

Short Communication

Comparative *in vitro* inhibitory effects of cold extracts of some fungicidal plants on *Fusarium oxysporium* mycelium

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Accepted 15 July, 2008

Cold extracts of five fungicidal plants (*Aloe barbadensis*, *Azdrichtha indica*, *Nicotiana tabacum*, *Tridax precubens* and *Carica papaya*) were screened for *in vitro* inhibitory effects on *Fusarium oxysporium*, using 25, 50, 75 and 100% concentrations of each extract. The four different concentrations of *N. tabacum* completely inhibited radial growth of *F. oxysporium* mycelium *in vitro*, 75 and 100% concentrations of *A. barbadensis* had a similar effect, while only the 100% concentration of *A. indica* completely prevented the fungal growth. Generally *C. papaya* and *T. precumbens* showed fungicidal properties, but they could not completely inhibit radial mycelia growth of the fungal pathogen at any of the concentration levels tested. *N. tabacum* was found to be most fungitoxic as exhibited by all the concentration levels used.

Key word: *In vitro*, plant extracts, *Fusarium oxysporium* mycelium.

INTRODUCTION

Many fungi have been identified by various workers as causal organisms of various plant diseases. Adebajo and Onesirosan (1986) isolated *Colletotrichum gloeosporoides* as a fungal pathogen infecting minisettes through infested yam tubers. Ikotun (1989), also isolated several fungi associated with rot of yam tuber.

Chemical control measures have been tested and found effective in the control of plant diseases. Coursey (1961) reported protecting yam against rot with borax (5% aqueous solution), copper 8-hydroxyquinolinolate (4% aqueous Solution), and lime wash.

Plumbley (1985) and Ogundana and Denis (1981) listed some other fungicides sensitive against some rot-causing pathogens of plants. Nwankiti et al. (1990) highlighted some protective fungicides that have been found effective in controlling some plant diseases, but he remarked that those chemicals were expensive and not usually affordable. Kamel and Mangla (1987) indicated

that "the continuous and indiscriminate use of chemicals in agriculture posed the problem of acute and chronic toxicity to man". The use of chemicals are of great concern to man; they can cause death through poisoning, accumulate in man, concentrate in food chains, because they are often not easily biodegradable, they cause resurgence and resistance in pathogens and pest populations, and destroy parasites, predators and flower pollinators. However in Nigeria today, subsistence farmers, not to mention large-scale farmers, depend on chemical toxicants indiscriminately used on their crops. Therefore, there is a need to look for long-lasting and reliable solutions that respect the requirements of man and environment.

The use of pesticides of plant origin has been suggested by some workers as alternatives to synthetic chemicals, in order to counter the potential hazards and pollution problems associated with the use of synthetic chemicals (Amadioha and Obi, 1998, 1999; Amadioha, 1998; 2000). Therefore, the efficacy of plant extracts needs to be investigated. Sawdust extract from Camwood has been used to control yam tuber rot caused by

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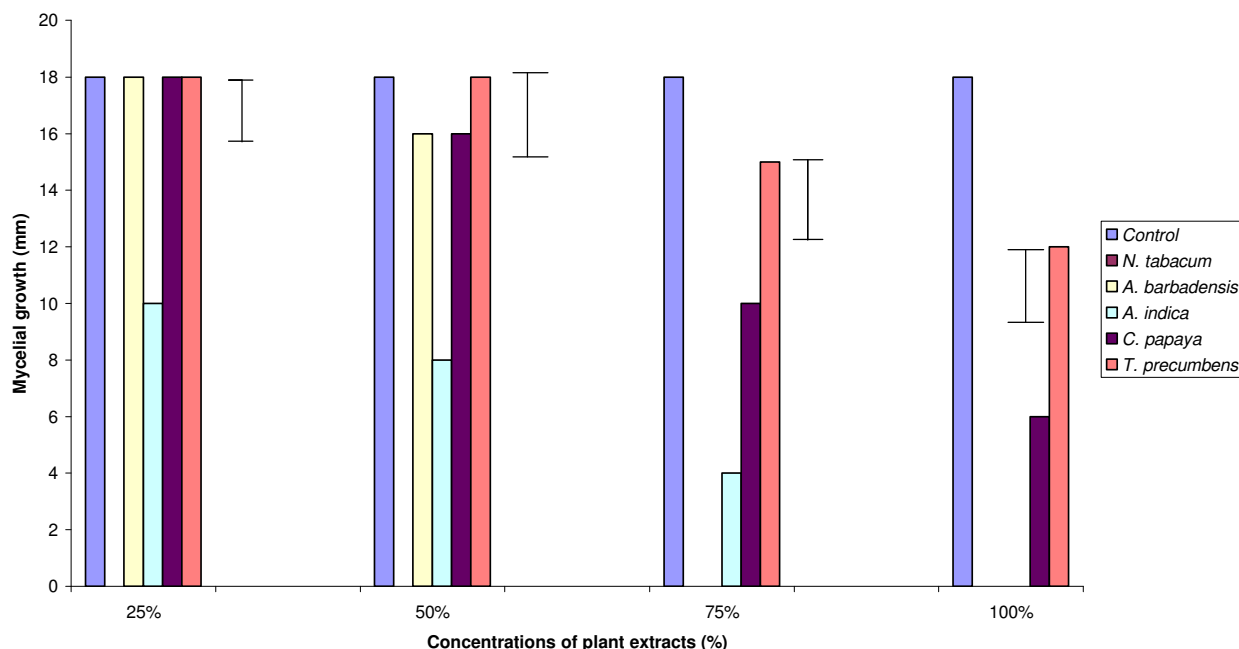


Figure 1. *In vitro* inhibitory effects of cold plant leaf extract on mycelia growth of *F. oxysporium*. I = LSD (P = 0.05).

Sclerotium rolfsii (Ejечи and Ilondu, 1999). Al-Abed et al. (1993) have shown the importance of natural chemicals as possible source of phytotoxicity; they are systematic and easily biodegradable, as revealed in separate works by Amadioha and Obi (1998, 1999), Olufolaji (1999) and Amadioha (2000).

The aims of this research therefore are to compare the effect some selected plant extracts on *Fusarium oxysporium* mycelia growth *in vitro* and identify the concentrations of the plant extracts that are fungicidal.

MATERIALS AND METHODS

Five plants (*Aloe barbadensis*, *Azadirachta indica*, *Nicotiana tabacum*, *Tridax precubens* and *Carica papaya*) were collected from the premises of Kogi State University, Anyigba. Stock of *Fusarium oxysporium* was collected from the Department of Biological Sciences Laboratory, Kogi State University, Anyigba.

Potato Dextrose Agar (PDA) was the medium used. Pure culture of the fungal isolate was maintained by aseptic transfer to a freshly prepared PDA medium. Sterile PDA medium were used to test the mycelia growth rate of the fungi. A disc of the fungus pure culture (4 mm diameter) was introduced into sterile PDA medium in Petri dishes with two intersecting lines at the bottom (to determine the centre of the plate). The inoculated plates were incubated for 7 days at room temperature ($25 \pm 3^\circ\text{C}$). This was replicated in three Petri dishes. The diameter of the radial growth of the fungi were measured at the end of incubation period and then used to determine the radial growth rate of the fungus.

From the powder sample, *A. indica*, *T. precumbens*, *C. papaya*, and *N. tabacum* cold water extractions were made; while the succulent leaves of *A. barbadensis* were used directly. The cold extracts were obtained by adding 10, 20, 30 and 40 g of the powder of each plant leaf to 100 ml of sterile distilled water in 250 ml

beaker. This was left for 24 h, and then subsequently filtered through four fold of sterile cheese cloth. These preparations gave 25, 50, 75 and 100% crude aqueous extract.

The bioassay of the different plant extracts was carried out by determining the effects of their concentrations on radial growth as described by Amadioha and Obilor (2002). This was carried out in sterile Petri-dishes of 9 cm diameter, containing PDA. The fungitoxic effects of each plant extract were tested on the fungi by growing the fungi on the PDA medium containing 1 ml of 25, 50, 75 and 100% of each plant extract, separately spread on the surface of the solidified PDA Petri-dishes. A disc of 4 mm diameter (using a sterile cork-borer) of each pure culture of the isolated fungi was placed on the thin film formed on the PDA just at point of intersection of two lines at the bottom of each Petri-dish. Three plates were treated each with the extract of each plant. The control experiments had distilled water in place of plant extracts; the treatments and control were incubated for seven days at room temperature. The diameter of the radial growth of the fungi were measured at the end of incubation period and then used to determine the percentage inhibition of each extract using the formula:

$$\text{Mycelia growth inhibition (\%)} = [(dc-dt) / dc] \times 100 (\%)$$

Where dc = average diameter of fungal colony in the control, and dt = average diameter of fungal colony in treatment group.

Statistical analysis

Descriptive statistics of variables measured are presented as mean values in bar charts. In order to test whether or not there were significant differences between the plant extracts used in treating the fungal pathogen and the control group, the smallest difference between means (least significant difference [LSD]) of mycelia growth inhibition was computed by post hoc tests to obtain multiple comparisons between treatments. The results obtained were sub-

jected to statistical analyses using one-way ANOVA at 5% level of significance. The software used was SPSS version 10.

RESULTS AND DISCUSSION

The radial growth of (*F. oxysporium*) completely covered the Petri dishes by the 7th day of incubation (45 mm). It was generally observed that *C. papaya* and *T. precumbens* showed fungicidal properties compared to the control tests, but none of their tested extracts concentrations was able to completely inhibit radial mycelia growth of the fungi pathogens within the period of incubation (Figure 1).

The results of aqueous plant extracts on PDA medium showed that *N. tabacum*, *A. indica*, *A. barbadensis*, *T. precumbens* and *C. papaya* extracts were fungitoxic to the fungi pathogen but not all were efficacious in controlling fungal radial growth of the tested fungi using the different plant extracts concentrations (100, 75, 50 and 25% concentrations). All the tested *N. tabacum* extract concentrations (100, 75, 50 and 25%) completely inhibited radial mycelia growth of *F. oxysporium*; 100 and 75% concentrations of *A. barbadensis* had similar effects, while only the 100% concentration of *A. indica* completely inhibited radial mycelia growth of *F. oxysporium* (Figure 1).

N. tabacum was found to be most fungitoxic as exhibited by all the concentration levels used. *A. barbadensis* ranked second in fungicidal property, while *A. indica* is 3rd among the five tested plants. At 25%, *A. indica* was significantly more fungicidal than *A. barbadensis*, *C. papaya*, and *T. precumbens*. With 50%, no difference inhibitory effect was observed between extracts of *A. barbadensis*, *C. papaya*, and *T. precumbens*. However, at 75%, there was significant difference between *A. barbadensis*, *C. papaya*, and *T. precumbens*. It was only at 100% concentration that *C. papaya* was better than *T. precumbens*.

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