Journal of Biopesticides and Environment
ISSN: 2449-1284 (Online); 2449-1292 (Print)
http://www.biopjournal.com

ASSESSMENT OF THE INHIBITORY EFFECTS OF FOUR ANTAGONISTS FROM COCOA SOILS AGAINST THREE FUNGAL COCOA PODS ISOLATES IN ABIA STATE, NIGERIA.

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ABSTRACT

Four antagonists, *Trichoderma harzianium*, *Aspergillus niger, Aspergillus flavus* and *Rhizopus stolonifer* isolated from cocoa plantation soils in Abia State were evaluated for inhibitory actions against three fungal pathogens from cocoa pods; *Phytophthora megakarya, Fusarium decemcellulare, Colletotrichum ignotum* in the plant pathology laboratory of the National Root Crops Research Institute, Umudike. The rate of inhibition was evaluated by calculation of percentage reduction of the radial mycelia growth by the antagonists. The result showed that all the antagonists were able to cause significant ($P \leq 0.05$) reduction in the radial mycelial growth of the fungi. *Trichoderma. harzianium* were the most suppressive against the tested fungi on PDA media at 28 ± 2^0C. Spores of *T. harzianium, A. niger* were found exclusively growing over the colonies of the test organisms. Also interacting fungal mycelia mutually inhibited each other with more overgrowing the colony of the other, with a narrow zone of inhibition of about 0.5 – 1.5mm wide formed between the colonies. Two of the organisms tested *F. decemcellulare* and *C. ignotum* showed this reaction. Similarly, mycelia of *R. stolonifer* and *T. harzianium* significantly also overgrew the colony of *P. megakarya.*

Keywords: Antagonists, cocoa, soils, fungal isolates, *radial* mycelial growth, PDA media

1. INTRODUCTION

In Nigeria, agriculture plays a strategic and important role in reviving the national economy and cocoa (*Theobroma cacao* L.) remains one of the major export crops and a non-oil foreign exchange earner in Nigeria (ICCO, 2009), with a world production of about 43% (ICCO, 2000). One of the major constraints responsible for low yield in cocoa production in most cocoa growing- countries is the severity of insects pest and pathogens especially mirids and fungal pathogen such as *Phytophthora megakarya* and *P. palmivora*. *Phytophthora megakarya* which incites black pod disease of cocoa is a soil borne pathogen and mainly attacks the cocoa pods (Mpika *et al.,* 2009). A large number of microorganisms such as fungi, bacteria, algae etc co-exist in the rhizosphere, and they differ from one another, taxominically, physiologically and in some other respects, in the ability of attacking plants (Saharan and Nehra, 2011).

Often no single factor could result to disease infection but, rather other associated organisms with favourable environmental conditions and the host. Biological control of plant diseases has proven to be durable in its effects and has the advantage of not requiring repeated periodic applications as is the case with chemical pesticides. It is thus, potentially better suited for use particularly in developing economies (Okigbo and Ikediugwu, 2000). However, biological control does provide an attractive and environmentally friendly option to numerous studies of plant disease. (Mpika *et al.,* 2008) have examined biological control of *Phytophthora megakarya* and *P. palmivora* in cocoa, using microbial antagonists such as *Bacillus spp., Aspergillus tamari, Aspergillus gigentus, Botryodiplodia theobromae, Pencillium purperescens* and *Pseudomonas fluorescens,* with some success *Trichoderma spp, Pseudomonas spp, Aspergillus spp, Bacillus spp* etc in the soil are widespread throughout the world and have been recognized as the most successful biocontrol agents for soil borne pathogens (Galindo, 1992). Several modes of action have been described, including

competition for nutrients, antibiosis, induced resistance, mycoparositism, plant growth promotion and rhizosphere colonization capability (Hassanein *et al.,* 2006; Siddiqui and Aklitar, 2007; and Bailey *et al.,* 2008).

The objective of the study therefore, was to assess the in-vitro control of three fungal isolates from cocoa pods by four antagonist isolated from cocoa soils, on fresh PDA media.

2. MATERIALS AND METHODS

Soil samples were collected from cocoa plantation soils in Abia State within the cocoa growing communities. Surface litter was first cleared and soil randomly collected with a trowel from an area of 0 – 15cm depth within the plantations. Samples from each community were composited, thoroughly mixed and brought back to the laboratory in separate clean polyethylene bags. Each soil samples was air-dried by thinly spreading it out on a laboratory bench at room temperature of 27^0C – 29^0C for 5 days, and sieved (2mm). Twenty-five grams of each soil sample was placed in graduated cylinder and sterile distilled water was added to make up a total volume of 250mls. Each of the suspensions formed was poured in 1 litre flasks and stirred before shaking mechanically for 30 minutes (i.e. poured plate methods, Ikotun and Adekunle, 1995). 1ml of dilutions were plated on PDA media and then replicated three times. The Petri plates were incubated for 48hrs at 28 ± 2^0C after which they were observed for patterns of growth of microorganisms that were present. To isolate antagonistic fungi, the incubated plates of the soil dilution were observed for 3 – 4 days after which the cultures were populated mainly by fungal species. The antagonists were isolated, sub-cultured until pure cultures were obtained. Pure cultures were properly identified using I.M.I (International Mycological Institute) monographs of the description of fungal pathogens, as well as Barnett and Hunter (1999) on description of illustrated genera of imperfect fungi (5^{th} edition) these were all used after viewing them on light microscope.

The pairing of *P. megakarya*, with *T. harzianium, A. niger, A. flavus* was carried out on Petri dishes containing molten PDA media. A segment of 5mm disc of the mycelium was taken around the edge of the cultures of each fungus. These, segments (mycelia disc of *P. megakarya – T. harzianium, A. niger, A. flavus, R. stolonifer), F. decemcellulare (T. harzianium, A. niger, A. flavus, R. stolonifer), C. ignotum (T. harzianium, A. niger, A. flavus, R. stolonifer),* were placed 2cm apart on the Petri dishes containing the PDA media. The control treatment was 5mm disc of the pure cultures plated on Petri plates without the bioagents. The incubation was done at 28 ± 2^0C and the treatment replicated three times on a Completely Randomized Design (CRD) and analysis was carried out using Gen-Stat Discovery Edition 3.

3. RESULTS

The result showed that the four antagonists (*T. harzianium, A. niger, A. flavus, R. stolonifer*) assessed for their inhibitory action against the three test fungi were suppressive on PDA media at 28 ± 2^0C. Spores of *T. harzianium, A. niger* were formed exclusively over the colonies of the test organisms. *Trichoderma harzianium* isolate was the most suppressive bioagent followed by *A. niger* and *R. stolonifer* which were less suppressive. A narrow zone of inhibition of about 0.5 – 1.5cm wide was formed between the colonies and this reaction was showed mostly by *F. decemcellulare* and *C. ignotum*. Similarly, the result showed that all the antagonists used were able to cause significant reduction in the radial mycelia growth of the tested fungi and *T. harzianium* recorded the highest radial mycelia growth. Mycelia of *R. stolonifer* and *T. harzianium* significantly ($P \leq 0.05$) overgrew the colony of *P. megakarya* and accounts for more than 50% of the rhizosphere fungi grown on PDA media at 28 ± 2^0C.

4. DISCUSSION

All the antagonists (*T. harzianium, A. niger, A. flavus* and *R. stolonifer*) studied *in-vitro* were found to be suppressive to the test fungi, based on the reduction in growth area of the pathogens. *Trichoderma harianium* significantly ($P\geq0.05$) differed, and effectively inhibited the mycelial growth of the test fungi

when paired together on PDA media. Similarly, spores of *T. harzianium* and *A. niger* grew over the colony of *F. solani, C. ignotum* five days after inoculation. Also mycelium of *R. stolonifer* and *T. harzianium* were noted to be more antagonistic against the three test fungi when compared to *A. flavus*.

This result confirmed the report by Harman (2006) who stated that *Trichoderma* strains are antagonistic to some phytopathogenic fungi due to its ability to suppress the diseases they cause. Shoresh, *et al.,* (2010) also observed that Trichoderma also uses several biocontrol mechanisms such as mycoparasitism, antibiosis, and competition for space and nutrients and also is able to promote growth. Benitez, *et al.,* (2004), Wokocha and Okereke (2003) further stressed that *Trichoderma* has the ability to produce lytic enzymes that can act in a synergistic way thereby increasing its antagonistic action. According to Howell, (2003), Okigbo and Ikediegwu (2000) the fungistatic activity observed in *Trichoderma* and observed that the pathogen has the ability to colonize and compete for nutrients on culture media. Antibiotic and proteins by *T. harzianium* have been described as a complex of several cryptic species (Chaverri, *et al.,* 2003). Oyeyiola and Husseni (1992) reported that *A.niger* has a high sporulating nature and this coupled with their ability to grow well on laboratory media.

Furthermore, *Trichoderma spp, Pseudomonas spp, Aspergillus spp, Baccillus spp* etc according to Bailey, *et al.* (2008) have been recognized as the most successful biocontrol agents for soil pathogens. Similarly, *R. stolonifer* also showed a significant difference in reducing the mycelial growth rate of *F. decemcellulare* which was slightly lower than *A. flavus* when compared to *P. megakarya* which had the highest radial growth. According to Okigbo and Ikediugwu (2000), biological control of plant diseases has proved to be durable in its effect and, has the advantage of not requiring repeated periodic applications as is the case, with chemical pesticides. It is thus potentially better suited for use particularly in developing countries. Adbel-Monaim (2008) reported that *T. harzianum, A. sp, Penicillium sp*. were found to be antagonistic to some pathogenic fungi, based on the reduction in growth area of pathogens.

CONCLUSION

The assessment of the inhibitory effect of the antagonists therefore showed that all the antagonists were suppressive to the test fungi, based on the reduction in growth area of the pathogen on PDA media at 28±2^{0}C. *T. harzianium* and *A. niger* significantly ($P<0.05$) inhibited the radial mycelia growth of the three test fungi when compared to *R. stolonifer* and *A. flavus* on *F. decemcellulare* and *C. ignotum*. In summary *T. harzianium* has shown to be the most effective bioagent in the control of *P. megakarya, F. decemcellulare* and *C. ignotum* isolated from cocoa pods.

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ACKNOWLEDGEMENTS

We are grateful to the Executive Director of the NRCRI, Umuahia for his permission to use the Plant Pathology Lab of the Institute and to Dr. C. Onyeka for his assistance during the course of this laboratory work. We also appreciate the entire staff of Farm Unit of the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike for allowing us to use the screen house.

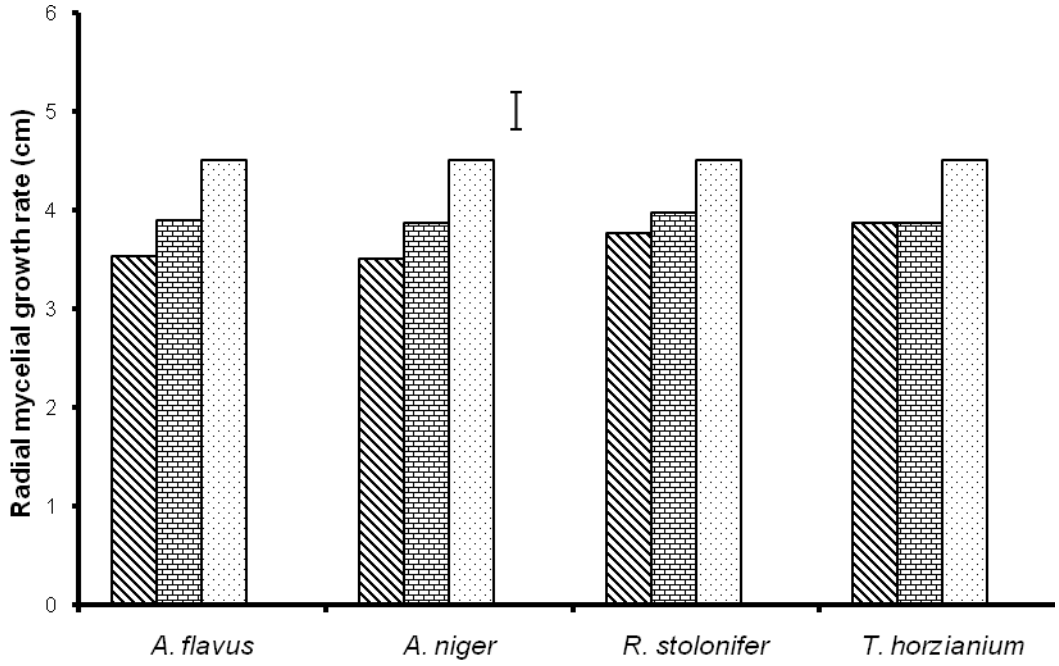

Fig. 4.4: Effects of different antagonists on three fungal isolates (*F. decemcelluare, C. ignotum* and *P. megakarya* from cocoa pods 7days after inoculation

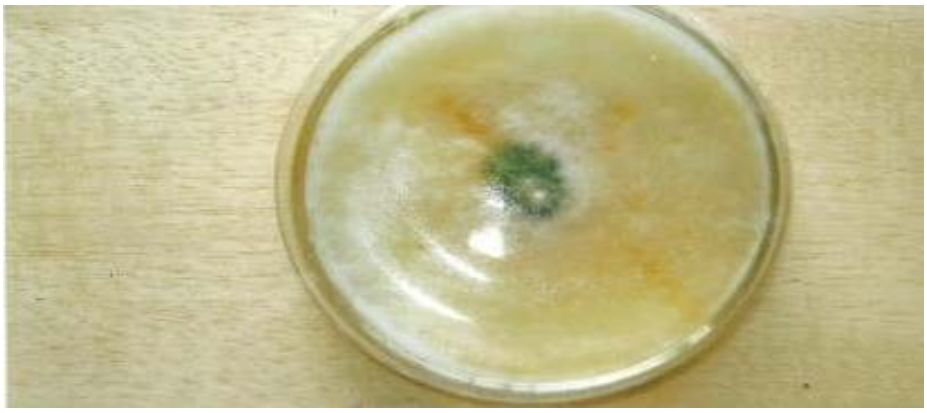

Plate 1a: *P. megakarya* reinoculated with *T. harzianium* (Mag x ½)

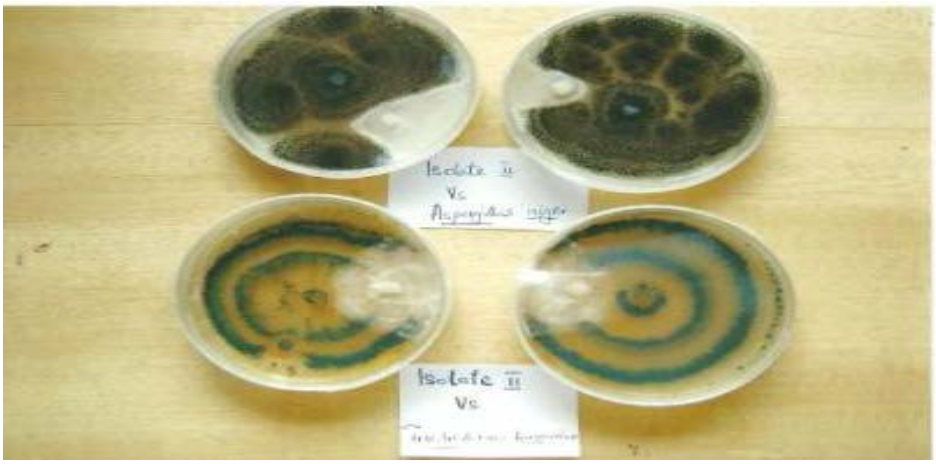

Plate 2a: Biocontrol (*C. ignotum* + *A. flavus*)
b: Biocontrol (*C. ignotum* + *T. harzianium*) 5days after inoculation at 28±2^{0}C Mag: (X 3).

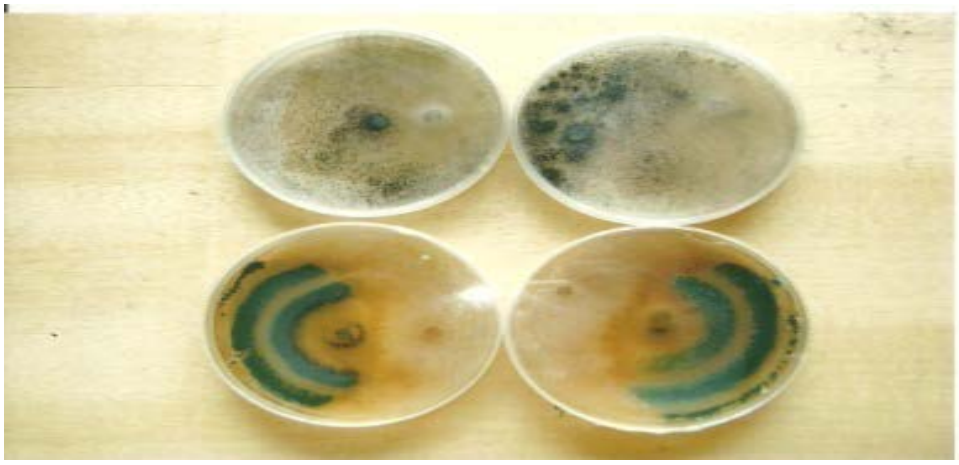

Plate 2a: Biocontrol (*P. megakarya* + *A. niger*)
b: Biocontrol (*P. megakarya* + *T. harzianium*) 5days after inoculation at 28±2^{0}C. Mag: (X3)

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