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COMPARATIVE STUDIES OF TWO BIOPESTICIDAL CONTROL TACTICS AGAINST *Pythium myriotylum* CAUSATIVE AGENT OF COCOYAM ROOT ROT DISEASE IN UMUDIKE, SOUTHEASTERN NIGERIA.

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ABSTRACT

Two biopesticidal disease management tactics namely: Use of plant extract and *Trichoderma harzianum* against *Pythium myriotylum* incitant of root rot disease of Cocoyam in Umuahia, Southeastern Nigeria were investigated and compared. Ethanol extractions of leaves of *Azadirachta indica* (neem), *Hyptis suaveolens* (bush tea), and *Carica papaya* (pawpaw), rhizomes of *Zingiber officinale* (ginger) and seeds of *Garcina kola* (bitter cola) gave significant 5% probability level increase in the radial mycelia growth inhibition of the test pathogen. The rhizomes of ginger gave the highest value of 90.33% at the extract concentration of 40% as compared to paw paw leaves extract that gave the lowest value of 25% . In the poisoned food agar test, at 100% concentration of crude filtrate of 20 day old *Trichoderma harzianum*, percentage mycelial growth inhibition of *Pythium myriotylum* 4 day after incubation was 65%. Both tactics exhibited sufficient antifungal efficacy, but botanicals were higher. By this, Both biopesticides provide a promising replacement for costlier and environmental unfriendly chemical fungicides.

Keywords: *Pythium myriotylum*, cocoyam root rot, Botanicals, *Trichoderma harzianum*, mycelia growth inhibition.

1. INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium (L) Schott)* is a herbaceous, monocotyledonous crop of the family Araceae, cultivated in rural parts of America, the Caribbeans, West Africa and the Pacific (Goenaga and Chardon, 1995,). The edible part-its underground stem (Corms and Cormels) are rich sources of carbohydrate and protein. The protein content is higher in total essential amino acids (5.47%) and sulphur than other staple foods such as yam (4.45%) or cassava (2.84%) (Kay, 1987). Nigeria is the largest producer of Cocoyam in the world with estimated annual production of 5.45million metric tons (FAO, 2007). However, Cocoyam production dropped from year 2000 due to several field and storage diseases (FAO, 2007), but poor knowledge of proper management practices has further made the situation more critical (Goenaga and Chardon, 1995; Ekanayake *et al.*, 1998).The most important fungal disease of Cocoyam is the cocoyam root rot disease caused by *Pythium myriotylum* (Pacumbaba *et al.*, 1992, Wokocha and Aduo 2012). The disease is characterized by premature leaf yellowing, reduced leaf size and number, root decay and pruning and total plant death (Tambong *et al.*, 1999, Wokocha and Aduo 2011). *Pythium myriotylum* is mainly an oomycete soil saprophyte with a fairly wide host range resulting in diseases such as pre and post emergence damping off of seedlings, root rot or wilting (Farr *et al.*, 2007). Like most soil borne fungal pathogens, the management of *Pythium myriotylum* incited Cocoyam root rot disease in difficult using chemical control methods (Nzietchueng, 1983), breeding of commonly acceptable disease resistant cultivars has not been possible (Wokocha and Aduo 2012). The use of biological control agent and methods (biopesticides) as alternative of disease management in isolation or as a component of integrated disease management package is being investigated. Biopesticides also known as

biological pesticides are pesticides derived from natural materials such as animals, plants or microorganism (Leahy *et al.,* 2014). Typically biopesticides have unique modes of action and are considered reduced risk pesticides and are grouped into three classes:- biochemical pesticides, microbial pesticides and plant incorporated protectants. The use of *Trichodema spp* as biocontrol agents in both axenic and field soil systems on a wide range of plant/pathogen combinations has been demonstrated (Geremia *et al.,* 1993, Harman *et al.*, 2004, Harman and Shoresh 2007). Various workers have exploited the use of natural plant extracts and essential oils to control different foliar, soil borne and postharvest storage diseases of crops (Oluma and Garba 2004, Wokocha and Nwaogu 2008, Amadioha 2012). Natural plant extracts have been suggested as a good alternative to synthetic chemicals due to their rich source of bioactive chemicals that are selectively against specific target species and gradually biodegrade to nontoxic products. The aim of this investigation was to compare the toxicities of common medicinal plant extracts with filtrates of antagonist *Trichoderma harzianum*, against *Pythium myriotylum* expressed in mycelium growth inhibition.

2.0 MATERIALS AND METHODS

2.1 Sources of plant materials and preparation of plant extracts

Plant materials used in this study included leaves of *Azadirchta indica A. Juss* (neem), *Hyplis suaveolens Poir* (Bush tea bush) and *Carica papaya L.* (Pawpaw); seeds of *Garcina* kola Heckle (bitter cola) and rhizomes of *Zingiber officinales Rosce* (ginger). The bitter cola seeds and ginger rhizomes were obtained from the Umuahia main market while the other materials where collected from the Michael Okpara University of Agriculture, Umudike premises and identified by Prof. Ekeleme (Weed Scientist). The various plant materials were separately washed under tap water and disinfected by immersing in 0.1% sodium hypochlorite solution for 30 seconds, washed again in three changes of sterile distilled water. They were cut into small pieces and air-dried before grinding using a portable mechanical grinder (Thomas Wiley Mill model ED-5) according to Wokocha and Okereke (2005). For extraction using 70% ethanol (solvent), each weighed out sample of the plant material (5g, 10g, 20g, 30g and 40g) was soaked separately in 100ml of the solvent in 250ml conical flash. The flasks were intermittently shaken and allowed to stand for 6 hours at room temperature (28 ± 2^0C). The contents of each flask were filtered off separately into clean 250ml beakers through fourfold cheese cloth. The filtrate represented ethanol extract concentration of 5%, 10%, 20%, 30% and 40% and stored briefly in the refrigerator at 10^oC before use.

2.2 Fungal Isolates

The virulent isolate of the Cocoyam root rot fungus-*P. myriotylum* was collected from the infected roots of the corresponding diseased host plant in the production fields in Umudike. Infected root sections were surface sterilized with 1% commercial bleached (sodium hypochlorite solution for 1 minute, rinsed in three changes of distilled water and dried between sterile filter papers before been plated on fresh Potato dextrose agar (PDA) in 9.00cm petri dish. The antagonist organism- *T. harzianum*, was isolated from the rhizosphere soil of the infected Cocoyam plants in the field according to Wokocha and Aduo (2012). A ten-fold serial dilution of 1 gram of rhizosphere soil was used. 1 milliliter of the suspension was inoculated on fresh PDA plate and incubation was at 28 ± 2^oC for five days.The obtained fungal isolates were earlier identified according to their unique morphological and cultural characteristics as described in Barnett and Hunters (1987). Pure cultures of *Pythium myriotylum* and *Trichoderma harzianum* were then maintained on Potato Dextrose Agar (PDA) medium at 10^0C for further studies.

2.3 Effect of different concentrations Of Ethanol extracts of five plant materials on the mycelia growth of *Pythium myriotylum*.

One milliliter volume of each extract at 5%, 10%, 20%, 30% and 40% concentrations was pipetted separately and asceptically into 20ml of cool molten potato dextrose agar medium inside 9.00cm petri dish. Each medium was gently swirled in order to achieve uniform dispersion of the extract, then allowed to solidify for 1hour. A 5mm disc of *P. myriotylum* was taken from the advancing edges of 5 days old culture of the fungus was asceptically placed in the centre of each petri dish. Mycelia growth was determined by measuring linear growth of the fungus daily

along three diameters with a transparent rulers plant extract free PDA plates similarly inoculated at 28±2^{0}C for 10days. Each treatment had three replicates and the experiment was laid out in a Completely Randomized Design (CRD). The percentage inhibition of mycelia growth was calculated by using the formular of Pandey *et al.*, (1982) as follows:

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where dc = mean diameter of fungal colony in control plate,

dt = mean diameter of fungal colony in treated plate.

2.4 The poisoned food agar technique using crude *Trichoderma harzianum* metabolites.

The method of Eziashi *et al.,* (2007) was modified and adopted. Five millimeter of mycelia plugs of 5 days old pure culture of *T. harzianum* was asceptically transferred into each of the sterile one litre Erlenmeyer conical flask containing sterilized 500ml potato dextrose broth medium. The flasks were covered with sterile non absorbent cotton wool and the inoculated medium incubated at room temperature 28±2^{0}C for 20 days. The culture filtrates were harvested by filtering twice through Whatman No. 1 filler paper. The culture filtrates of *T. harzianum* were carefully diluted with sterile distilled water to give concentrations of 20%, 50%, 70% and 100% (v/v) solutions respectively. Five milliliters volume of culture filtrates of each concentration were placed in sterile petri dish into which 20ml of cool molten PDA was poured. The dish was gently swirled to achieve even dispersion. After the agar had solidified for 2 hours mycelial disc of *P. myriotylum* (5mm diameter) was placed in the centre of the agar plates and incubated for 10days at 28±2^{0}C. The control experiment consisted of 5mm mycelia disc on plain potato dextrose agar on which was added 5ml of sterile water. The percentage inhibition in the radial mycelia colony growth formulars of Pandy *et al.,* (1982) was used and calculated.

2.5 Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using Genstat Discovery Edition 3 2013. Means were separated using Fischers Least Significant Difference (LSD) according to Wokocha and Aduo (2012)

3. RESULTS

Extracts of all the 5 plant materials exhibited varying degrees of mycelial growth inhibition. There was a significant ($P \leq 0.05$) difference in their yield results against *P. myriotylum* (fig. 1). Rhizomes of *Z. officinales* gave the highest value of 90.33% at 40% concentration and the least value of 3.67% mycelial growth inhibition with *C.papaya* at the lowest concentration value of 5%. There was a serial increase in mycelia concentration among the plant materials for example; ginger with increase in concentration from 5% to 40%, percentage mycelial growth inhibition increased from 32.67 - 90.33% or 3.67 – 24.53% percentage inhibition respectively in pawpaw. However, among the different plant materials only ginger, bitter cola and neem yielded up to 50% and above. percentage inhibition at 30 and 40% extract concentration. Pawpaw and Bush tea yielded far less below 50% mycelial growth inhibition at highest concentration having 24.53 and 31.73% respectively. On the other hand, the 20 day old *T. harzianum* culture filtrate effect on *P. myriotylum* showed the highest percentage inhibition of radial mycelial growth of 66.5% occurred at 100% filtrate concentration 4 days after incubation (table 1). There was also a serial increase in the inhibitory activity with increase in filtrate concentration for example at 100% growth inhibition of 66.5% decreased to 56.6% and 50.3% at 70 and 50% culture filtrate concentrations respectively but a serial decrease in the period (age) of incubation. The highest concentration of culture filtrate of 100% after 4 days of incubation gave the optimum growth reduction of 66.50% until there was a steady drop till 48% after 10 days incubation. Filtrate concentrations of 70 – 100% recorded up to 50% and above growth inhibition after 7 days of incubation, 9 days with 100% filtrate concentration but only after 4 days with 50% concentration.

4. DISCUSSION

The difference in the inhibitory effect of the various extracts could be due to the qualitative and quantitative differences in the antifungal principles present in the materials. Wongkaew and Sinsiri (2014) observed the inhibition efficacy of most plant extracts are probably due to the presence of active constituents such as flavonoids, alkaloids, tannins, terpenes and some other phenolic compounds Raina *et al* (2005) identified oleoresin: the essential oil in ginger rhizome containing Zingiberone and Zingerone as active ingredients that act against microorganisms and pests. Comparing the efficacy of crude plant extracts of ringworm cassia (*Cassia alata L.)* and turmeric (*Curcuma Longa L.)* against important plant pathogenic fungi, Wongkaew and Sinsiri (2014) noted *C. longa* were significantly effective in comparison to commercial fungicide like copper oxychloride and mancozeb. The efficacy of *C.alata* was far less effective against mycelia growth of *Altenaria alternata, Colletotrichum gloeosporides, Fusarium oxysporium fsp lycopersici, Phytophthora infestans and Pythium spp.* The inhibitory activity of *T. harzianum* filtrates could be due to the production of some volatile and nonvolatile metabolites (EL-Kattatny *et al.,* 2001). The decreasing effects of the older cultures could be due to the degradation of the metabolite caused by the activities of the fungus, and increase in stalling materials in the growth medium with time. Growth inhibition of fungal pathogens by *Trichoderma* metabolites has been reported by several workers (Claydon *et al.,* 1987; Huang *et al.,* 1995; Sivan *et al.,* 1984). Rahman *et al.,* (2009) in a comparative assessment of various *Trichoderma* isolates against the pathogen *Ceratocystis paradoxa* causing pineapple disease of sugarcane observed the *T. harzianum* yielded the highest percentage inhibition radial growth value of 75 – 84% at 80% concentration on the 4th day. Eziashi *et al.,* (2007) reported that *C.paradoxa* mycelia growth was inhibited at high concentration of 70% and 100% of metabolites by *T.polysporus* and *T.viride* respectively. From these investigations, plant extracts have shown sufficient antifungal efficacy coupled with their ease of preparation. By this, they provide a promising opportunity for a replacement of chemical fungicide. The antagonist fungus- *T. harzianum* metabolite also yielded a marked inhibitory effect though less than the plant extract. However with their ease of application of both biocontrol tactic-soil drenching for the plant extracts and the possibility of incorporation of *T. harzianum* with organic matter as single operation or as a component of integrated disease management program, these would likely address the devastating impact of cocoyam root rot disease in cocoyam production. *Trichoderma spp* have been found to induce systemic resistance as demonstrated in both axenic and field soil systems on a wide range of plant/pathogen combinations (Harman *et al.,* 2004, Yedidia *et al.,* 2003).

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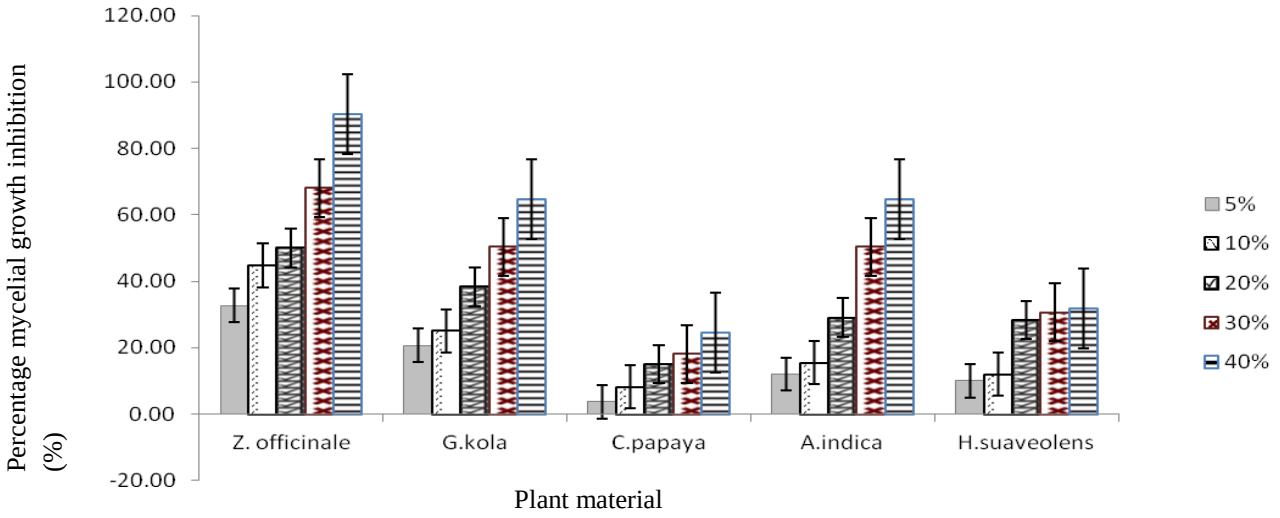

Figure 1: Effect of different concentrations of crude ethanol extract of five plant materials on the radial mycelial growth of *Pythium myriotylum* incubated on PDA for 7 days at 28±2^{o}C.

Table 1: Effects of different concentrations and ages of *Trichoderma harzianum* on the mycelial growth of *Pythium myriotylum* in culture (Temperature - 28 ± 2°C).

Concentration of Culture filtrate (%)	**Percentage inhibition of mycelial growth (%) / incubation (days)**						
	4	**5**	**6**	**7**	**8**	**9**	**10**
100	66.50	63.30	60.1	54.55	52.28	50.30	48.63
70	56.57	54.33	52.17	50.20	48.50	44.13	40.23
50	50.25	48.67	47.36	44.77	42.79	40.23	38.49
20	34.33	32.20	28.20	26.10	24.30	20.70	15.80
Mean	51.86	49.63	46.96	43.91	41.97	38.84	35.73
LSD ($_{0.05}$)	0.506	0.348	0.362	0.276	0.277	0.348	0.333

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