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Preventive and curative control of sclerotium rot disease of cocoyam cormel (*Colocasia esculenta* [L., Scott]) using plant extracts and *Trichoderma koningii*

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ABSTRACT

Aqueous leaf extracts from air-dried samples of *Carica papaya*, *Chromolaena odorata,* and *Azadirachta indica* were screened for antifungal properties against *Sclerotium rolfsii* at 50, 70, and 100% concentrations and *Trichoderma koningii* (4.9×10^6 spores/ml) *in vivo*. Dithane M45 at 2% concentration was used as positive control for better comparison. The aqueous extracts and *T. koningii* were applied as preventive and curative methods by spraying fresh and healthy cormels with *S. rolfsii* (3.75×10^5 spores/ml). The results showed that Mancozeb and *T. koningii* had significant effect (preventive and curative) on disease severity and percentage weight loss. Furthermore, preventive and curative application of *T. koningii* significantly reduced disease severity. *C. papaya* at 50% concentration (preventive method) had the highest disease severity (3.8%), as to the control (4.0%). The extracts and dithane M45 were more effective as curative, while *T. koningii* was better as preventive method. The result on percentage weight loss showed that *C. odorata (*70% concentration) had the highest (49.61%), followed by *C. papaya* (50%) with 47.85%, and the least was *A. indica* (23.77%) at 70%. *C. odorata* and *A. indica* 50% and 100% concentrations, respectively, significantly reduced weight loss, while *C. odorata* (100% concentration) and the control gave the highest percentage weight loss in the curative method. Conclusively, plant extracts and *T. koningii* were more effective in controlling the disease and reduction in weight loss than mancozeb.

1. INTRODUCTION

Cocoyam is a perennial monocotyledonous and herbaceous plant of the family Araceae. It is an important staple food in many developing countries in Africa, Asia, and the Pacific [1]. In Sub-Saharan Africa, cocoyam production is essentially by small-scale, resource-poor farmers (mostly female) with minimal agricultural input. Cocoyam is consumed mostly by the low-income earners and the economically vulnerable groups [1]. The two most commonly cultivated species are *Colocasia esculenta* (the red type or taro) and *Xanthosoma sagittifolium* (the white type or tannia). In Nigeria, cocoyam is mainly cultivated for the edible corms as a source of carbohydrate to supplement yam and cassava as well as for medicinal purposes [2,3]. Cocoyam ranks third in importance after cassava and yam among the root and tuber crops cultivated and consumed in Nigeria. Cameroon, Ghana, and Nigeria account for over 60% of the global cocoyam production [1]. Cocoyam tolerates shady environments, often intercropped with perennial cash crops such as cocoa, bananas, and oil palms.

Harvested cocoyam is stored by different methods to extend the shelf life for use in the next planting season and 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 [4]. Organisms associated with cocoyam rot in Nigeria include *Aspergillus flavus, Penicillium digitatum, Botryodiplodia theobromae, Sclerotium rolfsii, Fusarium solani,* and *Erwinia carotovora* [5]. In particular, cocoyam cormel rot caused by *S. rolfsii* is common. *S. rolfsii* Sacc. is distributed in tropical and subtropical regions of the world with high temperatures [6]. Symptoms due to *S. rolfsii* in host plants include seed rot, seedling blight, collar rot, stem rot, and wilt resulting to economic losses. Management of *S. rolfsii* is difficult due to its wide host range and the ability of sclerotia to survive between 3 and 4 years in the soil [7]. The goal of plant disease management is to reduce the economic and esthetic damage caused by plant diseases. Chemical control of rot diseases in crops is feasible but not without identifiable problems such as chemical residues, biodegradation, phytotoxicity, development of resistance in target organism, and high cost [8].

Natural plant products are potential alternatives to chemical fungicides in plant disease management. *Carica papaya* (family: Caricaceae) is an

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unbranched small tree cultivated in the tropical regions for the edible fruits. *C. papaya* is used as food and in medicine. *C. papaya* contains various types of biologically active compounds with the two important compounds as chymopapain and papain. [9,10] reported that the extracts of papaya leaves inhibited the growth of *Rhizopus stolonifer*. Formulations of *C. papaya* roots, *Mangifera indica* leaves, *Citrus limon* fruit, and leaf have also been reported to possess antibacterial activity against species of *Salmonella* [11]. *Azadirachta indica* (Neem, family Meliaceae) is a tropical tree commonly found in Asia and Africa, but native to India, Bangladesh, Pakistan, and Myanmar [12]. Its local names include Dogon Yaro (Nigeria), Azad Dirkht (Persian), and Neeb (Arabic). Several authors have reported the medicinal uses and phytotoxicity of *A. indica*, where extracts from the seed have been reported to greatly reduce conidial germination in several fungi [13]. *Chromolaena odorata* (family Asteraceae) is a perennial weed shrub of crops and pastures found mainly in West Africa and South Asia [14]. A decoction prepared from the aqueous extract of the leaves of *C. odorata* has been licensed for clinical use in Vietnam. [14] reported that aqueous leaf extracts of *C. odorata* showed antimicrobial and anticoagulation effects in clinical studies. The same leaf extracts have been used as cough remedy and as an ingredient with leaf extracts of guava and lemongrass for the treatment of malaria. [15,16] reported that fresh leaves have been traditionally used in most tropical countries like Vietnam for the management of burn wounds, dentoalveolitis, soft tissue wounds, leech bite, and skin infection. There are confirmed reports that leaves and stems of *C. odorata* contain steroids, essential oils, flavonoids, and triterpenes [17]. The antagonistic activities of *Trichoderma koningii* against plant pathogenic fungi are well-documented [18]. However, reports on their efficacy on the control of rots caused by *S. rolfsii* are sparse. Therefore, the objective of the present study is to evaluate the antifungal properties of leaf extracts of *C. papaya, C. odorata* and *A. indica*, *T. koningii* (a biocontrol agent), and standard fungicide - Dithane M-45 (Mancozeb) in the management of cocoyam cormel rot disease caused by the pathogen *S. rolfsii*.

2. MATERIALS AND METHODS

2.1. Source of Materials

Fresh and healthy cocoyam cormels and cormels showing symptoms of post-harvest rot were obtained from Mile 1 market located at the city center in Port Harcourt, Nigeria, in sterile polythene bags. Dithane M-45 commonly called mancozeb was also obtained from School to Land Authority of the Rivers State Government. Fresh leaves of *A. indica, C. papaya,* and *C. odorata* were obtained from a village in Etche Local Government Area of Rivers State, Nigeria and authenticated in the Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, where voucher specimens of the plants were deposited. *T. koningii* was graciously supplied by the Advanced Plant Pathology Laboratory, International Institute for Tropical Agriculture (IITA), Ibadan.

2.2. Preparation of Plant Extracts and Pathogenicity Test

Fresh leaves of *C. papaya, C. odorata,* and *A. indica* were dried at room temperature for 1 week and later ground into very fine powder using laboratory mortar and pestle and stored separately in sterile plastic containers. An aqueous solution of the dried plant samples was prepared by mixing 1 g of each leaf powder with 100 ml of sterile distilled water, stirred vigorously and kept for 24 h. The extracts were then decanted, filtered through a Whatman filter paper and used within 24 h of preparation. The 100% concentration from each extract was serially diluted to obtain 50% and 70% concentration. Cocoyam cormels showing symptoms of rot were surface sterilized in 5% sodium hypochlorite and rinsed thrice in sterile distilled following the method of [19]. Approximately 2 mm size bearing infected and healthy portions of the cocoyam cormel were cut using a sterilized scalpel, surface sterilized with 5% sodium hypochlorite, rinsed in sterile distilled water, dried between sheets Whatman filter paper and inoculated onto Potato Dextrose Agar in Petri dishes. The plates were incubated at 28 ± 2°C for 4 days and the resulting fungal growth was sub-cultured by transferring hyphal tips from the edge of each colony onto fresh PDA plates to obtain a pure culture of the organism. Pathogenicity test was done by cutting about 5 mm mycelial disk from a 6-day-old pure culture of *S. rolfsii*, mixed with 3.9 g PDA broth in 100 ml sterile distilled water and incubated for 7 days. Then, the mixture was filtered using Whatman filter paper and transferred into 50 ml distilled water containing 10% glucose, properly agitated, and sprayed on fresh surface sterilized healthy cocoyam cormels and kept at 28 ± 2°C. Symptoms of rot started appearing 7 days after inoculation and *S. rolfsii* was reisolated from the infected cormel with physical properties confirming the pathogen.

2.3. *In Vivo* Tests and Disease Severity

The aim of this trial was to examine the preventive and curative effects of the plant extracts, *T. koningii* and the synthetic fungicide on disease severity and weight loss in cocoyam cormels due to *S. rolfsii*. The treatments consisted of three plant extracts at (50, 70, and 100%) concentrations, dithane M45 (mancozeb), and *T. koningii* laid out in a completely randomized design. The curative method was done by growing three mycelial disks (5 mm each) of *S. rolfsii* from a 6-day-old pure culture for 7 days in a PDA broth containing 17 g of PDA mixed in 400 ml sterile distilled water. The culture was filtered using Whatman filter paper and transferred into 300 ml distilled water containing 10% glucose. The mixture was properly agitated and sprayed on the freshly cut surface of healthy cocoyam cormels at 10 ml per cormel. After 2 days, 50, 70, and 100% concentration of each plant extract were sprayed on the cormels. The preventive assay was performed in the same way, except that the plant extracts were applied 3 days before the inoculation of the pathogen. For the fungicide mancozeb, 10 ml was measured from a 2% concentration and applied on the freshly cut surface of healthy cocoyam cormels, 3 days after and before the application of the pathogen, to constitute the curative and preventive assays, respectively. In the case of the biocontrol agent, 5 mm disk of a 6-day-old pure culture of *S. rolfsii* (pathogen) and *T. koningii* (biocontrol agent) was grown separately in PDA broth containing 3.9 g of PDA mixed in 100 ml sterile distilled water for 7 days. Each culture was filtered separately in Whatman filter paper and transferred into 50 ml distilled water containing 10% glucose. The mixtures were properly agitated and 3.75×10^5 spores/cell of *S. rolfsii* was sprayed on freshly cut surface of healthy cocoyam cormels using hemocytometer. Furthermore, 4.9×10^6 spores/ml of *T. koningii* was sprayed on the cormels before and after 3-day following the application of the pathogen to constitute the "curative" and "preventive" methods, respectively. Cormels sprayed with *S. rolfsii* only served as negative control. All the treatments were kept at laboratory temperature for 14 days and replicated thrice.

The severity of the infection was scored on a 5-point scale of 0-4, 14 days after incubation; where: 0 = no infection; 1 = slight infection; 2 = moderate infection (50% of cormel infected); 3 = severe infection (75% of cormel infected); and 4 = complete rot (100% infection),

according to [3]. Furthermore, percentage weight loss of cormel, 14 days after inoculation was calculated as:

% weight loss = (IW-FW/IW) × 100/1

IW = Initial weight before inoculation

FW = Final weight after inoculation.

All data obtained were statistically analyzed using analysis of variance and means separated using Duncan's multiple range test at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1. Disease Severity

The results indicate that the plant extracts, mancozeb (dithane M45) and *T. koningii* had varying significant preventive and curative effects on disease severity and percentage weight loss in cocoyam cormels infected with *S. rolfsii*. As expected, disease severity was highest in the negative control compared to other treatments (Fig. 1). The application of *T. koningii* as a preventive measure significantly reduced disease severity. It also had the same effect when applied as curative measure, but this was not significantly different from other methods of control (Fig. 1). A higher disease severity was observed in *C. papaya* at 50% concentration comparable to the control. The result further shows that the plant extracts and the fungicide were more effective as curative, while *T. koningii* was more effective in the preventive method. The differential activities of preventive and curative effects of plant extracts, essential plant oils, and fungicides on plant pathogens have been reported [14]. Furthermore, the activities of plant extracts and *T. koningii* as both protective and curative biocontrol agent have been reported by other workers [20,21]. [14] reported the protective effects of neem seed oil and dinocap (fungicide) on *Erysiphe cichoracearum*, the causal pathogen of okra powdery mildew in detached leaf-disk assay and in potted plants in Egypt. The same authors showed that neem seed oil showed a high curative effect and decreased disease severity, improved plant growth and pod yield in okra. The low disease severity recorded in *T. koningii* for this work especially as preventive method, suggests that this antagonist should be applied before the appearance of symptoms under field conditions for effective management of *S. rolfsii* in cocoyam cormels. In general, curative method reduced *S. rolfsii* severity than preventative method in all treatments, but for the biocontrol agent (Fig. 1). The efficacy of *T. koningii* as a biocontrol agent in the management of cocoyam cormel rot caused by *S. rolfsii* was <25% of the cormel showed signs of rot. Furthermore, *A. indica* (100% concentration) had disease severity score of 2, while severity score for *C. papaya* and dithane M-45 was 3, in each case (Plate 1). Thus, *T. koningii* reduced cocoyam rot compared to the other treatments. The metabolites produced by *T. koningii* with antagonistic property against plant pathogens could serve as alternative method to the popular fungicides used in the control of cocoyam diseases.

3.2. Weight loss Assessment

The effect of the various treatments applied either as curative or preventive method on percentage weight loss in cocoyam cormel rot is shown in Fig. 2. The inoculated control had the highest (46.51%) percentage weight loss of in both methods. Aside the negative control and *C. odorata* at 100% concentration, weight loss was lower in the curative than preventive method assays. The application of *C. odorata* at 50% and *A. indica* at 100% concentrations as curative method resulted to significant reduction in cormels weight, while *C. odorata* at 100% concentration and the negative control gave the highest percentage weight loss. There were no significant differences in the other treatments (Fig. 2). However, this was completely different from the preventive method where no significant difference was observed in all the treatments including the control. However, *C. odorata* at 70% concentration gave the highest (49.61) percentage weight loss. This was followed by *C. papaya* at 50% concentration with 47.85% and the least (23.77%) was *A. indica* at 70% concentration (Fig. 2). Therefore, the curative method revealed the effects of the

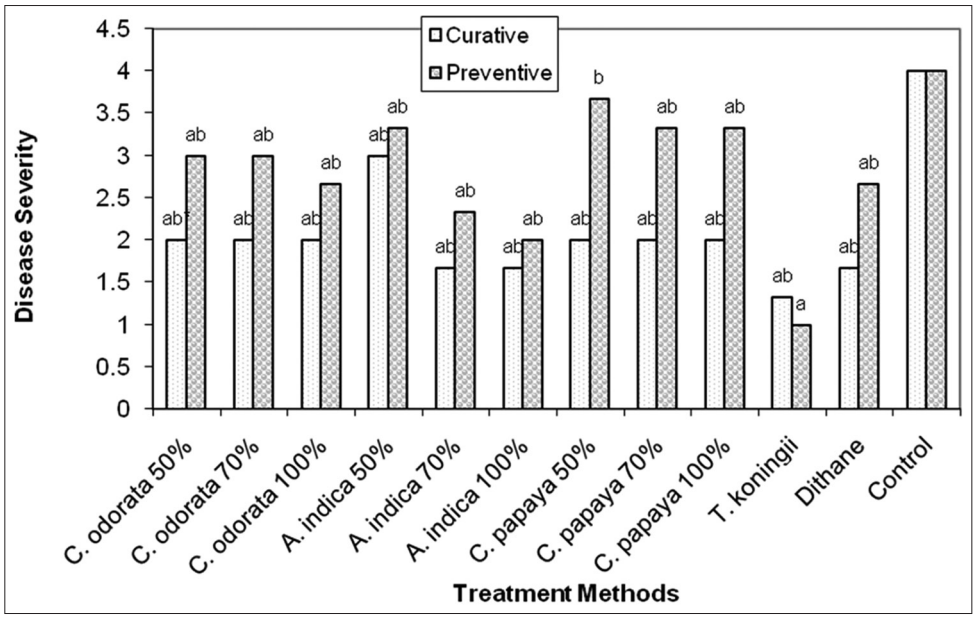

Fig. 1: Curative and preventive effects of leaf extracts of *Chromolaena odorata*, *Azadirachta indica*, *Carica papaya*, *Trichoderma koningii*, and dithane M45 on rot development in cocoyam cormels after 14 days incubation. *Mean values per treatment followed by the same letter are not significantly different ($P \leq 0.05$).

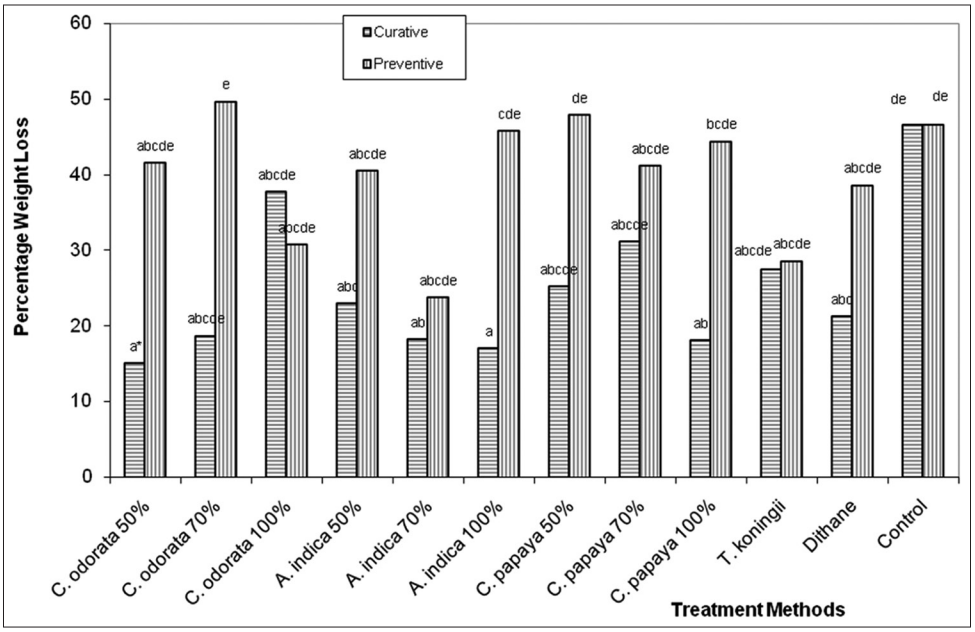

Fig. 2: Effects of curative and preventive treatments on percentage weight loss of cocoyam cormels after 14 days incubation. *Mean values per treatment followed by the same letter are not significantly different ($P \leq 0.05$).

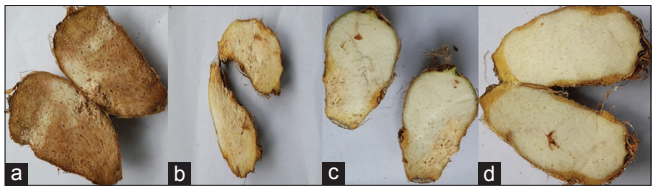

Plate 1: Cocoyam cormel showing different stages of rot. (a) 100% rot (4), (b) 75% rot (3), (c) 50% rot (2), (d) 25% rot (1).

different management methods on the weight of cormels affected by rot [22]. Reported that *Cassia alata* reduced weight loss and rot in cocoyam cormels in Enugu State, Nigeria. The significant reduction in percentage weight recorded in *A. indica* indicates that the plant possesses some positive bioactivities on rot causing organisms in cocoyam. Similar results on the reduction of weight loss using ashes from the bark of kola nut tree, neem tree, and inflorescence of oil palm have been reported [23].

4. CONCLUSION

Our study confirms that *C. papaya, C. odorata, A. indica,* and *T. koningii* possess potential inhibitory effect on the mycelia growth of *S. rolfsii*, the causal agent of cocoyam cormel rot. However, cormels treated with *T. koningii* gave the best performance in rot reduction. Therefore, *C. papaya, C. odorata, A. indica,* and *T. koningii* can be used to control *S. rolfsii.* Their effectiveness could be incorporated to form an integrated disease management strategy to control this pathogen. This will also reduce the risk of pesticide residues build-up and pathogens resistance to fungicides.

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