Efficacy of Carbendazim and other Synthetic Fungicides on Taro Leaf Blight Disease caused by *Phythophtora Colocasiae*

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KEYWORDS

Cocoyam leaf blight,
Fungicides,
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ABSTRACT

The results of the serial concentrations of different synthetic fungicides, phytopathogenic fungi and their interactions on isolates from cocoyam had significant effects ($P \leq 0.05$) on the percentage inhibition and mycelia growth. P. colocasiae (12.50 mm) treated with hexaconazole had the lowest mycelia growth rate followed by Botryodiplodia theobromea (21.28 mm) with fludioxonil and carbendazim (24.03 mm) while Rhizopus spp (85.83 mm) and F. solani (78.798 mm) on metaxyl recorded the highest mycelia growth. The effects of different environmental stress, fungi and their interaction on mycelia growth showed significant difference on the organisms, the stress and their interactions. No mycelia growth were seen when B. theobromea were subjected to H_2O_2 (5.00 mm), followed by P.colocasia (14.67 mm).

In conclusion, fungicides found to be effective for the control of cocoyam leaf blight in order of merit were carbendazim, fludioxonil, hexaconazole and mancozeb. Metaxyl recorded no or little effect on the inhibition of mycelia growth of all the four fungi organisms isolated from cocoyam as the organisms have developed resistance to it. It should be used in mixture with other fungicides with different mode of actions for example mancozeb.

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INTRODUCTION

Cocoyams, *Colocasia esculenta* are important perennial food crops cultivated for their edible roots (Owusu-Darko *et al*., 2014). The crop is primarily grown in tropical and sub-tropical countries and is one of the major food crops in Nigeria (Aniekwe, 2015, Omeje *et al*., 2015).

Despite their importance and usage, their production in Nigeria are heavily affected by plant diseases especially fungi including; *Phyllosticta colocasiophila*, *Phytophthora colocasia, Fusarium spp, Phythium spp, Aspergillus niger, Sclerotia rolfsii* and *Botryodiplodia theobromae* (Anukworji *et al*., 2012; Omeje *et al*., 2015) but the most devastating fungus is *P. colocasia* which causes leaf blight. Symptoms are small, brown, water-soaked lesions on the leaves which enlarge and coalesce into large lesions with yellow exudate, defoliation and death.

The infection of taro leaf blight has reduced taro production rate by 30-40%, leading to heavy reduction in quality and quantity of corm and hindering the commercialization of taro corm product in many taro producing countries (Bandyopadhyay *et al*., 2011; Mbong *et al*., 2013). Control measures need to be adopted to reduce the effect of taro leaf blight (TLB) below its economic injury level and hence increase taro production. TLB has been controlled using cultural strategies including field sanitation, roguing, clean planting stocks, and use of resistant varieties (Aggarwal and Mehrotra, 1987; Nelson *et al*., 2011) such as *Trichoderma* species, *Rhizobacterium* species) (Chukwu and Enyiukwu, 2021). However, due to the drastic reduction in yield of taro, the use of fungicide is justified to ensure quick response.

Fungal infection has been worldwide estimated to reduce yield of crops to 20% if not treated, thus use of fungicides has become wide spread recently in Agriculture (Rohr *et al*., 2017). Fungicide controls the fungal infection during the establishment and growth of a crop, it enhances the productivity of crop and decrease defects (Bandyopadhyay *et al*., 2011). Some fungicides that can be used to control taro leaf blight disease includes single or compound synthetic fungicides such as mancozeb, diflolatan, and ridomil (metalaxyl) or copper (Aggarwal and Mehrotra, 1987; Cox and Kasimani, 1988).

To improve the efficiency of production, this fungus must be controlled and managed through the use of some synthetic fungicide benzimidazole.

Benzimidazoles is a group of fungicides commercially available as benomyl, carbendazim (MBC), thiophanate-methyl, thiabendazole and fuberidazol, known for their broad-spectrum activities against several fungal pathogens namely ascomycetes, some basidiomycetes and deuteromycetes (Leadbeater, 2014). These fungicides can be applied to cereals, fruits, vegetables and vines and also used in postharvest handling of crops (Duan *et al*., 2014; Y. Duan et al., 2019; Oliver and Hewitt, 2014) against *Cercospora spp*., *F. spp*, *Botrytis cinerea Colletochrichum spp*., powdery mildew, *Erysiphe spp* and *Oidium spp* crops (Duan *et al*., 2019; Oliver and Hewitt, 2014). Carbendazim is the most common and widely used to manage crop diseases in cereals, roots and fruits (Zhou *et al*., 2016). The combination of carbendazim and diethofencarb is a good control measure against *B. cinerea* although the appearance and spread of the two fungicides caused problems (Leroux and Fritz, 1984).

Benzimidazoles interrupt pathogen energy metabolism through their selective bindings with high affinity to pathogen β-tubulin and inhibition of microtubule polymerization resulting to destruction of cell structure and death of pathogen. They are the important inhibitors of β-tubulin polymerization against several plant pathogenic fungi such as *B. cinerea, Cercospora spp*., *C. spp*., *F. spp*., *E. spp* and *O. spp* (Duan *et al*., 2019). This study was therefore, to determine the efficacy of synthetic fungicide carbendazim on the leaf blight of cocoyam caused by *P. colocasiae* with the following objectives to:

(a) Determine the sensitivity and resistance of *P. colocasiae* to carbendazim.
(b) Assess the effect of carbendazim and other fungicides on other fungi organisms associated with cocoyam
(c) Evaluate the effect of *P. colocasiae* and other fungi organisms isolated from cocoyam on environmental stress

MATERIALS AND METHODS

Experiment sites

Laboratory experiments were conducted at the Department of Crop Science, Teaching and Research Farm, Faculty of Agriculture, University of Nigeria, Nsukka.

Collection of P. colocasiae isolates

Cocoyam leaves with leaf blight symptoms were collected from Department of Crop Science research farm, University of Nigeria, Nsukka. The leaves were disinfected with 0.5% (vol/vol) of Naclo for 1 minute and 75% (vol/vol) ethanol for 30s, rinsed three times with distilled water and cultured in potato dextrose agar (PDA) plates PDA which contains 200g of boiled potato tubers, 20g of dextrose and 15g of agar with distilled water per litre, amended with streptomycin sulfate (98% a.i., 50 μg/ml; Solarbio Science & Technology Co., Ltd., Beijing, China) and incubated for 3 days. Mycelia growth emerging were cut from the colony margins and transferred to fresh PDA plates. Pure cultures of the isolates were collected by sub-culturing on PDA under 12/12h light/darkness photoperiod using near-ultraviolet (NUV) light at 25 ^{0}C. a single conidium of the isolates obtained were maintained PDA slant at 4 ^{0}C for further use.

Fungicides and media

All fungicides of pure technical grade were used for the experiment. Fludioxonil (97.9% a.i.), carbendazim (98% a.i), metalxyl, mancozeb and hexaconazole (98.7% a.i.) were dissolved in methanol to obtain 10^4 (μg) ML^{-1} of the stock solution were preserved in the dark at 4 ^{0}C. PDA were prepared with 200g of potato, 20 g of glucose and 16 g of agar L^{-1} of distilled water where Potato dextrose broth (PDB) used for these experiments also has the same composition but lacks agar.

Evaluation of sensitivity of *P. colocasia* to carbendazim

The sensitivity and resistance test of *P. colocasia* and other fungi organisms isolated from cocoyam were estimated using mycelia growth assay. The other fungi organisms were *Fusarium solani, Rhizopus spp* and *Botryodioplodia theobromea*.

Mycelia plugs (5-mm in diameter) were prepared from one-third outside of the active margin of colony, transferred to new PDA plates and used for the experiment treated with carbendazim, mancozeb, metalaxyl, fludioxonil and hexaconazole at the serial concentrations of 0, 10, 20, 40, 80 and 100 μg/ml and incubated at 25 0C for 6 days. The diameters were investigated when the colonies without fungicide approached to eight cm. Data on mycelia growth in terms of colony diameter were obtained by averaging the diameters at perpendicular directions from which 5-mm in diameter were subtracted. The percentages of growth inhibition was calculated and arcsine-transformed prior to statistical analysis using the percentage transformation formula (Inhibition rate = ARSIN (SQRT (ab/100))*180/3.1415926) prior to statistical analysis. The treatments were replicated three times for each strain of the six pathogens and experiments performed two times.

Virulence test assay

To determine whether the organisms associated with cocoyam leaves can cause infection, virulence test assay were done on healthy cocoyam leaves.

Mycelia plugs (5mm in diameter) from a 6 days old PDA culture of each isolates were placed on the surface of the fruits over artificial wound for easy penetration of the pathogen, keep moist with a piece of moistened absorbent cotton and incubated in a growth chamber at 25 0C under 12-h photoperiod and 85% relative humidity. After 6 days, lesion diameters were measured as the mean of two diameters (cm) at perpendicular angles. The experiment was done two times with three replications

Osmotic stress assay

Mycelia plug (5mm in diameter) was taken from the edge of a 5 days old colony and transferred to PDA amended with 1.2 M NaCl, 1.2 M KCl, 0.5 M $CaCl_{2,}$ 0.3 mg/ml Congo red and 32µg/ml H_2O_2. PDA without osmoticum served a control; each treatment was replicated three times and incubated at 25 ^{0}C for seven days. The percentages of growth inhibition were calculated and arcsine-transformed prior to statistical analysis as they were not evenly distributed. The experiment was conducted two times.

Experimental design

The experiments were laid out as factors in a completely randomized design (CRD) with three replications. Factor A were the different plant fungi, factor B were the fungicides and factor C were different concentrations.

Statistical analysis

Data were analyzed using a GENSTAT statistical software package, 12.0 Release 4.23 (Payne, 2009). Colony diameters, mycelia weights and lesion areas were estimated in a completely randomized design (CRD) with three replications. Means were compared with Fisher's protected least significant difference at 5% probability level as outlined by (Obi, 2002).

RESULTS

Evaluation of sensitivity of *P. colocasia* to carbendazim and other fungicides

The results showed that serial concentrations of different synthetic fungicides, phytopathogenic fungi and their interactions had significant effect ($P \leq 0.05$) on the percentage inhibition and mycelia growth of isolates from cocoyam. Five fungicides at concentrations: 0.10, 20, 40, 80 and 100 µg/ml evaluated *in vitro* against *P. colocasiae, F. solani, R. spp* and *B. theobromea* exhibited wide range of mycelia growth and inhibition of the pathogens. *P. colocasia*e (12.50 mm) treated with hexaconazole had the lowest mycelia growth rate followed by *Botryodiplodia theobromea* (21.28 mm) with fipronil and carbendazim (24.03 mm) while *Rhizopus spp* (85.83 mm) and *F. solani* (78.798 mm) on metaxyl recorded the highest mycelia growth (Fig 1). Low mycelia growths were seen on all fungal isolates tested on concentration 100 µg/ml when compared with the untreated plates (Fig 2). Concentration 100 µg/ml of carbendazim (7.00 mm) and mancozeb (5.00 mm) recorded the lowest mycelia growth (Fig 3). The organism *B. theobromea* at concentrations 10, 20, 40, 80 and 100 µg/ml of carbendazim and 80 and 100 µg/ml of mancozeb did not grow likewise *P. colocasiae* at concentrations 10 -100 µg/ml of hexaconazole and 20 -100 µg/ml of carbendazim. Concentrations 40 – 100 µg/ml of carbedazim did not allow rhizopus to grow (Fig 4). The results revealed that all the five fungicides tested at concentrations 40 -100 µg/ml significantly inhibited mycelial growth over untreated plates. Furthermore, the percentage mycelia growth inhibition of carbendazim and mancozeb increased with increased in concentration of fungicides tested. Highest percentage inhibitions of *P. colocasiae* were observed on hexaconazle (83.33%) and carbendazim (75.97%). However, fungicides carbendazim, fipronil, hexaconazole and mancozeb were considered to be good for the inhibition of *B. theobromea* and *P. colocasiae*. The effects of different environmental stress, fungi and their interaction on mycelia growth showed significant difference on the organisms, the stress and their interactions. No mycelia growth were seen when *B. theobromea* were subjected to H_2O_2 (5.00 mm), followed by *P.colocasia* (14.67 mm) (Fig 5).

Table 1: Mycelia growth rate and virulence test assay of *P. colocasia* , *B.theobromea, R. spp* and *solani*

Fungi	**Virulence assay**	**mycelia weight**
Rhizopus spp	8.5 b	0.023
B. theobromea	10 b	0.104
F. solani	19.17 a	0.062
P. colocasia	10 b	0.453
F-LSD $_{(0.05)}$	3.863	NS

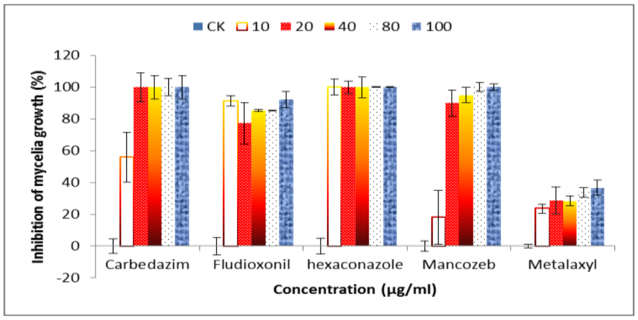

Fig 1: Effects of different synthetic fungicides and their concentration on the percentage inhibition of mycelia growth of *Phytophthora colocasiae.*

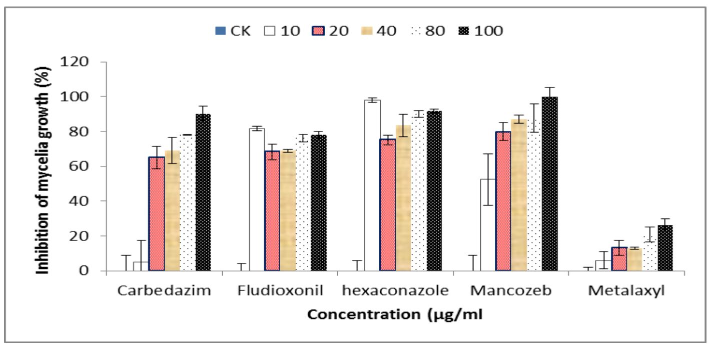

Fig 2: Effects of different synthetic fungicides and their concentration on the percentage inhibition of mycelia growth of *Fusarium solani*.

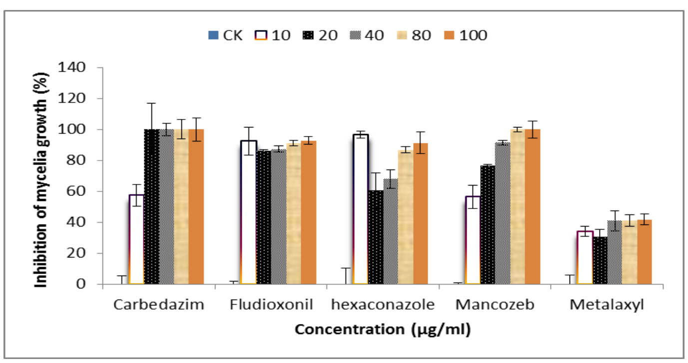

Fig 3: Effects of different synthetic fungicides and their concentration on the percentage inhibition of mycelia growth of *Botryodiploma theobromea.*

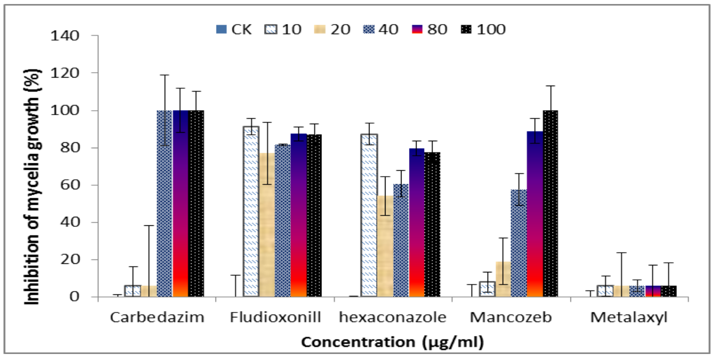

Fig 4: Effects of different synthetic fungicides and their concentration on the percentage inhibition of mycelia growth of *Rhizopus spp.*

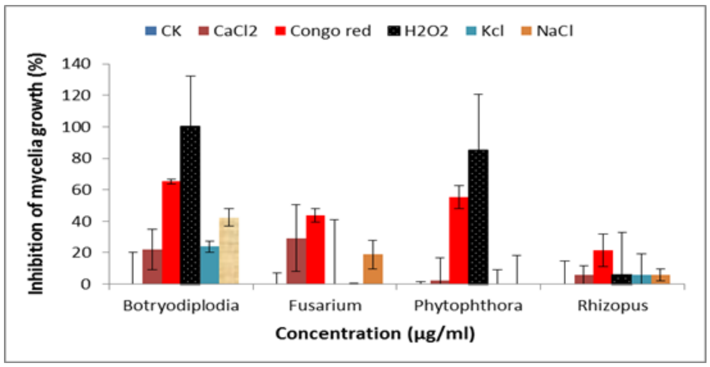

Fig 5: Effects of different environmental stress on the percentage inhibition of mycelia growth of different fungi organisms isolated from cocoyam.

DISCUSSION

The results revealed that all the five fungicides tested at concentrations 40 -100 µg/ml significantly inhibited mycelial growth over untreated plates. Furthermore, the percentage mycelia growth inhibition of carbendazim and mancozeb increased with increased in concentration of fungicides tested. Highest percentage inhibitions of P. colocasiae were observed on hexaconazle (83.33%) and carbendazim (75.97%). However, fungicides carbendazim, hexaconazole , mancozeb, and fludioxonil were considered to be more effective in the inhibition of *B. theobromea* and *P. colocasiae.*

Early research on the mechanism of action of carbedazim dwell on DNA and RNA synthesis which were described as secondary and primary effects respectively (Davidse, 1995;Davidse, 1986). In arresting nuclear division of fungi, carbendazim fungicide gives a striking resemblance to secondary plant metabolite colchicine, which disrupt mitosis and meiosis in animal and plant cells by inactivating the spindle (Zhou *et al*., 2016). The biochemical analysis of mechanism of action of carbendazim indicate the antimitotic activity of carbendazim in fungi facilitated through binding to fungal tubulin (Davidse, 1995; Zhou *et al*., 2016). They are greatly important in controlling many plant pathogenic diseases of crops. Carbendazim is a strong inhibitor of tubulin polymerization, antifungal in action and interfere with parasites energy metabolism through their selective bindings with high affinity to pathogen β-tubulin resulting to destruction of cell structure and death of pathogen (Chen *et al*., 2009; Lacey, 1990; Prichard, 1970a, 1970b; Schmit, 2013; Tejada *et al*., 1987; Y. Zhou *et al*., 2016). Hexaconazole is used widely for the management of anthracnose, powdery mildew, late blight, early blight, downy mildew and grey mildew diseases on crops due to its broad spectrum activity (Zhou *et al*., 2017).

Metalaxyl spectrum of activity broadened to control more diseases when it is used with other fungicides such as dithiocarbamate, phythalimides or copper fungicides and at the same time thus buildup of resistant fungal strains may be delayed and prevented (Sukul and Spiteller, 2000; Yao *et al*., 2009). Metalaxyl inhibits ribosomal RNA synthesis through RNA polymerization by acting on the polymerase 1 complex of rRNA synthesis which is the target site (Agrios, 2005). Resistance isolates of metalaxyl were detected in *Pseudoperenospora cubensis, P. infestans, P. tabacina* and *P. viticola*, therefore it was recommended that it should use in mixture with other fungicides with different mode of actions for example mancozeb (Agrios, 2005)

The effects of different environmental stress, fungi and their interaction on mycelia growth showed significant difference on the organisms, the stress and their interactions. No mycelia growth were seen when *B. theobromea* were subjected to H_2O_2 (5.00 mm), followed by *P.colocasia* (14.67 mm).

In conclusion, fungicides found to be effective for the control of cocoyam leaf blight in order of merit were carbendazim, fludioxonil, hexaconazole and mancozeb. Metaxyl recorded no or little effect on the inhibition of mycelia growth of all the four fungi organisms isolated from cocoyam as the organisms have developed resistance to it. It should be used in mixture with other fungicides with different mode of actions for example mancozeb.

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