot experiments.

RESULTS AND DISCUSSION

Significant differences were noticed in disease reduction compared to check when *T. harzianum* was applied to soil as WBSD preparation or on seeds as spore suspension (Table 1). Disease reduction was maximum (92.58%) when *T. harzianum* was applied @10g/ kg soil followed by seed coating @ 2.43×10^{10} spores/ml (83.00%). Increased rate of WBSD preparation resulted in an increasing trend in disease control with all the doses tested. However, increased trend in disease control with increased spore concentration of the antagonist was observed above 3.0×10^{9} spores/ml concentration. ED_{50} and ED_{75} values for WBSD preparation were calculated as 2.5g and 4g/kg soil, respectively. Management of *S. rolfsii* through antagonist soil application and seed treatment were earlier reported (Mukhopadhyay *et al.*, 1992; Upadhyay and Mukhopadhyay, 1986).

Vitavax at and above 0.3μg/ml and Vitavax-200 at and above 0.5μg/ml could inhibit the *S. rolfsii* growth completely (100%)*in vitro* (Table 2). Other fungicides needed higher doses for complete inhibition. Similar trend was registered in pot experiments with fungicide as seed coating (Table 3). Vitavax-200 and Vitavax rendered maximum protection (77.55 and 71.94%, respectively) to groundnut seedlings. Ziride and Thiram were found least effective (13.90 and 25.30%, respectively). ED_{50} and ED_{90} values of fungicides against *S. rolfsii in vitro* were 70 and 200μg/ml, 0.13 and 0.21 μg/ml, and 0.8 and 0.26μg/ml, respectively for Thiram, Vitavax and Vitavax-200. Similar results on Vitavax efficacy were earlier reported (Prasad *et al.*, 1977).

Sensitivity of *T. harzianum* towards Vitavax, Vitavax-200 and Thiram was assayed at ED_{50} and ED_{90} concentrations calculated for S. *rolfsii*. Vitavax and Vitavax-200 had no effect on the growth of *T. harzianum* where as Thiram at both concentrations (70 and 200 μg/ml) was inhibitory. Upon prolonged incubation (7 days), the inhibitory effect of Thiram was nullified. Similar result was observed when the groundnut seeds were coated with fungicides and inoculated with antagonist (Table 4). In Vitavax and Vitavax-200 coated seeds, no inhibition zone was observed. However, in Thiram coated seeds inhibition zone was observed initially which upon prolonged incubation disappeared and the antagonist could cover the entire seed. Fungistatic effect of Thiram on antagonist isolates was earlier reported by Mukherjee (1987) and insensitivity of antagonist isolates towards Vitavax and Vitavax-200 was reported by Kaur (1989).

Dual culturing of *S. rolfsii* and *T. harzianum* with ED_{50} concentration of Vitavax or Vitavax-200 in the medium revealed that the antagonist could grow faster and occupy *S. rolfsii* growth in shorter time (5 days of inoculation) compared to unamended medium (7 days). With respect to Thiram amended medium, no over growth occurred even after ten days of inoculation.

Significant disease reduction was observed when the fungicide-coated seeds (at 0.1% concentration) were integrated with the antagonist applied to soil (ED_{50} @2.5g/kg soil and ED_{75} @4g/ kg soil concentrations) or on seed (at three spore concentrations namely 1.15×10^{8}, 1.15×10^{9} and 1.15×10^{10}spores/ml) except in case where antagonist and Thiram were applied together on seeds (Table-5). Complete protection (100 %) up to 35 DAS was observed when Vitavax or Vitavax-200 was integrated with any of the antagonist doses applied to soil. Integration of antagonist soil application and fungicidal seed coating resulted in synergistic effect on disease reduction compared to their individual applications. Thiram alone at 0.1% seed treatment showed very poor control (34.00%) and same treatment in combination with *Trichoderma* soil application required higher dose (4g/kg soil) for 100% control.

Table 2. Fungicidal efficacy on radial growth of *S. rolfsii in vitro*

Fungicide	Concentration (μg /ml)	Inhibition over check (%)
Thiram	10	0.00 (0.00)
	20	18.55 (25.53)
	30	19.44 (26.36)
	50	27.77 (31.82)
	100	66.66 (54.76)
Ziride	1	4.44 (11.45)
	3	6.66 (15.32)
	5	17.77 (24.65)
	7	18.11 (24.93)
	10	20.00 (27.34)
Calixin	1	20.55 (26.90)
	2	23.88 (29.23)
	5	41.88 (40.35)
	10	53.88 (47.23)
	20	81.66 (61.24)
Vitavax	0.1	44.44 (41.78)
	0.2	87.22 (69.13)
	0.3	100.00 (90.00)
	0.4	100.00 (90.00)
	0.5	100.00 (90.00)
Vitavax-200	0.1	59.44 (50.44)
	0.3	83.33 (65.88)
	0.5	100.00 (90.00)
	1.0	100.00 (90.00)
	2.0	100.00 (90.00)
CD (P=0.01)		(2.98)

Figures in parentheses are arcsine transformed values.

Table 3. Fungicidal efficacy against Sclerotium wilt of groundnut *in vivo*

Fungicide (0.2% seed coating)	Disease control over check (%)
Ziride	13.90 (15.91)
Thiram	25.3 (26.48)
Vitavax	72.94 (62.56)
Vitavax -200	77.55 (65.68)
Calixin	54.63 (47.68)
CD (P=0.01)	(2.5)

Figures in parentheses are arcsine transformed values.

Table 4. Fungicidal sensitivity of *T. harzianum* – *in vitro* seed coating method

Fungicide	Concentration (%)	Inhibition zone (diam in mm)
Thiram	0.05	-
	0.10	+ (30.00)
	0.15	+ (31.60)
	0.20	+ (35.00)
Vitavax	0.05	-
	0.10	-
	0.15	-
	0.20	-
Vitavax 200	0.05	-
	0.10	-
	0.15	-
	0.20	-
Check	0.00	-

\+ Inhibition zone formed

\- Inhibition zone not formed

Table 5. Integration effect of *T. harzianum* and fungicide on Sclerotium wilt

Treatment	Disease control over check (%)
Thiram + Th-3 @ 2.5g/kg soil (SA)	77.87 (66.51)
Thiram + Th-3 @ 4.0g/kg soil (SA)	100.00 (90.00)
Thiram + Th-3 @ 1.15×10^{10} spores/ml (ST)	19.97 (21.91)
Thiram + Th-3 @ 1.15×10^{9} spores/ml (ST)	53.33 (46.90)
Thiram + Th-3 @ 1.15×10^{8} spores/ml (ST)	26.66 (30.76)
Vitavax + Th-3 @ 2.5g/kg soil (SA)	100.00 (90.00)
Vitavax + Th-3 @ 4.0g/kg soil (SA)	100.00 (90.00)
Vitavax + Th-3 @ 1.15×10^{10} spores/ml (ST)	93.33 (81.15)
Vitavax + Th-3 @ 1.15×10^{9} spores/ml (ST)	80.00 (68.07)
Vitavax + Th-3 @ 1.15×10^{8} spores/ml (ST)	66.66 (60.00)
Vitavax-200 + Th-3 @ 2.5g/kg soil (SA)	100.00 (90.00)
Vitavax-200 + Th-3 @ 4.0g/kg soil (SA)	100.00 (90.00)
Vitavax-200 + Th-3 @ 1.15×10^{10} spores/ml (ST)	93.33 (81.15)
Vitavax-200 + Th-3 @ 1.15×10^{9} spores/ml (ST)	86.66 (72.29)
Vitavax-200 + Th-3 @ 1.15×10^{8} spores/ml (ST)	53.33 (46.92)
Th-3 @ 2.5g/kg soil (SA)	48.03 (43.72)
Th-3 @ 4.0g/kg soil (SA)	77.87 (66.51)
Th-3 @ 1.15×10^{10} spores/ml (ST)	66.66 (60.00)
Th-3 @ 1.15×10^{9} spores/ml (ST)	33.33 (34.97)
Th-3 @ 1.15×10^{8} spores/ml (ST)	33.33 (34.97)
Thiram alone	34.00 (35.67)
Vitavax alone	77.87 (66.51)
Vitavax-200 alone	77.87 (66.51)
CD (P=0.01)	(12.37)

Figures in parentheses are arcsine transformed values.

Th-3: *T. harzianum* isolate –3

SA: Soil application ST: Seed treatment
Fungicides are used @ 0.1% seed treatment.

Maximum disease reduction was registered when Vitavax or Vitavax-200 was integrated with *T. harzianum* @ 10^{10} spores/ml. At lower concentrations, the efficacy was on par with either fungicide or antagonist alone. Synergistic effect of antagonists and fungicides in reducing disease incidence was reported earlier (Upadhyay and Mukhopadhyay, 1986).

Integration of antagonist with Thiram (seed coating) was found detrimental to the disease management strategy because of fungistatic effect of Thiram on the antagonist. However, antagonist application to soil (as WBSD preparation) and Thiram (seed coating) resulted in synergistic effect on disease reduction indicating that the antagonist could occupy the rhizosphere zone better whereas Thiram on seeds could reduce other competitive micro-flora of the soil.

Vitavax-200 and Vitavax both when used alone at 0.1 percent as seed coat showed same (77.87%) disease control, while in combination with soil application of *T. harzianum* @ 2.5g/kg soil showed 100 per cent control. Vitavax contains 75 per cent active ingredient while Vitavax-200 contains only 37.5 per cent same active ingredient along with 37.5 per cent Thiram. These results indicate, that soil application of *T.harzianum* in combination with Vitavax-200 seed treatment can provide greater degree of synergistic effects as compared to Vitavax. There is a substantial decrease in dose of both the fungicides in Vitavax-200 indicating synergistic effects in case of their combination also.

Thus the present investigations revealed that *T. harzianum* could be successfully integrated with Vitavax or Vitavax-200 to manage Sclerotium wilt of groundnut even at lower doses. Though integration of Thiram (seed coating) and *T. harzianum* soil application was compatible and gave maximum protection to groundnut plants against *Sclerotium rolfsii*, seed treatment with both antagonist and Thiram was found incompatible and thereby not practically feasible.

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