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Preliminary study on the effects of carbendazim on fungi isolated from crop soil samples obtained from Afe Babalola University and Ado-Ekiti farm

Ekundayo EA[1], Ogunmefun OT[2], Shobanjo TO[2], Anuoluwa IA[1], Oso AO[3], Oluwafemi YD[1] and Akharaiyi FC[4]

[1]Department of Biological Sciences (Microbiology unit), University of Medical Sciences, Ondo, Nigeria
[2]Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Nigeria
[3]Department of Microbiology, Edo University, Uzaire, Nigeria
[4]Department of Chemistry, Ekiti State University, Ado-Ekiti

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Abstract

Fungi were isolated from five different soil samples of 5 crops obtained from Afe Babalola University, Ado – Ekiti farm on Potato Dextrose Agar (PDA) and PDA supplemented with 1% chitin at 25 ± 2°C. Twenty-one (21) fungi were isolated and identified as *Trichoderma viride, T. harzianium, Aspergillus niger, Penicillium digitatum* and *Epicoccum purpurascen.* Four isolates were obtained from pawpaw (*Carica papaya*), five (5) from *Moringa oleifera*, five (5) from plantain (*Musa parasidica*), three (3) from garden egg (*Solanum* spp.) and four (4) from okra (*Abelmoschus esculentus*). The isolates were also screened qualitatively for chitinase on PDA amended with 2% chitin, 0.05% yeast extract and 0.01% congo red. None of the isolates showed any zone of clearance. The effect of a fungicide, carbendazim, at four different concentrations on the rate of growth of the isolates was determined. *Trichoderma viride*, *T. harzianium*, and *Penicillium digitatum* were able to grow on different concentration of carbendazim. However, *A. niger*, *Epicoccum purpurascens* were susceptible to carbendazim. This study shows that *Trichoderma* species were most resistant to the fungicide, and the lower the concentration, the higher the rate of growth. Therefore, *T. viride* and a lower concentration of carbendazim can be used in integrated pest management.

Keyword – Carbendazim – Chitinase – Integrated pest management – Susceptible – *Trichoderma* species

Introduction

Soil serves as a reservoir for many microbial communities of plants and herbs. Microbial composition and functioning change soil quality by decomposition of organic matter, recycling nutrients, and biological control (Stefanis et al. 2013). Fungi are widely distributed in the environment, especially in all types of soil (Boer et al. 2005, Mukherjee et al. 2006) and play an important role as major decomposers in the soil ecosystem (Ng 2004, Seth et al. 2016). They are

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Corresponding Author: Esther A. Ekundayo – email – eekundayo@unimed.edu.ng

one of the dominant groups present in soil, which strongly influence ecosystem structure and functioning and thus playing a key role in many ecological services (Orgiazzi et al. 2012). Fungi benefit most plants by suppressing plant root diseases and promote healthier plants by attacking plants pathogens with fungal enzymes. Fungi also use antagonism to reduce competition by producing antibiotics, suppressing other microorganisms from growing. They produce many vitamins which promote plant growth. However, some fungi act as plant pathogens which cause most of the diseases occurring in agricultural and horticultural setups (Agrios 2009), causing significant economic losses and undesirable characteristics of the plant commodities (Agrios 2005). This, therefore, necessitates the use of fungicides.

Fungicides are chemical agents that inhibit the growth of fungi or fungal spores. They can either be contact, translaminar or systemic and are used both in agriculture and to fight fungal infections in animals (Latijnhouwers et al. 2000). Flucycloxuron and diflubenzuron are used as pesticide to control pests (Rouabhi et al. 2009). Carbendazim is most widely used benzimidazole fungicide in controlling diseases caused by fungi (Kalwasinska et al. 2008) and it is the major degradation product of systemic fungicide such as benomyl (Xu et al. 2006). Fungicides have many side effects on natural non-target organisms. Some fungicides are dangerous to human health, such as vinclozolin, which has now been banned from use (Hrelia et al. 1996). It has been found that the persistent use of fungicides also could weaken the natural antagonistic activity (Lenteren & Woets 1988).

However, some microorganisms including *Trichoderma* have the capability of degrading xenobiotic compounds (Ezzi & Lynch 2005, Zhou et al. 2007, Tang et al. 2009). This present research was conducted to isolate fungi from some soil samples and determine carbendazim's effects on them.

Materials & Methods

Collection of samples

Soil samples were collected from the root of five different plants viz pawpaw, moringa, plantain, garden egg and okra from Afe Babalola University Ado-Ekiti, Ekiti State (ABUAD) farm in 2016. All the samples were collected in small sterile nylon and adequately labelled. Also, fungicide carbendazim was obtained from the same farm.

Isolation, characterization and identification of fungal species

Sevenfold serial dilution was made from all the samples. An aliquot (1mL) of each sample was plated in duplicates on sterile potato dextrose agar and incubated at 25^{o}C for 3 to 5 days using pour plate method. Also, fungal isolates were isolated on PDA was amended with 1% chitin obtained from Sigma Aldrich, USA. Colonies were selected from each plate and purified by subculturing into potato dextrose agar plates and were identified (Fawole & Oso 2001).

Chitinolytic activities of fungal isolates obtained from the samples

Chitinase index of the fungal isolates was assessed by measuring the clear zone produced by degradation of chitin in PDA plates supplemented with 2% chitin, 0.05% yeast extract and 0.01% congo red using the modified methods of (Valadares-Inglis & Azevedo 1997, De Boer et al. 2004). The zone of clearance around the well was measured after four days of incubation.

Effect of carbendazim on the mycelial growth of the fungal isolates

The potato dextrose agar media was prepared and autoclaved at 121^{o}C for 15 minutes. After cooling, various concentrations (0.005, 0.0025, 0.00125 and 0.000625) of fungicides were added. A sterilized needle was used to transfer mycelial mat of the fungus on to the plates and was incubated at 27^{o}C for 3 to 5 days. Fungal cultures without the fungicide served as control (Dhingra & Sinclair 1985).

Results

The fungal species isolated from the different soil samples were 21 and identified as *Penicillium digitatum, Aspergillus niger, Trichoderma harzianium, T. viride* and *Epicoccum purpurascens*. *Penicillium digitatum* was obtained from all the rhizosphere samples except garden egg. Also, *A. niger*. *T. viride* and *T. harzianum* were obtained from all the soil samples while *E. purpurascens* was obtained from only moringa and plantain soil samples. The percentage occurrence is presented in Table 1.

Table 1 Percentage occurrence of fungi obtained from soil samples of some crops.

Rhizosphere samples	**Fungal isolates**				
	Penicillium digitatum	***Asgergillus niger***	***Trichoderma viride***	***Trichoderma harzianum***	***Epicoccum purpurascens***
Pawpaw	+	+	+	+	-
Okra	+	+	+	+	-
Moringa	+	+	+	+	+
Plantain	+	+	+	+	+
Garden egg	-	+	+	+	-
Occurrence (%)	80	100	100	100	20

Key: + = Present; - = Absent

Chitinolytic activities of the isolates

No zone of clearance was observed from the fungal isolates cultured on PDA amended with yeast extract, chitin and congo red.

Effect of carbendazim on the mycelial growth of the fungal isolates

Penicillium digitatum, Aspergillus niger, Trichoderma harzianium isolated from pawpaw soil sample were susceptible to carbendazim at all the concentrations used. However, there was mycelial growth of *Trichoderma viride* at 0.25% and 1.25% of carbendazim from day 3 to 5 (Fig. 1).

Epicoccum purpurascens and *A. niger* obtained from moringa soil showed no growth after 3 to 5 days. Mycelial growth of *Penicillium digitatum* was observed at 0.00125% and 0.000625%. Growth was observed at all concentrations for *T. viride* and *T. harzianium*. The mycelial growth rate ranged from 2.00 to 3.95mm (Fig. 2).

Epicoccum purpurascens, Penicillium digitatum Trichoderma harzianium, Aspergillus niger isolated from plantain soil showed no growth after 3 to 5 days. However, *Trichoderma viride* obtained from the same source was resistant to carbendazim at all concentrations (Fig. 3).

Aspergillus niger and *Penicillium digitatum* obtained from okra soil showed no growth after 3 to 5 days. *Trichoderma harzianium* growth was observed at 0.00125% and 0.000625%. The growth was measured at 3 to 5 days as 2.10 and 3.5cm, respectively. The mycelia growth of *T. viride* growth was observed at all concentrations (Fig. 4).

None of the isolates obtained from garden egg soil was resistant to carbendazim at all concentrations.

Discussion

Fungi are important components of the soil microbiota and play a focal role in nutrient cycling by regulating soil biological activity. In this present study, fungi were isolated from 5 different soil samples. This is a testament to the ubiquity of microorganism. Due to high sporulation capacity, *Aspergillus* and *Penicillium* have been found in most agricultural soils. *Penicillium* spp. have been shown to produce fungal and bacterial antibiotics while *Aspergillus* spp. produce different kinds of toxins such as aflatoxins, achrotoxins etc (Gaddeya et al. 2012). These toxins may prevent the growth of other fungal species. *Trichoderma* spp. are free-living fungi that

are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. They produce or release various compounds that induce localized or systemic resistance responses in plants (Islam et al. 2011).

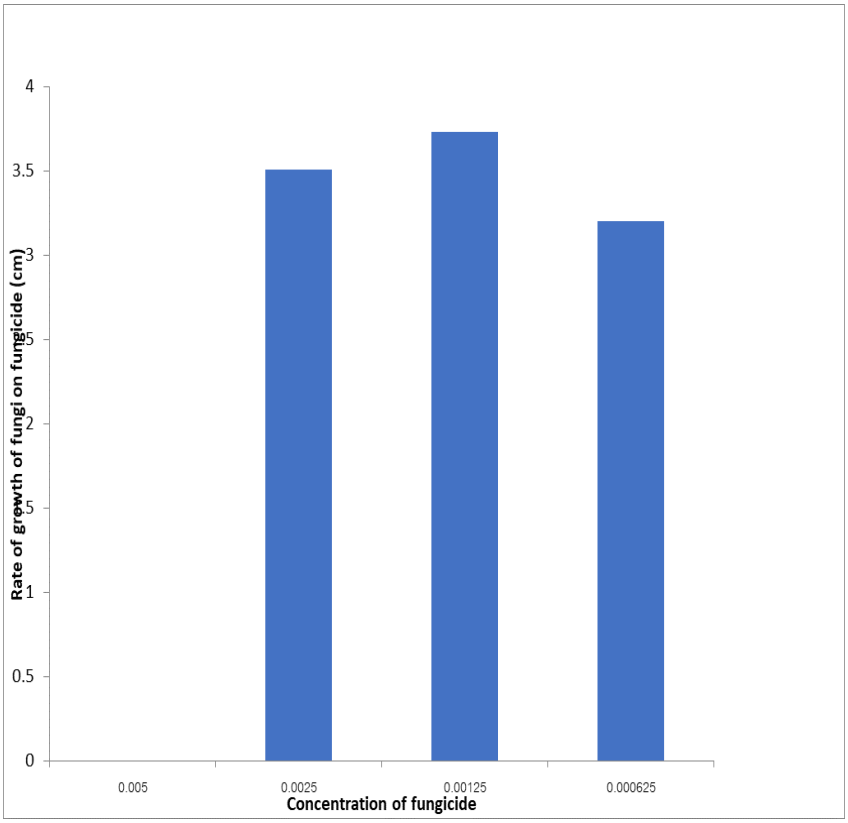

Fig. 1 – Rate of growth of *Trichoderma viride* from pawpaw soil in carbendazim amended medium at 25ºC.

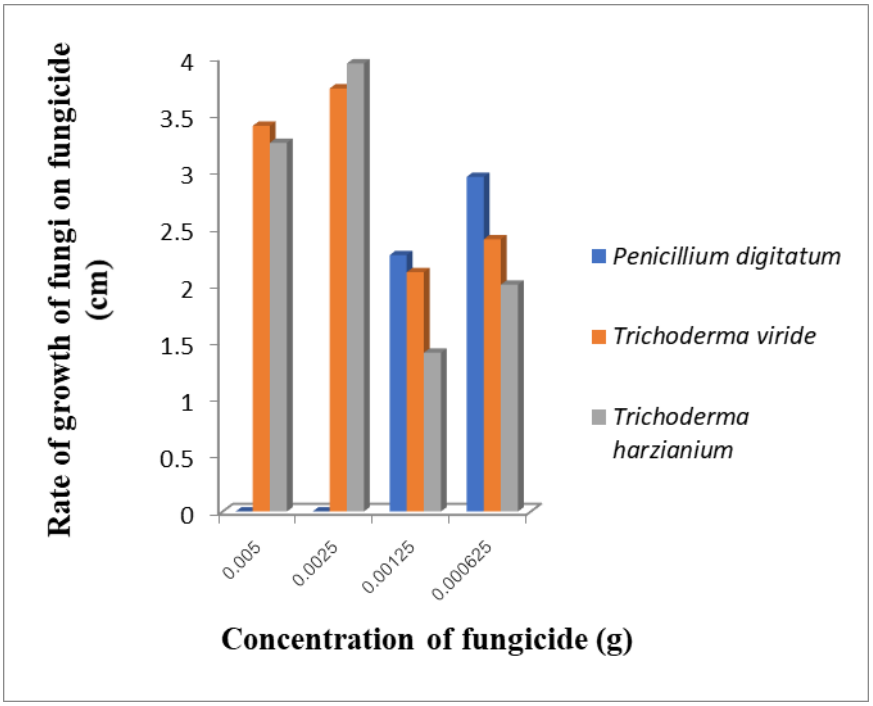

Fig. 2 – Mycelial growth of fungi isolated from moringa soil in carbendazim amended medium at 25ºC.

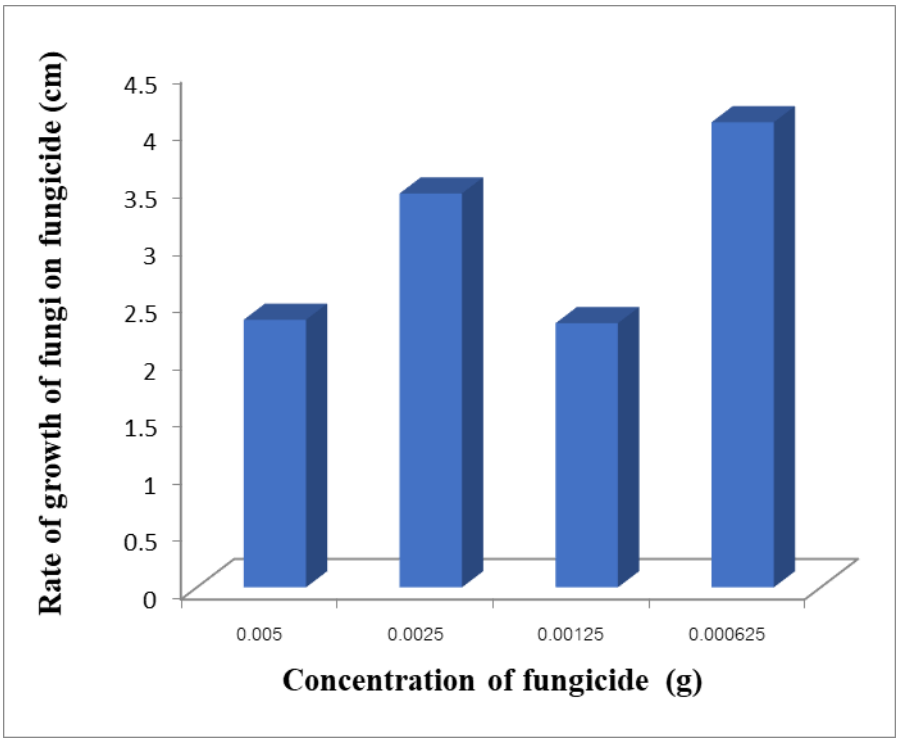

Fig. 3 – Rate of growth of *T. viride* obtained from plantain soil in carbendazim amended medium at 25°C.

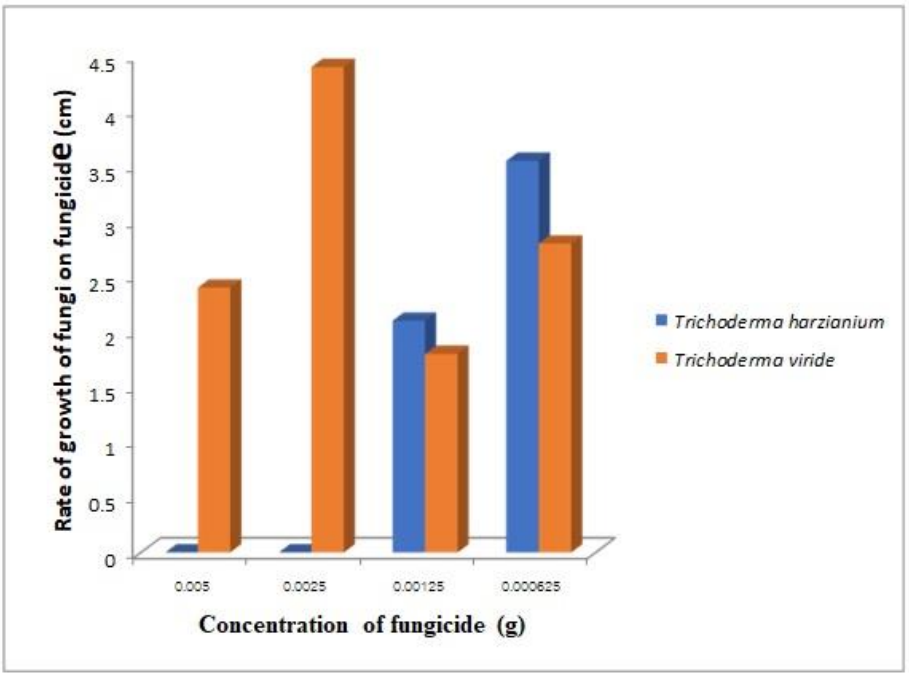

Fig. 4 – Rate of growth of *T. viride* and *T. harzianum* obtained from okra soil in carbendazim amended medium at 25°C

Three fungal species were able to grow on carbendazim amended medium. Most of fungal species that grew on carbendazim amended medium were from *Trichoderma* species. Goldman et al. (1993) and Mukherjee et al. (1999) have successfully obtained *T. viride* and *T. pseudokoningii* strains tolerant to chemical fungicides. The resistance mechanism of some fungi to chemical fungicides is due to genetic mutations, which reduces the susceptibility to the fungicides and decreases their efficacy (Yan & Dickman 1996, Deyle et al. 1997, Yamamoto & Baird 1999).

Fungicide resistance is a stable, inheritable adjustment by a fungus to a fungicide, resulting in reduced sensitivity of the fungus to the fungicide. Resistant isolates are less affected or not inhibited by applying a fungicide (Ma & Michailides 2005). Ruocco et al. (2009) explained that the ability of *Trichoderma* to withstand relatively high concentrations of a variety of synthetic and natural toxic compounds, including its own antibiotics, depends on efficient cell detoxification mechanisms supported by a complex system of membrane pumps. It is well known that the genome of *Trichoderma* includes ABC transporters (ATP binding cassette (ABC) transporters), which are members of a protein superfamily that effluxes drugs from cells of target organisms. Thus, transporters may provide a mechanism of protection against cytotoxic drugs and xenobiotic agents. The natural function of ABC transporters in plant pathogenic fungi may relate to transport of plant-defense compounds or fungal pathogenicity factors (De Waard 1997). The ABC transporters may explain the natural tolerance of fungicides on *Trichoderma*, and their ability to survive in extreme environments successfully.

Aspergillus niger, Epicoccum purpurascens could not grow on carbendazim at all concentrations. This shows that carbendazim could inhibit the synthesis of β-tubulin (Pfeil & Dellarco 2005).

C**onclusion**

This study highlights the effect of fungicide on fungi from five different soil samples. It was found out that three fungal species were resistant to carbendazim. Among these fungal species, *Trichoderma* species were found to be most resistant to the fungicide. This present study recommends that carbendazim could be used to control *Aspergillus niger* provided the fungicide will have no negative effect on non-target organisms. Also, since *Trichoderma* species showed resistance to carbendazim, the two can be used together in integrated pest management scheme.

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