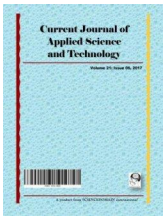

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Comparative Toxicity of Copper – Based Fungicides against *Phytophthora megakarya*; a Causal Agent of Black Pod Disease of Cocoa

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Authors' contributions

This work was carried out in collaboration among all authors. Author DOA designed the study, wrote the protocol and the first draft of the manuscript. Authors AOA, BAO and ABF managed the literature searches, sourced for the isolate and carried out in-vitro assay. Authors AOA and OOK collated the data, performed the statistical analysis and improve the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study determines the estimated toxicity and efficacy of copper-based fungicides use to control *Phytophthora megakarya*.
Study Design: Toxicity of fungicides against *Phytophthora megakarya* was determined *in-vitro* using mycelial growth inhibition.
Methodology: Isolates of *P. megakarya* were collected from cocoa experimental plots in Cocoa Research Institute of Nigeria, Ibadan, South Western Nigeria. Three active ingredients: Cuprous oxide, Copper hydroxide and Copper hydroxide + metalaxyl in fungicides retailed in open market were assayed *in-vitro* at 0.5 µg/ml, 1.0 µg/ml, 1.5 µg/ml, 2.0 µg/ml and 2.5 µg/ml of active ingredient against mycelial growth of *P. megakarya;* pathogen of black pod disease. The antifungal

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index and effective concentration at which mycelial growth was inhibited by 50% (EC_{50} value) was calculated for pathogen/fungicide combination and probit analysis.
Results: Toxicity of fungicides against *P. megakarya* using the EC_{50} showed significant variation as estimated from the mycelial growth inhibition. Highest mycelial inhibition (85.25%) of *P. megakarya* was recorded at 2.5 µg/ml of Copper hydroxide + metalaxyl with estimated EC_{50} value of 0.18 µg/ml and highest toxicity was recorded in Copper hydroxide + metalaxyl while Cuprous oxide was least toxic against *P. megakarya*. The toxicity responses of these Copper-based fungicides against *P. megakarya* vary with active ingredients and Copper hydroxide + metalaxyl gave the highest fungitoxic effect.

Keywords: Black pod; disease; effective concentration; antifungal index; EC_{50}.

1. INTRODUCTION

The genus *Phytophthora* is one of the most important plant pathogens worldwide, and many economically important crop species including cocoa are susceptible. Among the numerous pathogens of cocoa (*Theobroma cacao* L.), the species of *Phytophthora*, notably *P. palmivora, a cosmopolitan pathogen* and *P. megakarya*, which is restricted to West Africa, cause serious losses [1]. *Phytophthora* pod rot (black pod) as one of the major diseases of cocoa account for over 40% annual loss of cocoa [2]. Black pod is an important disease of cocoa that causes very serious losses. The disease affects the pods, beans, flower cushions, leaves, stems and roots [3]. *P. megakarya* is found in Cameroon, Nigeria, Togo, Ghana, Côte d'Ivoire, Gabon and Equatorial Guinea and has become the main yield-limiting factor in cocoa production in the sub region [4]. The menace of *P. megakarya* on cocoa is of great concern to cocoa farmers and scientists.. However [5] reported rainfall, high relative humidity, and low temperature as known factor that create favourable humid conditions for the development of the black pod disease. Yield loss in cocoa was largely due to black pods representing 64.1% of total yield loss [6]. The control of black pod disease is a major challenge for world cocoa cultivation, several methods have been adopted by farmers to control diseases caused by *P. megakarya* in cocoa and use of copper–based fungicides is the most effective and popular means of controlling black pod disease of cocoa among Nigerian farmers. The use of copper-based fungicide in Nigeria is as old as the crop in the country, it is reasonably effective but the high cost of chemical control in Africa poses a serious challenge to peasant farmers who produce over 50% of the world cocoa. Fungicides with active ingredients such as Copper hydroxide, Cuprous oxide and Copper hydroxide+metalaxyl have been used effectively to control black pod disease in Nigeria. These fungicides used on cocoa represent a substantial input of toxic substances in the environment, and residues present in cocoa beans are important risk factors for consumers. Therefore, the search for active ingredients which are more effective, environment-friendly and lower mammalian toxicity, still remains a difficult task and greatly needed in the field of agricultural fungicide. The aim of this study is to provide an independent source of information about the toxicity of some active ingredients against this major pathogen of cocoa.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of *Phytophthora megakarya*

Isolation of *P. megakarya* was done from naturally infected cocoa pods harvested from the experimental plots of Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. The diseased pods were washed with distilled water, blotted dry and were then surface sterilized with 70% ethanol. The active growing edges of infected parts of the pods were cut into 3 mm segments, surface sterilized in 10% sodium hypochlorite for 2-3 minutes and flooded with three changes of sterile water. The inocula were blotted dry on a sterile Whatman No 1 filter paper and inoculated on acidified V_8 juice agar routinely prepared in the Petri dish on the laboratory bench and the plates were incubated at 24 ±2°C for 7 days. The seven-day old pure culture of the pathogen was observed and the sporulated cultures were kept for further study.

2.2 Assay of Fungicide Toxicity

Three selected active ingredients: Cuprous oxide, Copper hydroxide and Copper hydroxide + metalaxyl in fungicides retailed in open market were assayed *in-vitro* against mycelial growth of

P. megakarya. The concentrations of the active ingredients tested against *P. megakarya* were 0.5 µg/ml, 1 µg/ml, 1.5 µg/ml, 2.0 µg/ml, and 2.5 µg/ml and each concentration was assayed by food poison method [7], with 2 ml added to about 20 ml of sterilized V_8 agar in 85 mm Petri dish and vortex. Each concentration of the active ingredients with medium in Petri dishes was replicated three times and arranged in a Completely Randomized Design (CRD). Five millimeter (5mm) disc from a seven-day old pure culture of *P. megakarya* was inoculated in the solidify poisoned medium and the plates incubated at 24±2°C for 7 days. The diameter of the mycelial colony was measured and the antifungal index (%) calculated [8]. Mycelial growth on the fungicide-amended medium was measured as a percentage against control, fungicide concentrations inhibiting mycelial growth by 50% (EC_{50}) were determined for *P. megakarya* and data on fungicide concentrations and antifungal index were processed by probit analysis [9].

3. RESULTS AND DISCUSSION

The results of the toxicity of the active ingredients against *P. megakarya in-vitro* are presented in Table 1. Copper fungicides can be described as insoluble compounds, yet their action as fungicides and bactericides is due to the release of small quantities of copper (Cu2+) ions when in contact with water [10–11]. The recommended fungicides for the control of black pod disease in Nigeria are all copper--based which are grouped as either contact or systemic fungicides. Investigation on the active ingredients of fungicides showed different toxicity as concerns the mycelial growth inhibition of *P. megakarya*.

Formulations of inorganic Cu, most commonly as copper hydroxide and copper sulphate, are used as agricultural pesticides to control fungi, bacteria, and in some instances, invertebrates and algae. As a result, water insoluble copper compounds are used as fungicides [12]. The percent growth inhibition as expressed in the antifungal index showed the highest inhibition of 40.80% *P. megakarya* when assayed in 2.5 µg/ml of cuprous oxide and the concentration that inhibit 50% mycelia growth (EC_{50} value) was 4.08 µg/ml (Fig. 1).

The EC_{50} value of copper hydroxide against growth of *P. megakarya* was 2.46 µg/ml and the antifungal index of 25.40% was recorded at 2.5 µg/ml of the same active ingredient (Fig. 2). Copper hydroxide is more water soluble at low pH (high acidity) and it is applied in spray solution such as water at a pH above 6 to avoid phytotoxicity [13].

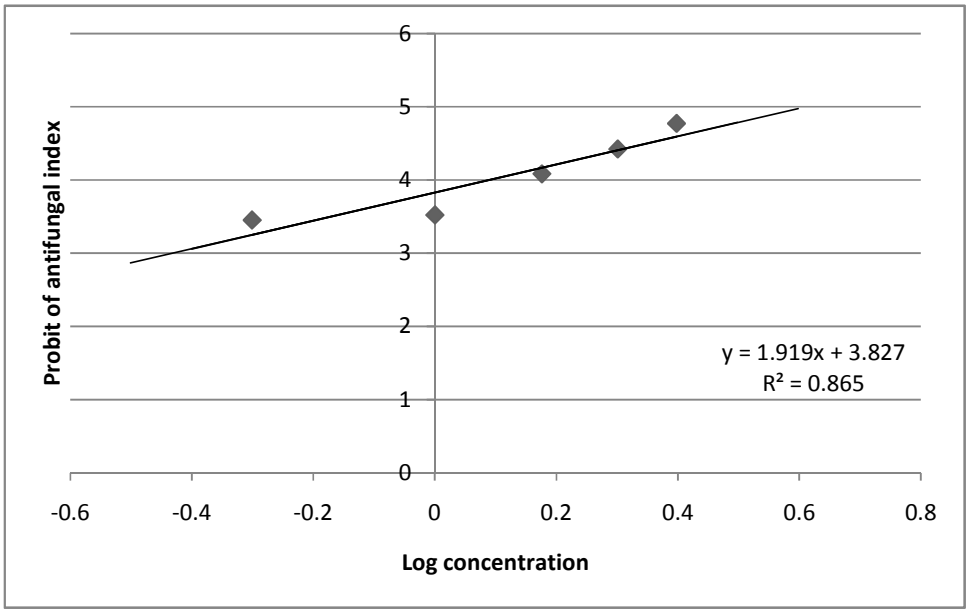

Fig. 1. Scatter plot and linear regression of EC_{50} values for Cuprous oxide

Table 1. Antifungal indices and toxicity of active ingredients against *P. megakarya*

Active ingredient (µg/ml)	Log. Conc.	Cuprous oxide		Copper hydroxide		Copper hydroxide + metalaxyl	
		Antifungal index (%)[a,b]	Probit	Antifungal index (%)[a,b]	Probit	Antifungal index (%)[a,b]	Probit
0.5	-0.3010	6.34	3.45	0.00	0.00	61.93	5.31
1.0	0.0000	7.55	3.52	14.20	3.92	63.75	5.36
1.5	0.1761	17.82	4.08	14.50	3.96	72.81	5.58
2.0	0.3010	28.25	4.42	18.30	4.08	78.01	5.77
2.5	0.3979	40.80	4.77	25.40	4.61	85.25	6.08
EC_{50} (µg/ml)		4.08		2.46		0.18	

[a]*Data collected after 7 days of incubation.*
[b]*Each experiment was performed three times and the data were averaged (n = 3)*

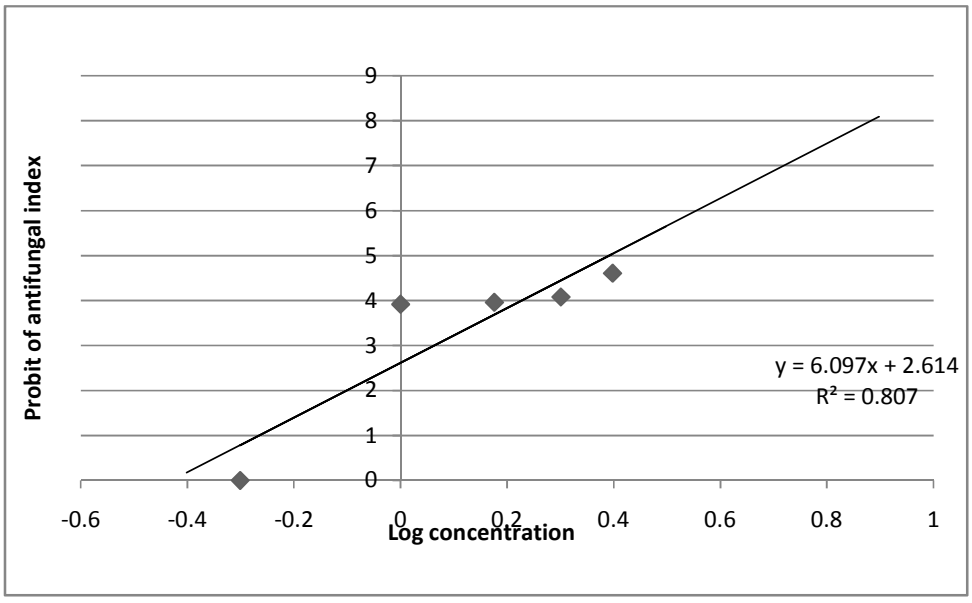

Fig. 2. Scatter plot and linear regression of EC_{50} values for Copper hydroxide

The toxicity of copper hydroxide + metalaxyl against *P. megakarya* showed significant variation in the mycelial growth of the pathogen of black pod disease compared with other active ingredients assayed. The 0.5 µg/ml of copper hydroxide + metalaxyl showed 61.93% mycelial inhibition of *P. megakarya* which was higher than 6.34% and 0.00% inhibition recorded in the same concentration of cuprous oxide and copper hydroxide respectively. The highest antifungal of 85.25% mycelial inhibition of *P. megakarya* was also recorded at 2.5 µg/ml of copper hydroxide + metalaxyl and EC_{50} value of this active ingredient was estimated as 0.18 mg/ml (Fig. 3).

The highest toxicity of the three active ingredients against *P. megakarya* was recorded in copper hydroxide + metalaxyl while cuprous oxide was least toxic showing highest EC_{50} value though the antifungal index at 2.5 µg/ml was higher than copper hydroxide. The toxicity on *P. megakarya* growth increases with decrease in EC_{50} value with exception of copper hydroxide and the most effective is highly toxic against the pathogen of black pod disease. Concentrations of copper based fungicides that are reported as toxic vary; critical factors include the organism, whether acute or chronic toxicity was determined and the extraction method [14]. Bacteria, fungi, and mollusks are generally the most sensitive to Cu compared with flowering plants and vertebrate animals [15–16].

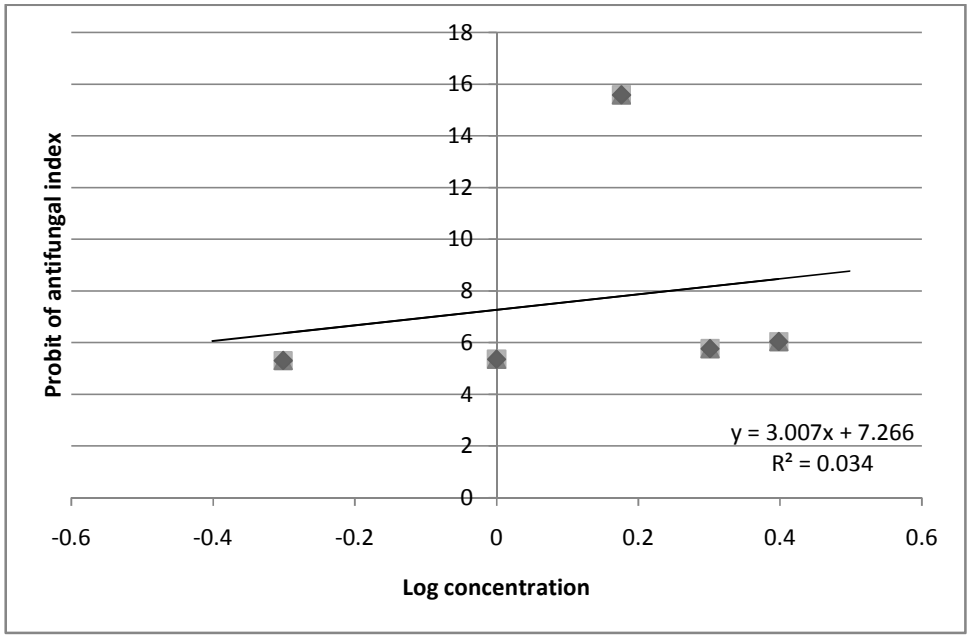

Fig. 3. Scatter plot and linear regression of EC_{50} values for Copper hydroxide + metalaxyl

4. CONCLUSION

Copper based fungicides are still very important regarding disease control in cocoa production even though the demand for organic food is on the increase. The toxicity responses of these fungicides against *P. megakarya* vary with active ingredients; Copper hydroxide + metalaxyl showed high fungitoxic effect while others showed less toxicity against this pathogen. The information provided will be valuable in reduction of chemical usage for safety of the ecosystem, reduce the risk of chemical residue and trials need to be conducted to consolidate these findings under field conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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