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Effects of Some Tropical Plant Extracts, *Trichoderma harzianum* and Captan on the Damping-off Disease of Tomato Induced by *Sclerotium rolfsii*

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Abstract: Aqueous extracts of ginger, neem seed, bitter cola and leaves of *Hyptis suaveolens* as well as captan and *Trichoderma harzianum* were evaluated in the greenhouse for their efficacy as soil drenches in the reduction of the damping-off disease of tomato incited by *S. rolfsii.* The experiment was laid out in a 6x3 factorial in a completely randomized design replicated five times. The results showed that soil drenching two days before inoculation with *S. rolfsii* gave disease reduction values which were similar in the treatments: *T. Harzianum* (67.1%), neem seed (62.4%), *H. suaveolens* (60.8%), captan (60.4%), bitter cola (60.1%) and ginger (57.4%). When soil drenches were applied on the same day as the inoculation, *T. harzianum,* captan and extracts of neem seed, *H. suaveloens* leaves and ginger significantly differed ($p<0.05$) from the bitter cola extract and the disease reduction values ranged from 40-60%. At two days after inoculation, neem seed extract, captan and *T. harzianum* significantly ($p<0.05$) reduced the disease giving disease reduction values of 53.3, 57.5 and 64.7%, respectively. Generally, the plant extracts showed selective fungi toxicity and were not phytotoxic to the tomato seeds.

Key words: *Sclerotium rolfsii*, tropical plant, tomato, *Trichoderma harzianum*

INTRODUCTION

The damping-off disease of tomato (*Lycopersicon esculentum* Mill) caused by *Sclerotium rolfsii Sacc* is an economically important disease of tomato and other vegetable crops in the Nigerian savanna[1,2]. The soil borne pathogen has been described as one of the most important on crop plants worldwide attacking over 500 different plant species[3], particularly in the Warm, moist tropical regions of the world. The fungus is visible on the surface of the soil or at the base of infested plants as whitish ropy mycelia mat which later produces reddish or dark-brown sclerotia depending on the biochemical composition of the fungal strain[4]. Considerable losses due to damping-off are incurred annually by tomato growers in Nigeria[5].

The cost of seed treatment and damage to the soil environment has made it necessary to seek other methods of damping-off control. The chemical control of damping-off disease in most vegetable crops is both impractical and uneconomical[6]. Although fungicides such as PCNB, captan, calixin which have been used for seed dressing and other chemicals such as methyl bromide and chloropicrin as soil fumigants to the control of damping-off organisms is expensive, environmentally hazardous and difficult to adopt in low subsistence agriculture in West Africa[4,7], there is therefore the need for the use of cheaper, environmentally friendly and readily available alternative materials such as bio-agents and extracts of medicinal plants for the control of the damping-off disease.

The present investigation was designed to evaluate the efficacy of some plant extracts, *Trichoderma harzianum* and captan for the control of the damping-off disease of tomato incited by *S. rolfsii* in the greenhouse.

MATERIALS AND METHODS

Isolation of *S. rolfsii:* Infected tomato stems obtained from the field were washed free of soil, cut into 5 mm segments including the advancing margins of infection. The segments were surface disinfected in 0.5% sodium hypochlorite solution for 5minutes and rinsed in three changes of sterile water. The segments were then dried in between sheets of sterile filter paper and plated on fresh Potato Dextrose Agar (PDA) medium at the rate of three segments per plate. The dishes were incubated at 25-28°C and examined for 7days. Three subcultures were made to obtain pure cultures of isolate.

Isolation of *Trichoderma harzianum:* *T. harzianum* isolate employed in this investigation was obtained from field soil. 15 mL of cool molten potato dextrose agar was

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poured into sterile 9.0 cm Petri dishes and 3 g of the field soil were inoculated onto the PDA medium, which was gently swirled and allowed to settle and solidify. The inoculated medium was incubated at 25-28°C for three days.

Isolates of *T. harzianum* growing on the medium were sub-cultured to obtain pure cultures. *Trichoderma harzianum* was identified by reference to Barnett and Hunter[8].

Preparation of plant extracts: The following plant materials were used for the preparation of extracts: Neem (*Azadirachta indica)* seeds, ginger (*Zingiber officinale)* rhizomes, bitter cola (*Garcinia cola*) seeds and leaves of *Hyptis suaveolens*. They were sun-dried for 7 days and ground using a mechanical grinding machine. Twenty grammes of each of the ground materials were soaked separately over night in 100 mL of cold water in 250 mL conical flasks. The suspension was hand-shaken and filtered into a clean container using whatman no. 1 filter paper and then passed through a membrane filter (0.2 um) to avoid microbial contamination.

Preparation of inocula: Maize-meal inocula of *S. rolfsii* used for pot inoculation and *T. harzianum* used as an antagonist in the greenhouse were prepared as follows: three hundred grammes (300 g) of ground maize were suspended in 500 mL of distilled water containing 15 g of glucose[9]. The suspension was dispensed in 1 liter conical flasks and autoclaved at 120°C for 30 mins. The flasks were allowed to cool to room temperature before each flasks was inoculated separately with seven, 5 mm discs of young (3 to 4-day old) cultures of *S. rolfsii* and *T. harzianum*. The were incubated for five days at 25-28°C. The content of the flasks were aseptically removed using flame sterile metal spatula into clean trays, covered with double-layered clean cheese cloth and sun dried for seven to eight days. Dry inocula were stored at room temperature until needed.

Phytotoxicity tests: Twenty tomato (Roma VF) seeds were soaked overnight in 15 mL of the different plant extracts and then plated in sterile 9.0 cm Petri dishes containing sterile whatman no. 1 filter paper and incubated at room temperature for five days. Germinated seedlings per plate were recorded daily for five days.

Greenhouse studies: One hundred and twenty plastic pots (14 cm diameter) were filled with heat sterilized field soil at the rate of 1.4 kg per pot. The field soil was a sandy-loam with pH 4.81; 0.1% total N and 1.6% organic carbon. Inoculation of potted plants with the pathogen was carried out at the following frequencies: Same day as treatment, two days before and after the application of plant extracts, captan and *T. harzianum* and tomato (Roma VF) seeds were sown in treated soil at the rate of 10 per pot. Potted soils were inoculated by carefully scattering seven grammes of *S. rolfsii* inoculum by hand on the soil surface and covering with sterile soil to a depth of 5 cm. Two hundred milliliters of the aqueous plant extracts and captan ($1gl^{-1}$) were applied as soil drenches while seven grammes of maize-meal inoculum of *T. harzianum* were used. Pots inoculated without treatment and non-inoculated pots served as control. The potted soil were randomly arranged and watered regularly. The experiment was repeated twice with five replications and seedlings counts were made 21 days after sowing. Percentage disease reduction were calculated according to the formula of Kataria and Grover (1976) as cited by Pandey and Dubey[10].

Experimental design and statistical analysis: Each experiment was repeated twice with five replicates in a 6x3 factorial in a Completely Randomized Design (CRD). Analysis of variance was carried out and significant differences among means were determined using least significant differences.

RESULTS AND DISCUSSION

Phytoxicity tests: Results of the phytotoxicity tests show that plant extracts did not affect seed germination adversely during the test period. Percent seed germination ranged from 84-90%, indicating that the plant materials do not posses toxic substances that could inhibit tomato (Roma VF) seed germination.

Greenhouse studies: Significant ($p<0.05$) difference occurred in the ability of treatments and inoculation regimes to reduce the disease. Percentage disease reduction of 57-62% was achieved when the extracts were applied two days before inoculation. Similar results were obtained with *T. harzianum* and captan (Table 1). The level of disease reduction was higher at this inoculation regime than all other inoculation regimes employed in this investigation. This showed that extracts of bitter cola, gingers, *H. suaveolens* and neem seed probably exerted some antifungal protective action over tomato seeds when applied two days before inoculation. These findings confirm the observation on Olufolaji[11] on the preventive properties of neem extracts on *Choanephora cucurbitarum* incitant of the lambs tail rot disease of *Amaranthus* Sp. Similar disease reduction were also recorded by the extracts of ginger, *H. suaveolens*, neem seed as well as captan and *T. harzianum* when applied same day as the Inoculation of the fungus given a disease reduction range 52-66%. Their effects significantly ($p<0.05$) differed from the effect

Table 1: Effects of four plant extracts, Captan and *T. harzianum* on the damping-off of tomato seedlings, 21 days after inoculation with *S. rolfsii* in the greenhouse at 27-29°c

	(%) Disease reduction			
	Time of application			
Treatment	2 days before inoculation	Same day as inoculation	2 day safter inoculation	Mean
Bittercola	60.1	40.3	30.6	43.7
Captan	60.4	55.3	57.5	57.7
Ginger	57.4	52.1	30.0	46.5
H. suaveolens	60.8	52.1	33.0	48.6
Neem seed	62.4	57.9	53.3	57.9
T. harzianum	67.1	66.4	64.7	66.0
Mean	61.3	54.0	44.8	

Data are average of five replications in two separate experiments

LSD (0.05) treatment	=	12.6	SED (treatment)=	6.3
LSD (0.05) Inoc. Date	=	8.9	SED (Inoc. Date)=	4.5
LSD (0.05) T x 1	=	N.S	SED (T x 1)=	11.0

N.S=Not Significant

of bitter cola extract, which gave lower disease reduction value of 40.3%.

Percentage disease reduction was lowest when the treatments were applied two days after inoculation. The disease reduction value ranged from 30-64%, with neem seed extract, captan and *T. harzianum* achieving the desired disease reduction (Table 1). This demonstrates that only neem seed extract competed favorably with captan and *T. harzianum* as an eradicant against *S. rolfsii* infection. The effect of *T. harzianum* though not significant (P>0.05) when compared with captan at this inoculation date was numerically higher, thus providing the effectiveness of the bio agent over the fungicide against the fungus. This agrees with the call by a number of workers on the use of bio agents as a safer and viable alternative to fungicides in plant disease control.

Since these extracts have also been shown to be innocuous to tomato seeds in this study and since efficiency increases with dosage[10] higher concentrations could result in greater disease reduction, therefore could be recommended as seed dressing agents against *S. rolfsii* infection. There were also evidence of stimulation of tomato seedling growth in pots treated with the bio agent and the plant extracts as observed in the study, there now exists the opportunity to utilize these control agents in biological control programmes against *S. rolfsii* infection.

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