ANTIFUNGAL ATTRIBUTES OF PLANT EXTRACTS, TRICHODERMA KONINGII AND DITHANE M-45 ON SCLEROTIUM ROLFSII - THE CAUSAL ORGANISM OF CORMEL ROT OF COCOYAM

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ABSTRACT

In vitro studies were carried out on the control of cocoyam (Colocasia esculenta [Lin., Scott]) cormel rot caused by Sclerotium rolfsii using plant extracts, a synthetic fungicide (Dithane M45) and Trichoderma koningii, a biocontrol agent. Air-dried aqueous leaf extracts of Carica papaya, Chromolaena odorata, Azadirachta indica at various concentrations (50, 70, and 100%) and T. koningii significantly inhibited the mycelial growth in Sclerotium rolfsii. However, C. odorata was more effective on the mycelial growth compared to the other extracts and the standard fungicide (Dithane M-45) at the 5% probability level. Generally, T. koningii was the most effective when compared to other control agents. –The antagonistic ability of T. koningii against the pathogen was overwhelmingly significant in dual culture. Therefore, both C. odorata and T. koningii should be tried under field conditions to further confirm their inhibitory potential against Sclerotium rolfsii.

Key Words: Plant extracts, T. koningii, S. rolfsii, antagonist, Chromolaena odorata, Azadirachta indica, biocontrol

INTRODUCTION

Cocoyam (Colocasia esculenta Lin, Scott) is a perennial monocotyledenous and herbaceous plant of the family Araceae. It is mainly cultivated for the edible corms, although other parts of the plant such as the leaves, petioles and flowers are used for both medicinal and culinary purposes (Eze and Maduewesi, 1990). Cocoyam ranks third in importance after cassava and yam among the root and tuber crops cultivated and consumed in Nigeria. Currently, Nigeria is the world’s leading producer of cocoyam (taro), accounting for up to 3.7 million metric tonnes annually. Various lines of ethnobotanical evidence suggest that cocoyam originated from South Central Asia, probably India in the Malay peninsula (Kolchaar, 2006). Wild forms exist in various parts of Southeast Asia (Purseglove, 1972). Harvested cocoyam is stored by different methods to extend their shelf life for the next planting season and for subsequent use as food.
Post-harvest loss of root and tuber crops has been a very serious problem to farmers as more than 40% of their harvest may be lost because of decay (Olurinola et al., 1992). Generally, rotting starts from the field and progresses in storage (Enyiukwu et al. 2004). Organisms associated with cocoyam rot in Nigeria include Aspergillus flavus, Penicillium digitatum, Botryodiplodia theobromae, Sclerotium rolfsii, Fusarium solani and Erwinia carotovora (Onuegbu, 1999). Lack of good storage methods limits the availability of cocoyam throughout the year (Chukwu et al., 2008). Conventional storage methods such as traditional cocoyam barn-heaping of the cocoyam under shade and storing inside pits have been found to be ineffective due to high percentage losses. Cocoyam is vegetatively propagated by its corm and cormels which guarantees the genetic fidelity of the materials. However, it also serves as the means by which bacterial, fungal and viral diseases are spread.

Among the diseases commonly reported to infect cocoyam is the cormel rot caused by Sclerotium rolfsii and some other micro-organisms particularly fungi and bacteria. Sclerotium rolfsii Sacc is distributed in tropical and subtropical regions of the world where high temperatures prevail (Arunasri et al., 2011). The fungus has a wide host range, affecting about 500 plant species in about 100 families including groundnut, green bean, potato, sweet potato, onion, garden bean, pepper, tomato, cocoyam and water melon (Aycoc, 1966). Sclerotium rolfsii produces abundant white, fluffy, branched, septate mycelia with clamp connections only on the main hyphae and has a fan-like spread. The mycelia normally form white tufts which later give rise to smooth, hard and dark brown sclerotia which may be spherical or irregular in shape and resembles the mustard seed at maturity (Arunasri et al., 2011). The sclerotal structure has been studied by several workers as having three layers of an outer rind, a middle cortex and an inner medulla. The size of sclerotia was reported to vary from 0.1 mm to 3.0 mm (Prakash and Singh, 1976; Ansari and Agnihotri, 2000 and Anahosur, 2001). Symptoms produced by S. rolfsii in host plants include seed rot, seedling blight, collar rot, stem rot and wilt. These resulted to great economic loss in affected crops namely: groundnut (Arachis hypogeaa), 25% of seedling mortality (Ingale and Mayee, 1986) and 30% of crop losses in tomato (Thiribhuvanamala et al. 1999).

The use of chemicals has helped in the control of rod diseases in crops, but not without identifiable problems such as chemical residues, biodegradation, phytotoxicity, development of resistance in target organism, high cost, non availability and environmental concerns to man and animals (Okigbo and Odurukwe, 2009). As an alternative, considerable efforts are now directed at exploring the potentials of botanicals in plant disease control. Aside being readily available, affordable and environmental friendly, botanicals have the advantage of non-phytotoxicity and easily biodegradable compared to synthetic fungicides (Akuesh et al., 2002; Okigbo and Nmeka, 2005; Okigbo and Omoda-miro, 2006). Also some antagonistic fungi such as
Trichoderma sp. have been found to play a major role in the biological control of some plant diseases. Several strains of Trichoderma have been developed as biocontrol agents against fungal diseases of plants (Harman et al., 2004). Therefore, the objective of the present study is to evaluate the efficacy of various concentrations of leaf extracts of Carica papaya, Chromolaena odorata and Azadirachta indica, culture filtrate of Trichoderma koningii (a bio-control agent) and standard fungicide- dithane M-45 (Mancozeb) for the control of Sclerotium rolfsii, the causal pathogen of cocoyam cormel rot under laboratory conditions.

MATERIALS AND METHODS:

Collection of materials: Cocoyam (Colocasia esculenta Linn. Scott) cormels with symptoms of post harvest rot as well as fresh healthy cormels were all obtained from the Mile 1 market Portharcourt, Nigeria; the synthetic fungicide- Dithane M-45 (Mancozeb²) was obtained from the School to Land Authority, Port Harcourt and leaves of Azadirachta indica Carica papaya, and Chromolaena odorata were collected at Etche Local Government Area also in Port Harcourt, Rivers State Nigeria. The leaf samples were authenticated in Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, Nigeria where voucher specimens of the samples were deposited. Pure culture of Trichoderma koningii was obtained from the Advanced Plant Pathology Laboratory of the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria.

Preparation of plant extracts and isolation of pathogen: Fresh leaves of C. papaya, C. odorata and A. indica were dried at room temperature (28±2°C) for 14 to 18 days and later ground into a very fine powder and stored in a sterile plastic container at room temperature. A stock solution of each extract was obtained by mixing 1 gram in 100 ml of Sterile distilled water, stirred vigorously and kept for 24 hours. The extracts were then decanted, filtered through a No.1Whatman filter paper and used within 48 hours. The isolation of the pathogen was carried on cocoyam cormels showing symptoms of the disease following Ritchie, (1991). Approximately 2 mm infected tissues were cut, including healthy and infected portions of the cocoyam cormel using a sterilized scalpel, and inoculated on to Petri dishes containing sterilized potato dextrose agar (PDA). The inoculated plates were incubated at temperature of 28±2°C for four days and examined for fungal growth.

Pure culture and pathogenecity test: Following fungal growth, subcultures were prepared to obtain a pure culture. The resulting pure culture was used for characterization and subsequent identification of the fungi isolates with the assistance of Advanced Plant Pathology.
Laboratory of International Institute of Tropical Agriculture (IITA) Ibadan Nigeria and identification guides (Sulton, 1980; Barnett and Hunter, 2008). To test for pathogenicity, a 5 mm mycelia disc from a 6-day old pure culture \( S. \) rolfsii was cultured for 7 days in a PDA broth containing 3.9g of PDA mixed in 100 ml sterile distilled water. The culture was filtered using Whatman filter paper and transferred into a 50 ml distilled water containing 10% glucose. The mixture was properly agitated and sprayed on fresh surfaced sterile uninfected cocoyam cormels. The cormels were kept at a temperate of 28±2°C. The organism was confirmed after 7 days of inoculation and re-isolation from the infected cormel.

**Effects of plant extracts and dithane M-45 on the radial growth of Sclerotium rolfsii:**
The stock solution of the leaf extracts were diluted to 50%, 70% and 100% and used in the *in vitro* experiment. Exactly 39g of PDA powder was dispensed in 1000ml of sterile distilled water in a conical flask whose mouth was covered with non-absorbent cotton wool and wrapped with aluminum foil. This was autoclaved at a temperature of 121°C and pressure of 15 atm for 15 minutes. Also, 2 ml of the different concentrations (50, 70 and 100%) were mixed with 10 ml PDA and poured in Petri dish to further reduce the concentration to 40, 60 and 90%, respectively. Then, the medium was uniformly mixed and allowed to cool and solidify. For the positive control, 2 ml of 2% of dithane M-45 (Mancozeb®) was mixed with 10ml PDA, poured into Petri dish and allowed to solidify. The control experiment had no leaf extract treatment. The experimental layout was Completely Randomized Design. A 5mm disc from a 4-day old culture of the pathogen was placed at the centre of each plant extract-PDA mixture, as well as the controls. The plant extract-PDA mixture was designated as *Carica papaya* potato dextrose agar (CPPDA) for *Carica papaya*, *Chromolaena odorata* potato dextrose agar (COPDA) for *Chromolaena odorata* and *Azadirachta indica* potato dextrose agar (AIPDA) for *Azadirachta indica*. Two intersecting lines were drawn at the bottom of each plate to determine the centre of the plate. A total of 30 plates were treated and each treatment replicated thrice. All the inoculated plates were incubated at room temperature and the radial growth (cm) of the fungus was measured daily for three days. The percentage growth inhibition was measured as follows:

\[
\% \text{ mycelial growth inhibition} = \frac{(DC-DT)}{(DC)} \times 100/1
\]

where:
DC = is the farthest radial distance of pathogen in the control plates.
DT = is the farthest radial distance of pathogen in the plant extract-PDA mixture plates.

**Effect of Trichoderma koningii on the radial growth of Sclerotium rolfsii:**
The antagonistic activity of *T. koningii* against *S. rolfsii* was carried out *in-vitro* using the dual culture method on PDA media. Thus, four different inoculation methods were employed in which a 5 mm diameter mycelial disc of the antagonist and 4 day old mycelia of the pathogen were placed simultaneously (SIM) at the centre and the opposite ends of Petri dish SIM C and B, respectively. In the second method preventive (PRV), the same amount of the antagonist was inoculated on PDA close to the centre of Petri dish 24 hours before the inoculation of the pathogen and labeled PRV. In the curative (CUR) method, the pathogen (*S. rolfsii*) was placed in the media 24 hours before the inoculation of the antagonist. The control (CTR) consisted of a single culture of *S. rolfsii* only without antagonist. The percentage inhibition of mycelial growth of *S. rolfsii* by *T. koningii* was calculated for all the treatments follows:

\[
\text{% mycelial growth inhibition} = \frac{(\text{DC} - \text{DT})}{\text{DC}} \times 100/1
\]

Where DC = is the farthest radial distance of Pathogen in control plate.

DT = is the farthest radial distance of Pathogen in plate in the presence of the antagonistic fungus.

The experiment was laid out in a completely randomized design repeated four times and data collected were subjected to Analysis of Variance (ANOVA) with the aid of SPSS computer software statistical package (IBM Version 23.0). Means separation was carried out using Duncan’s Multiple Range Test (DMRT) at 5% level of significance.

**RESULTS AND DISCUSSION:**

**Effect of plant extracts and *Trichoderma koningii* on the mycelial growth of *S. rolfsii***

Extracts of *C. odorata* at 50, 70 and 100% concentrations inhibited mycelial growth in *S. rolfsii* at 10.20%, 12.55% and 29.02% respectively, compared to dithane M-45 which had 5.09% inhibition (Figure1). Inhibition of mycelia was lowest in *A. indica* and *C. papaya* in all the concentrations tested ranging from 1.57 to 2.57% and this was significantly different from dithane M-45. However, *T. koningii*, was significantly effective in inhibiting mycelia growth in *S. rolfsii*, as indicated in the different control methods employed. Simultaneous inoculation of both the pathogen and the antagonist at the centre (SIM C) and preventive application (PRV) of the antagonist had more than 50% inhibition (51.37%) each, followed by simultaneous at both end of the plate (SIM B) and Curative (CUR) with inhibition percentage of 49.80 and 44.31, respectively. The antagonistic effect of *T. koningii* on the pathogen *S. rolfsii* using the dual culture method show *T. koningii* completely inhibited the growth of *S. rolfsii* in culture is shown (Plate 1 and 2). After 9 days of co-cultivation, the antagonist completely covered the pathogen. The presence of *S. rolfsii* as the causal agent of cocoyam cormel rot in storage in
Figure 1. Antifungal effects of *C. odorata*, *A. indica*, *C. papaya*, *T. koningii* and dithane M45 on the mycelial growth of *S. rolfsii*

* Mean Values per treatment followed by the same letter are not significantly different (*P* = 0.05) according to Duncan's Multiple Range Test (DMRT)

Plate 1. Dual culture showing antagonistic effect of *Trichoderma koningii* (*T.k* ) on *Sclerotium rolfsii* (*S.r*) (with arrow).
Plate 2. Dual culture showing antagonistic effect of *Trichoderma koningii* (*T.k*) on *Sclerotium rolfsii* (*S.r*) (with arrow).

Nigeria is well reported (Onwueme 1978; Eze and Maduevwi, 1990, Ugwuanyi and Obeta 1996; Nwachukwu and Osuji, 2008; Okigbo et al., 2009b) and confirmed in this study. The *in vitro* study showed that extracts from *C. odorata* inhibited mycelia growth in *S. rolfsii* compared to the other plant extracts. The inhibition potential of *C. odorata* can be due to high levels of fungitoxic substances like flavonoids, tannins and sapronin found in the extract, as earlier reported from extraction works on *C. odorata* leaves by Akinmoladun et al., (2007) and Okey, (2015). *Trichoderma koningii* completely inhibited the growth of the pathogen in culture. Similar findings on the antagonistic parasitism of *T. harzianum* on *S. rolfsii* have been reported (Elad et al., 1980, Olufolaj et al., 2014). Mycopara- chicasitism and competition for nutrients have been suggested as the ways by which *Trichoderma* spp. show antagonism (Jayasuriya et al., 1996, Olufolaj et al., 2014).

Conclusively, *C. odorata* and *T. koningii* possess potential inhibitory effects on the mycelia growth of *Sclerotium rolfsii*, the causal agent of cocoyam cormel rot. Their inclusion into an integrated disease management programme in the control of cocoyam cormel rot under field conditions is recommended.
REFERENCES


