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Full Length Research Paper

Antagonism of *Trichoderma viride* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*

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Trichoderma viride **was identified as a mycoparasite against** ***Ceratocystis paradoxa*****. When grown near the pathogen,** ***T. viride*** **was seen entwining around the pathogen mycelium. It was stimulated to produce branches that grew directly to the pathogen mycelium. Firm attachment on the pathogen conidia resulted in the penetration and successful growth of** ***T. viride*****. Some of the impregnated** ***C. paradoxa*** **were found death. Benlate solution and extracted water-soluble compounds from** ***Trichoderma*** **species were evaluated for the control of** ***C. paradoxa*****.** ***Trichoderma polysporum*** **significantly reduced the growth of** ***C. paradoxa*** **at high concentrations (100% and 70%) followed by** ***T. viride*****,** ***T. hamatum*****,** ***T. aureoviride*** **and benlate solution recorded average performances. At (50, 30 and 10%) low concentrations, they all recorded poor performances. Minimum inhibitory concentration by** ***T. viride*** **was 10%,** ***T. polysporum*** **25%,** ***T. hamatum*** **and** ***T. aureoviride*** **were 30% each, and benlate solution remained 50%.** ***T. polysporum*** **exhibited better control of the pathogen when compared with other extracted water soluble compounds from** ***Trichoderma*** **species and benlate solution.**

Key words: *Trichoderma*, benlate, extraction, water-soluble compounds.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) industry provides direct employment to about four million Nigerian people in about twenty oil palm growing states in Nigeria, and indirectly to other numerous people involved in processing and marketing (Ahmed, 2001). Despite the enormous potential of the oil palm, there is problem with soil borne fungus *C. paradoxa* causing black seed rot disease in oil palm sprouted seeds. The cause of the dry basal rot in adult palm, conducted by Robertson (1962), was shown to be due to the *Ascomycete*, *C. paradoxa* of which the imperfect stage is known as *Thielaviopsis paradoxa*. It is a soil inhabitant, widely distributed throughout the tropics of Africa and Asia and causes disease of several other crops (Rajagopalan, 1965).

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). *Trichoderma* species produce both volatile and non-volatile metabolites that adversely affect growth of different fungi (Bruce et al., 1984; Corley et al., 1994; Horvath et al., 1995 and Moses et al., 1975). Dennis and Webster (1971b) found that some *Trichoderma* isolates produced volatile components, which were inhibitory to the growth of other fungi. Acetaldehyde was identified tentatively as one of the metabolites of *T. viride* inhibitory to other fungi. Dennis and Webster (1971a) also found that many isolates of *Trichoderma* species produced non-volatile antibiotics, which were active against a range of fungi. The relative abilities of the three biotypes of *T. harzianum* to colonize compost in competition with *Agaricus bisporus* and their influence on *A. bisporus* growth may be associated with secondary metabolite production (Seaby, 1987). Harman, et al. (1980) and Nelson et al. (1988) reported the use of *T. hamatum* for the control of *Pythium* seed rot and *Rhizoctonia* root rot in pea.

Benlate (Methyl-1-Butyl-Carbonomyl-1-2-benzimidizole carbonate) is a relatively safe, most important broad-spectrum systemic fungicide against a large number of important fungal pathogens and it also suppresses mites.

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The recommended dosage of benlate is 5 parts per thousand (0.5% concentration). At this concentration, it is toxic to fungi but not toxic to mammals and less phytotoxic (Lever, 1990).

Although, benomyl is recommended for the control of *C. paradoxa* (Omamor, 1985), information on the use of bioagent for control is lacking. The paper present mycoparasitic activities of *T. viride* and effects of extracted water-soluble compounds from *T. viride*, *T. polysporum*, *T. hamatum*, and *T. aureoviride*, and compare them with commercial fungicide benlate against *C. paradoxa* causing black seed rot in oil palm sprouted seeds.

MATERIALS AND METHODS

The study was conducted in 2005 at botany and microbiology laboratories, University of Lagos, Nigeria. The fungal isolates were previously isolated by the authors from naturally diseased oil palm sprouted seeds collected from Nigerian Institute for Oil Palm Research.

Mycoparasitic activities of *Trichoderma viride* on *Ceratocystis paradoxa*

Coculture interactions between *T. viride* and *C. paradoxa* were examined with paired growths of colonies on PDA in 9 cm diameter Petri dishes. The medium was inoculated with a (no 2 cock borer) 4 mm diameter agar disc of both *T. viride* and *C. paradoxa* cut from the growing edges of three-day-old cultures on PDA. A sterilized glass slide (25x75 mm in size) was placed between the antagonist and pathogen in each Petri dish. Dual cultures were incubated at 28 ± 2°C.

After 6 to 8 days of incubation, the glass slides were removed from the media, stained with 0.2% Trypan blue in lactophenol to aid in the visibility of the mycelia and observed under light microscope (ZEISS West Germany) with an attached camera (Motic MCCamera) connected to the computer (Premio, Pentium 3) at 10 x ocular and 5 mm objective. The experiments were repeated twice.

Extraction of water-soluble components from *Trichoderma* species and benlate solution against *Ceratocystis paradoxa*

A water-soluble component(s) was extracted with butanol from the culture filtrates of *T. viride, T. polysporum, T. hamatum* and *T. aureoviride* grown for 14 days. Pooled culture filtrate (50 ml) was clarified through Whatman no 3 filter paper and extracted 2 times with 50 ml of butanol. The butanol extract (100 ml) were pooled dried in a water bath (22 liters Genlab limited) at 60°C for 7 to 8 h each day for three successive days. To determine the biological activity of the butanol extracts, the residues were dissolved each in 10 ml of sterile distilled water and from these solutions, 1 ml portion of each of the *Trichoderma* species extracts (100%) and the serial dilutions (70%, 50%, 30% and 10%) was pipetted into different wells (8 mm diameter) borne on the surface of solidified PDA plates (9 cm diameter) previously prepared to allow excess water evaporate (pH6). To another set of Petri dishes containing PDA, benlate solution (1 ml) of 1% (1 g benlate in 100 ml distilled water) 0.7% (0.7 g benlate in 100 ml distilled water), 0.5, 0.3, and 0.1% concentrations were added respectively. The PDA plates were bioassayed with *C. paradoxa* disc (6 mm diameter). The control plates had sterile distilled water pipetted (8 mm well) and covered with *C. paradoxa* agar disc (6 mm). All treated plates were compared with the control to calculate the percentage of inhibition or stimulation of the pathogen using the formula 1% = $[(C^2 – C^1) / C^2)]$ x 100, (Edington et al., 1971); where C^1 = means growth of *Trichoderma* specie; C^2 means growth of control. The lowest concentration of growth inhibition was serially diluted to obtain minimum inhibitory concentration (MIC) on the pathogen.

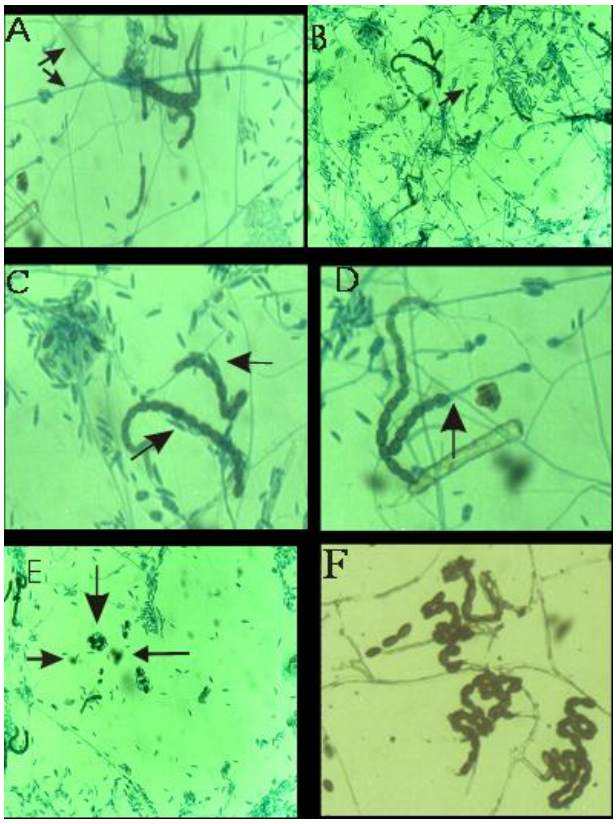

Figure 1. Photomicrograph showing mycoparasitism of *Trichoderma viride* against *C. paradoxa.*

RESULTS

Mycoparasitic activities of *T. viride* was observed on the mycelia of *C. paradoxa*. Mycelium intersections and the subsequent overlap of hyphae of both antagonist and the pathogen began to form after 3 to 4 days. Entwining around the mycelium was a characteristic response of *T. viride* to the pathogen after intersections (Figure 1A). When growing near the pathogen hyphae, *T. viride* often was stimulated to produce branches that were oriented towards the pathogen mycelium (Figure 1B). Penetration of the host and internal growth of hyphae was observed (Figure 1D). After the penetration and internal growth of *T. viride* hypae from the pathogen conidia, the pathogen mycelia appeared to stop growing (Figure 1D). The pathogen is characterized with long chain-tape of conidia (Figure 1F), but during intersections with *T. viride*, the pathogen chain-tape conidia were splitted into single or shorter chains of conidia (Figures 1C and E). The dislod-

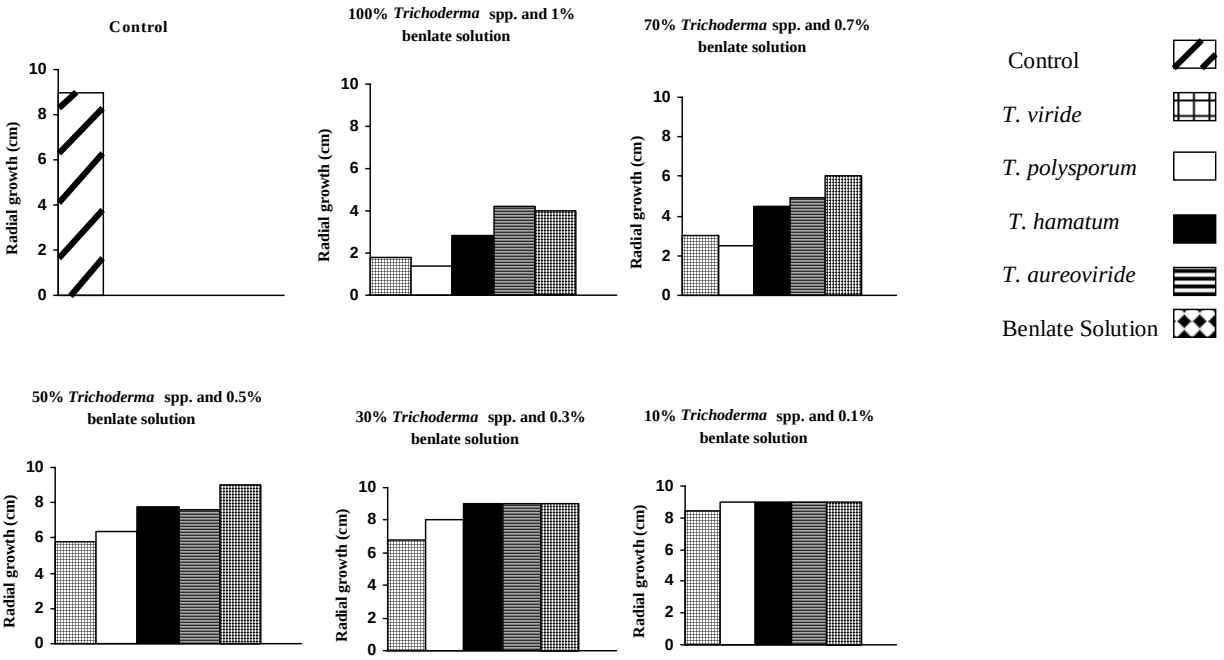

Figure 2. Average radial growth of water-soluble compound extracted with butanol from liquid culture of *Trichoiderma* species and benlate solution incorporated into PDA after 3 days incubation.

ged *T. viride* conidia as a result of the interactions were seen with firm attachment on the pathogen conidia (Figure 1C), some of the *C. paradoxa* conidia were found impregnated due to the penetration of *T. viride* spores (Figure 1E), thus causing the death of pathogen conidia (Figure 1E).

T. viride, T. polysporum, T. hamatum and *T. aureoviride*, and benlate solution responded to different concentrations of serial dilutions (Figure 2). *C. paradoxa* was reduced at high concentrations by *Trichoderma* species (100% and 70%) but benlate solution had average reduction (1% and 0.7%). At 100% and 70%, *T. polysporum* had high reduction when compared with *T. viride, T. hamatum, T. aureoviride* and the control (Figure 2). At low concentrations of 50%, 30% and 10%, *T. viride* had better reduction than the other *Trichoderma* species. At the same low concentrations, benlate, *T. hamatum* and *T. aureoviride* recorded poor performances that were similar to the one obtained in the control treatments.

Minimum inhibitory concentration (MIC) of *T. viride* against *C. paradoxa* was obtained at 10%. At 5% concentration, there was no MIC. *C. paradoxa* covered the 9 cm Petri dish plates at this concentration. *T. polysporum* (MIC) was obtained at 25%. At 20% concentration, the pathogen covered the 9 cm Petri dish plates. Benlate solution (MIC) also remained at 50%. *T. hamatum* and *T aureoviride* (MIC) also remained at 30%, further dilution to 25% permitted the growth of *C. paradoxa* to cover the Petri dish plates (Figure 3).

Arrow(s) **A**, entwining of long-chain of *C. paradoxa* by *T. viride* hyphae; **B**, *T. viride* phialospore was seen recognizing the presence of *C. paradoxa* conidia, initiating attachment; **C**, attachment of dislodged conidia of *T. viride* on *C. paradoxa*; **D**, successful penetration on *C. paradoxa* conidium, resulted in the growth of *T. viride* hyphae; and **E**, some of the impregnated spores of *C. paradoxa* were seen enlarged thus causing them to burst and death. The images were obtained with a 10 x ocular and 5 mm objective.

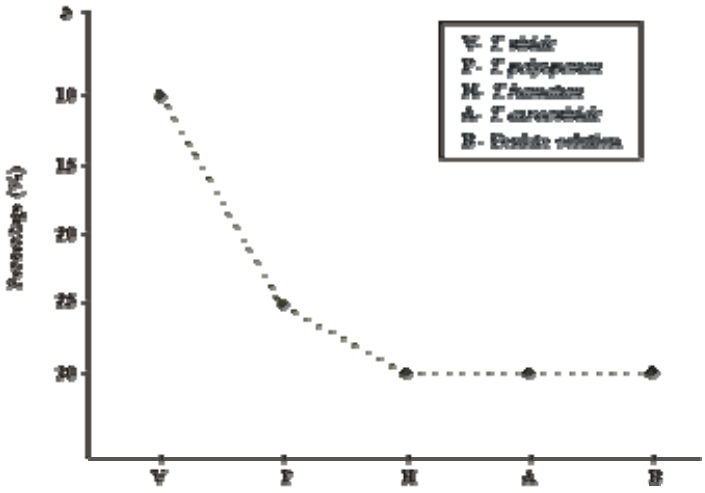

Figure 3. Average radial growths of minimum inhibitory concentrations (MIC) of water-soluble compounds extracted with butanol from liquid culture filtrates of *Trichoderma* species and benlate solution incorporated into PDA after 3 days incubation.

DISCUSSION

This work showed that *C. paradoxa* is vulnerable to attack by antagonist *T. viride.* The study demonstrated that the bioagent acted mainly by entwining, strangling and occasionally penetrating the pathogen hyphae. The results provided evidence that *T. viride* is a multifaceted process that requires the synergistic contribution of several mechanism, including entwining hyphae, spores attachment on its host, growing inside host conidia, splitting of host long conidia chains into single or shorter chains and subsequently causing death of host conidia.

One of the major events of antagonistic process was the apparent affinity of *T. viride* conidia on the conidia of the pathogen. The result supported the hypothesis that the antagonist is attracted to the host cells by an unknown mechanism that probably involves specific chemical stimuli (Whipps et al., 1988) or chemotropic growth (Chet, 1987).

The penetration of the pathogen conidia cell wall by *T. viride* may likely be due to alteration of the pathogen structure. It is likely that the firm attachment observed between conidia of the antagonist and *C. paradoxa* is mediated by a specific cell surface recognition, which in turn, triggers event that leads to host wall penetration. Cell surface molecules play an important role in cell to cell interactions in many biological systems (Inbar and Chet, 1994; Sequeira, 1985) and early recognition events, mediated by molecules with sugar-binding affinity, are known to be important determination in establishing the mycoparasitic relationship between *Trichoderma* species and their target hosts (Barak et al., 1985; Benhamou and Chet, 1993).

Studies on water-soluble compounds extracted from *Trichoderma* species, showed that all the *Trichoderma* species tested inhibited the growth of *C. paradoxa*. *T. polysporum* exhibited better growth inhibition at high concentrations than the other *Trichoderma* species. The effects of the inhibitory actions on *C. paradoxa* by *T. viride, T. polysporum* and *T. hamatum* appear to be associated with toxic volatile compounds produced by them. The actual effect and mechanism involved is not known, but *Trichoderma* spp. are known to produce a range of metabolites that may affects the growth of microorganisms and plants (Ghisalberti and Sivasithamparain, 1991).

It was obvious that *C. paradoxa* was inhibited at high concentrations of 100% and 70% by *T. polysporum* and *T. viride* but the pathogen growth was stimulated at low concentrations of 50%, 30% and 10%. The consistence of *T. polysporum*, dropped at low concentrations while *T. viride* picked up slightly at low concentrations. The reasons for the average performances of *T. hamatum* and *T. aureoviride* at high concentrations and their poor performances at low concentrations were not known.

The commercial fungicide benlate, at high concentrations had average growth inhibition of *C. paradoxa* when compared with the growth inhibitions by *T. polysporum* and *T. viride*. Benlate, *T. hamatum* and *T. aureoviride* were also found to be stimulating the growth of the pathogen at low concentrations of 50, 30 and 10%. However, these growth stimulations were less when compared with the control treatments. Minimum inhibitory concentration (MIC) of *T. viride* against *C. paradoxa* was higher than the other *Trichoderma* species. Considering the inhibition of *C. paradoxa* by *T. polysporum* at high concentrations, it was concluded that the antagonist exhibited better control of the pathogen.

The significance of this result suggests that water-soluble compounds extracted from these *Trichoderma* species were toxic and fungistatic to *C. paradoxa.* However, it has not been possible to extract the substances involved but they would be investigated for further studies.

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