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Inhibitory efficacy of some agrochemicals on mycelial growth of *Phytophthora cinnamomi* isolated from heart-rot disease of pineapple (*Ananas cosmosus* (L.) Merr.)

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Abstract

Some agrochemicals have been tested and found effective in plant disease control to improve food security. Growth inhibitory efficacy of four agrochemicals against *Phytophthora cinnamomi* isolated from heart-rot disease of pineapple (*Ananas comosus*) in naturally infested farm at Site I of Delta State University, Abraka was evaluated using poisoned food technique. The fungicides (fungu-force, mancozeb, maneb and mackecknie gold) at the concentrations of 25 -5000 ppm were evaluated *in-vitro* for their effect on the colony diameter of *P. cinnamomi* in pre-amended PDA medium. The fungicides showed response in inhibiting the growth with a dose dependent effect except for the fungu-force which totally inhibited the fungus at all concentrations tested. Complete inhibition was recorded for Fungu-force at 25ppm, Mancozeb at 1000ppm, Mackecknie gold at 4000ppm and Maneb at 5000ppm. The result of this study can be utilised to develop suitable application regime of these fungicides for trials on farmer's field in the control of heart-rot disease of pineapple and other crop diseases incited by this pathogen thereby improving food security.

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Introduction

The attempt by man to improve crop yield in order to produce enough food for consumption by the increasing population is a decision in right direction. The most important problems encountered in this attempt are how to drastically reduce or wholly prevent plant disease which is a continual battle. Chemical application is a highly effective technique to manage plant disease in agriculture (Adeniyi and Olufolayi, 2014). Some agrochemicals have been tested and found effective in plant disease control (Nene and Thapliyal, 1993). Certain protective fungicides although hazardous to the environment are still used for the control of fungal disease (Patel *et al.,* 2005; Ilondu, 2013).

Pineapple (*Ananas comosus* L. (Merr), is an important tropical field crop in regions such as Latin America, Asia and Africa on commercial basis (Kaneshiro *et al.,* 2008) and a herbaceous, perennial crop in the family of Bromeliaceae. It is the third most important tropical fruit in the world production after banana and *Citrus* (Bartholomew *et al.,* 2003). Nigeria is sixth on the list of world pineapple producers with about 800,000 tonnes per annum. *A. comosus* fruit is a rich source of vitamin A, B1, B6 and C, copper, manganese and dietary fibre (Office of the Gene Technology Regulator, (OGTR) (2008).

High concentration of Bromelain found in the ripe pineapple fruit is useful in confectionery and pharmaceutical industries as well as in diagnostic laboratories (Amao *et al.,* 2011). The pineapple leaves are a good source of fibre used in the production of Pina cloth (Kochhar, 1986).

Phytophthora cinnamomi is a soil-borne organism causing diseases of many crops including pineapple. Heart rot affects the basal leaf tissues and may cause rot of the fruit as well. The symptoms include rot of the basal tissues of the youngest leaves at the heart of the apical meristem. Such infected leaves may easily pull from the plant with a slight touch and as it advances may lead to total crop failure and subsequent yield reduction (Green and Scot, 2015). In this study, the efficacy of some agrochemicals against pineapple heart-rot pathogen was assessed.

Materials and methods

Test fungus

Phytophthora cinnamomi (Plate 1) was previously isolated and identified (Alexopalus *et al.,* 2002; Barnett and Hunter, 1999) from pineapple leaves with heart-rot disease symptoms (Plate 2) in a pineapple farm at Site 1, Delta State University, Abraka.

Fungicides

Mancozeb, Fungu-force, Maneb and Mackecknie Gold (Table 1) were purchased from Delta State Agricultural and Procurement Agency (DAPA) Ibusa near Asaba, Delta State.

Table1. Trade name, active ingredient and formulation of the fungicide evaluated in the study.

Trade name	Active Ingredient	Formulation
Dithane M_{45}	Mancozeb	80% WP
Fungu-force	Carbendazim + mancozeb	70% WP
Trimangol	Maneb	80% WP
Mackecknie Gold	Metalaxyl + Copper oxide	72% WP

In-vitro assay of fungicides

In vitro evaluation of the fungicides on the colony growth of *P. cinnamomi* was done through poisoned food technique (Taskeen *et al.,* 2011).

Stock solutions of the fungicides were prepared in sterile distilled water to get the required concentrations of 25, 50, 100, 200, 500, 1000, 2000, 3000, 4000 and 5000 ppm of the active ingredient (Ilondu, 2011). One millilitre of each level of concentration was aseptically incorporated into 20ml of cool molten PDA in each of the test-tube prior to the transfer to 9cm petri dishes.

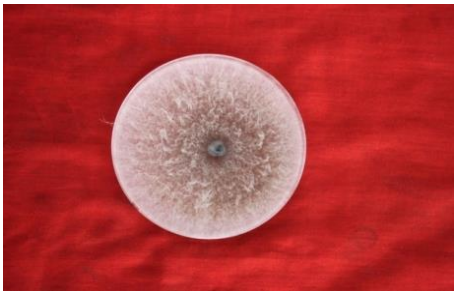

Plate 1. Culture of *P. cinnamomi*

Plate 2. Various degrees of heart-rot disease of pineapple in this study.

Each plate was inoculated with 4mm mycelia disc cut from the periphery of 5-day old culture of the test fungus. Three replicates were maintained for each concentration of each fungicide including the control (PDA without fungicide). The mean colony diameter was recorded after 5 days incubation at temperature (30±2°C) in a complete randomised design. The experiment was repeated twice. The toxicity of the fungicides on the growth of the fungus in laboratory assay was assessed by poisoned technique. The percentage inhibition of growth due to various fungicidal treatments at different concentration was computed by the method of Ilondu, (2013).

Data analysis

Data collected were subjected to analysis of variance (ANOVA) with statistical package for Social Sciences (SPSS, IBM Version 20) and means were separated using Duncan's Multiple Range Test at the probability level of ($P<0.05$).

Results and discussion

The linear growth of the fungus was considerably reduced by the fungicides with the degree of inhibition being directly related to increase in chemical concentration in the medium (Table 2).

The result showed that all the fungicides have *in vitro* toxicity to the pathogen but with varied effectiveness. The Fungu-force was the most prominent in its action on the pathogen with the minimum inhibition concentration of 25 ppm compared with the other fungicides. Complete inhibition was also observed at the concentration of 1000 ppm in Mancozeb and 4000 ppm in Mackecknie gold. The maximum inhibition of the test fungus was obtained in Maneb at 5000ppm concentration. Nwanosike and Adeoti (2002) opined that 100% inhibition of fungal growth is considered to be effective dose of a fungicide. The effectiveness of some of these fungicides on different plant pathogens has been observed (Chakrabarty *et al.,* 2013; Ilondu *et al.,* 2010; Ilondu, 2011, 2013; Patel *et al.,* 2005; Taskeen *et al.,* 2011).

Table 2. Effect of different concentrations (mg/ml) of four fungicides on the radial mycelia growth (cm*) and inhibition (%) of *Phytophthora cinnamomi in-vitro.*

Concentration (ppm)	Fungicides			
	Fungu-force	Maneb	Mackecknie Gold	Mancozeb
Control	4.30^{a} (0.00)	4.30^{a} (0.00)	4.30^{a} (0.00)	4.30^{a} (0.00)
25	0.00^{b} (100)	4.16^{a} (3.25)	3.53^{b} (17.90)	4.10^{a} (4.65)
50	0.00^{b} (100)	3.40^{b} (20.93)	3.33^{b} (22.56)	2.96^{b} (31.16)
100	0.00^{b} (100)	3.33^{b} (22.56)	2.80^{c} (34.88)	2.06^{c} (52.09)
200	0.00^{b} (100)	2.90^{c} (32.56)	2.43^{c} (43.49)	1.83^{c} (57.44)
500	0.00^{b} (100)	2.70^{c} (37.20)	1.96^{d} (54.41)	1.73^{c} (59.76)
1000	0.00^{b} (100)	2.36^{d} (45.12)	1.73^{d} (59.76)	0.00^{d} (100)
2000	0.00^{b} (100)	2.26^{d} (47.44)	1.43^{e} (66.04)	0.00^{d} (100)
3000	0.00^{b} (100)	1.73^{e} (59.76)	1.00^{f} (76.74)	0.00^{d} (100)
4000	0.00^{b} (100)	1.33^{f} (69.06)	0.00^{d} (100)	0.00^{d} (100)
5000	0.00^{b} (100)	0.46^{g} (89.30)	0.00^{d} (100)	0.00^{d} (100)

Values with the same superscript(s) in the same column are not significantly different at P > 0.05 by DMRT *Values are mean of three replicates; 0.00 = no growth/no inhibition; Fig.s in parenthesis are percentage growth inhibition

Conclusion

In this study, the best fungicide that could arrest the growth of *P. cinnamomi* is Fungu-force, followed by Mancozeb and Mackecknie gold. Therefore, the result of this study can be utilised to develop suitable application regime of these fungicides for trials on farmer's field in the control of heart-rot disease of pineapple and other crop diseases incited by this pathogen thereby improving food security. Further study is in progress with other chemicals and biocontrol agents to ascertain their efficacy in integrated approach to disease management.

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