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Evaluation of *Trichoderma harzianum*, Some Botanicals and a Fungicide on Sclerotium Wilt of Potted Tomato

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Abstract: The effect of *Trichoderma harzianum*, *Azadirachta indica* seed extract, pawpaw root extract, *Hyptis suaveolens* leaf extract and the fungicide captan on sclerotium wilt of tomato caused by *Sclerotium rolfsii* was investigated. *S. rolfsii* and *T. harzianum* on maize-meal were used to inoculate the soil around seedlings and aqueous extracts of the plant materials and an aqueous suspension of captan were used to drench the soil in pots. All treatments were observed to have profound effect on the disease, by reducing it, with *T. harzianum* having the highest disease reduction value of (80.3%) followed by *A. indica* (68.6%), captan (68.2%), *H. suaveolens* (60.8%) and *C. papaya* (33.6%). The effect due to *T. harzianum* was significantly different ($p = 0.05$) from others. The same trend was observed for disease severity. Pawpaw root extract had the least effect on all the disease parameters investigated, but its effect significantly differed ($p = 0.05$) from the control. Plant growth recorded significant difference ($p = 0.05$) among the treatments, with the pots treated with the plant extracts having the highest plant heights. *T. harzianum* , *A. indica* seed extract and *H. suaveolens* leaf extracts gave optimum effect and compared favorably with captan and could be considered to replace it as control agent(s).

Key words: *Trichoderma harzianum, Sclerotium rolfsii*, sclerotium wilt of tomato, botanicals, captan

INTRODUCTION

The effect of some synthetic fungicides on the environment and man, when used for plant disease control, has been deleterious (Olufolaji, 1999). Since the realization of this fact, scientists have launched the search for alternatives that would be environment and man friendly. Promise has been found in some antagonistic fungi (Lui and Baker, 1980; Okigbo, 2002) which are known to compete with pathogenic fungi so affect their spread and some botanicals (Naidu, 1988; Pandey and Dubey, 1994; Owalade and Osikanlu, 1999; Amadioha, 2003; Wokocha and Okereke, 2005) which inhibit the mycelia and sclerotia growth of some pathogenic fungi.

The antagonistic effect of *Tricoderma harzianum*, *T. viride* and *Bacillus subtilis* on some fungal plant pathogens (Henis *et al*., 1984; Abada, 1994; Okigbo, 2002; Godwin-Egein and Arinze, 2003, 2004) and its use as a biocontrol agent has been established. Also, *Azadirachta indica, Hyptis suaveolens, Carica papaya* and other botanicals have been used to control fungal pathogens (Olufolaji, 1999; Amadioha, 2000; Wokocha and Okereke, 2005). Wokocha and Ebenebe (1986) and Shahikant *et al.* (1989) reported the effectiveness of the synthetic fungicides, Captan and PCNB in controlling some soil-borne plant pathogenic fungi. The aim of this investigation was to study the antagonistic effect of *Trichoderma harzianum*, aqueous extracts of *Azadirachta indica* seeds, *Carica papaya* roots and *Hyptis suaveolens* leaves, when compared with synthetic fungicide, captan on the wilt disease of tomato incited by *Sclerotium rolfsii* in pots.

MATERIALS AND METHODS

Isolation of fungi species: *Sclerotium rolfsii* Sacc was isolated from naturally infected cowpea plants in the research farm of Michael Okpara University of Agriculture, Umudike, Abia State. Infected plant materials were cut into 5 mm pieces and surface sterilized with 0.5% sodium hypochlorite solution for 5 min and rinsed trice with sterile water. The pieces were dried with sterile filter paper and plated (3 pieces per plate) on fresh Potato Dextrose Agar (PDA) medium impregnated with streptomycin and incubated for 7 days at 28°C. Pure culture was obtained by sub-culturing three times. The

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isolate was then used to carry out pathogenicity test on tomato. Following Wokocha *et al.* (1986) maize-meal inoculum was prepared and 21-day old apparently healthy tomato plants were inoculated in pots containing heat-sterilized soil, by scattering the inoculum around the plant bases at 5 cm depth. Following this and subsequent isolation, Koch's postulates were carried out. Pure cultures of the final isolate were maintained on PDA slants in McCartney bottles in the refrigerator until required.

Trichoderma harzianum Rifai was also isolated from the same research farm, from organic matter rich soil within a 15 cm depth. It was isolated by plating soil directly on PDA. Three grams of soil was placed in 15 mL of molten, cooling PDA, swirled and allowed to solidify. The set up was incubated for 3 days at 28°C. Pure cultures were maintained on PDA. Inoculum preparation was the same as in *S. rolfsii* above, using the maize- meal method (Wokocha *et al.*, 1986). Dry maize meal inocula were stored at room temperature until needed.

Preparation of plant extracts: Neem (*Azadirachta indica*) seeds, pawpaw (*Carica papaya*) roots and bush-tea-bush (*Hyptis suaveolens*) leaves were thoroughly washed and sun dried until crisp. These were each ground into fine particles, using a grinding machine. Forty gram of the ground materials were each soaked in 100 mL of cold water in 250 mL conical flasks overnight, then filtered through Whatman No. 1 filter paper and again passed through a membrane filter (0.2 μm).

Pot inoculation: Four kilogram each of heat sterilized field soil was placed in 30 vapam sterilized pots measuring 22.5 cm in diameter. Fourteen day old apparently healthy tomato (Roma VF) seedlings were transplanted into the pots at the rate of 5 seedlings per pot and later thinned to two. The pots were either treated with maize-meal inoculum of *T. harzianum* (20 g/pot) or drenched with captan or aqueous plant extracts (200 mL/pot) 2 days before *S. rolfsii* inoculation. Captan suspension was made at the rate of 1g L^{-1}. Inoculation of pots were done by scattering 20 g of the maize-meal inoculum of *S. rolfsii* each around the bases of the plants at a depth of 5cm. Control pots were without treatments. The pots were arranged and watered regularly.

Disease severity and disease reduction were assessed 4 weeks after transplanting and the disease severity based on a scale 0-5, where: 0 = no visible disease symptom; 1 = less than 15% of stem circumference infected; 2 = 15-35% of stem infected; 3 = 36-49% of stem infected; 4 =50-75% of stem infected; 5 = more than 75% of stem circumference infected. Numerical ratings similar to these have been used by Wokocha (2001) to assess damage to some tropical plants by *S. rolfsii*. The effect of the treatments on the growth of the tomato seedlings was determined by measuring the height of the plants after 1 week. The experiments had five replicates and repeated twice in a Completely Randomized Design (CRD). The data was subjected to Analysis of Variance (ANOVA).

RESULTS

The cowpea isolate of *Sclerotium rolfsii* was pathogenic to and caused wilt in the test tomato plants. Disease was observed in all treatments as shown in Table 1. The disease was most severe in pawpaw root extract treated pots and this was not significantly different ($p = 0.05$) from those of the control pots. Least disease severity was observed in *T. harzianum* treated pots and they were not significantly different ($p = 0.05$) from those of *A. indica* and captan treated pots. But the effect due to *T. harzianum* was significantly different ($p = 0.05$) from those of *H. suaveolens* extract treated pots.

The same trend observed for disease severity was observed for disease reduction, as shown in Table 2, except that the effect *T. harzianum* (80.3%) had on disease reduction was optimum and was significantly different ($p = 0.05$) from all the others. No statistically significant difference ($p = 0.05$) was observed between A. indica (68.6%) and captan (68.2%) but their effects were significantly different ($p = 0.05$) from the pot treated with *H. suaveolens*. Least reductive effect, amongst the treatments, was shown by *C. papaya* extract (33.6%),

Table 1: Effect of *Trichoderma harzianum*, captan and aqueous extracts of *C. papaya* roots, *A. indica* seeds and *H. suaveolens* leaves on disease severity of tomato seedlings inoculated with *S. rolfsii*

Treatment	Disease severity
Trichoderma harzianum	1.2±037
Captan	1.8±0.20
Hyptis suaveolens	2.4 ±0.25
Azadirachta indica	1.8±0.34
Carica papaya	3.8 ±0.34
Control	4.4±0.37
LSD (0.05)	0.93

Data are average of 5 replications in 2 separate experiments

Table 2: Effect of *Trichoderma harzianum*, captan, aqueous extracts of *C. papaya* roots, *A. indica* seeds and *H. suaveolens* leaves on disease reduction of tomato seedlings inoculated with *S. rolfsii*

Treatment	% Disease reduction
Trichoderma harzianum	80.30±1.09
Captan	68.16 ±2.61
Hyptis suaveolens	60.82 ±0.71
Azadirachta indica	68.65 ±0.75
Carica papaya	33.62 ±1.09
Control	13.54±0.74
LSD(0.05)	3.91

Data are average of 5 replications in 2 separate experiments

Table 3: Effect of *Trichoderma harzianum*, captan, aqueous extracts of *C. papaya* roos, *A. indica* seeds and *H. suaveolens* leaves on the growth of tomato seedlings inoculated with *S. rolfsii*

Treatment	Plant height (cm)
Trichoderma harzianum	8.10±0.09
Captan	6.21±0.20
Hyptis suaveolens	8.63±0.18
Azadirachta indica	8.92±0.18
Carica papaya	7.57±0.18
Control	6.18±0.13
LSD(0.05)	0.48

Data are average of 5 replications in 2 separate experiments

which was significantly different (p = 0.05) from the effect observed in the control pots (13.5%), which had the lowest effect.

There were differences shown in growth, in the various treatments, as measured by plant height (Table 3). The differences were attributed to the effect various treatments had on the plants. *H. suaveolens* (8.63 cm) and *A. indica* (8.92 cm) were in the same category, showing no significant difference (p = 0.05) between them, while *T. harzianum* (8.10 cm) belonged to one category and *C. papaya* (7.57 cm) in another category. There were significant differences (p = 0.05) observed between the three categories. All the categories were significantly different (p = 0.05) from the plants drenched with captan (6.21 cm) and the control plants that had an average height of 6.18 cm.

DISCUSSION

The high disease severity and low disease reduction (Table 1 and 2, respectively) observed in the control pots and the very low disease severity and high disease reduction observed in the treatments, especially in *T. harzianum, A. indica* and *H. suaveolens* treated pots, indicated that the treatments affected disease development. The difference between *T. harzianum* and the others makes it a superior candidate for consideration for plant disease control activities. *A. indica and H. suaveolens* extracts competed favorably with the fungicide, captan in the disease reduction suggesting the presence of antifungal substances in the plant materials, which agrees with the results of other workers on pathogens of other crops (Olufolaji, 1999; Owolade and Osikanlu, 1999; Amadioha, 2000; Okereke and Wokocha, 2006). *Carica papaya* root extract on the other hand had the least effect on the pathogen and did not effectively control the disease (Table 2). This finding corroborates the earlier works of Wokocha and Okereke (2005) on the effect of this extract on the *in vitro* growth of *S. rolfsii*. The investigators showed that at 30% concentration of the extract, the inhibition effect on both mycelial growth and sclerotia germination of the pathogen was low. Even when the concentration was increased as shown in the present study, low disease reduction result was also obtained. The indication is that there is a relationship between the *in vitro* and *in vivo* antifungal activities of the plant extract. However, the inability of the *C. papaya* to effectively control the disease could be attributed to the plant part used as Amadioha (1998) had reported the presence of antifungal substances in the leaves against powdery mildew in pepper. There now exists the opportunity to utilize the leaf extract in plant disease management. Though the mycelial preparation and delivery system of the bio agent employed in the current study was comparatively simple, its acceptance and adoption by peasant farmers in the developing nations like ours remains a problem. This has therefore, left us with the option of screening of tropical plants with medicinal values for use in the plant disease management programs. Furthermore, one overriding consideration for the use of any disease control agent is its non-phytotoxic effect. This investigation has revealed that the plant extract as well as the bio agent employed in the study did not affect the growth of the tomato plants as observed in the plant heights (Table 3). Other workers (Pandey and Dubey, 1994; Ojo and Olufolaji, 2005) had earlier reported same effect. Pandey and Dubey, showed this phenomenon with *H. suaveolens* extracts when used against some soil-borne pathogens in potted tomato plants, while Ojo and Olufolaji, evaluated crude neem bark extracts against anthracnose diseased soybean seeds and observed same effect. Okereke and Wokocha (2006) also reported the non-phytotoxicity effect of *A. indica*, *Z. officinale*, *H. suaveolens* and *G. cola* extracts when used as seed dressing fungicides against the damping-off disease incited by *S. rolfsii*. The application of the treatments 2 days before inoculation with the pathogen may have aided in the disease reduction. It could be deduced that the extracts and the bio agent probably exerted some antifungal protective action over the tomato plants. It is therefore, plausible that the application of these extracts as soil drenches before cropping, in field infested with the pathogen could drastically reduce the amount of primary inoculum as well as the number of infection loci represented by sclerotia in the field. These, couple with their environmental friendliness have presented these agents for considerations as replacement for the synthetic fungicides against *S. rolfsii* infection in both biological and integrated control programs after thorough investigations.

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