

Toxicity of fungicides inhibitors to *Phytophthora megakarya* in cacao

Abstract

Aim: The study determines the estimated toxicity and efficacy of copper based fungicides.

Study Design: Toxicity of fungicides against *Phytophthora megakarya* was determined *in-vitro* using mycelial growth inhibition.

Methodology: Three fungicides: Cuprous oxide, Copper hydroxide and Copper hydroxide + metalaxyl were assayed at 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml of active ingredient against *Phytophthora megakarya*; pathogen of black pod disease. The antifungal index and effective concentration at which mycelial growth was inhibited by 50% (EC₅₀ value) was calculated for pathogen/fungicide combination.

Result: Toxicity of fungicides against *P. megakarya* using the EC₅₀ showed significant variation as estimated from the mycelial growth inhibition. Highest of 85.25% mycelial inhibition of *P. megakarya* was recorded at 2.5mg/ml of copper hydroxide + metalaxyl with estimated EC₅₀ value of 0.18mg/ml and highest toxicity was recorded in copper hydroxide + metalaxyl while cuprous oxide was least toxic against *P. megakarya*.

Keywords: Fungicide, toxicity, antifungal index, EC₅₀, *Phytophthora megakarya*

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.), these species of *Phytophthora*, notably *Phytophthora palmivora* with a worldwide distribution 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 fungal disease of cocoa that causes very serious losses. The disease affects the pods, beans, flower cushions, leaves, stems and roots [3]. *Phytophthora megakarya* has spread westwards from Cameroon through Nigeria and Togo, to the major cocoa producing countries of Ghana and Côte d'Ivoire, and southwards to 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 cacao is of great concern to cocoa farmers and scientists, but the processes underlying the emergence of *P. megakarya* on cacao are unknown. 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 cacao 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 study is aim to provide an independent source of information about the toxicity and effective concentration of some active ingredients against this major pathogen of cocoa.

Materials and Methods

Isolation and identification of *Phytophthora megakarya*: Isolation of *P. megakarya* was done from naturally infected pods collected from the experimental plots of Cocoa Research Institute of Nigeria (CRIN), 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 V₈ juice agar routinely prepared in the Petri dish on the laboratory bench and the plates were incubated at 24 ±2°C for 7days. The seven-day old pure culture of the pathogen was observed and the sporulated cultures were kept for further study.

Assay of fungicide toxicity: Three selected active ingredients: Cuprous oxide, Copper hydroxide and Copper hydroxide + metalaxyl were assayed *in-vitro* against mycelial growth of *P. megakarya*. The concentrations of the active ingredients tested against *P. megakarya* were 0.5mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml, and 2.5mg/ml and each concentration was assayed food poison method of [7] with 2ml added to about 20 ml of sterilized V₈ agar in 85mm Petri dish and vortex. 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 7days. The diameter of the mycelial colony was measured and the antifungal index (%) calculated according to [8]. Mycelial growth on the fungicide-amended medium was measured as a percentage against control, fungicide concentrations inhibiting mycelial growth by 50% (EC₅₀) were determined for *P. megakarya* and data on fungicide concentrations and antifungal index were processed by probit analysis [9].

Result and Discussion

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. This disease of cacao caused by *Phytophthora megakarya* and *P. palmivora* is the most important and devastating one in Nigeria. 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]. Researching active ingredients of the fungicides showed different toxicity as concerns the mycelial growth inhibition of *P. megakarya*. The results of the efficacy of the fungicide *in-vitro* are presented in table 1.

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

Active Ingredient (mg/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	0	61.93	5.31
1.0	0	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 (mg/ml)		4.08		2.46		0.18	

^aData collected after 7 days of incubation. ^bEach experiment was performed three times and the data were averaged ($n = 3$).

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 2.5mg/ml of cuprous oxide and the concentration that inhibit 50% mycelia growth (EC₅₀ value) was 4.08mg/ml (figure 1).

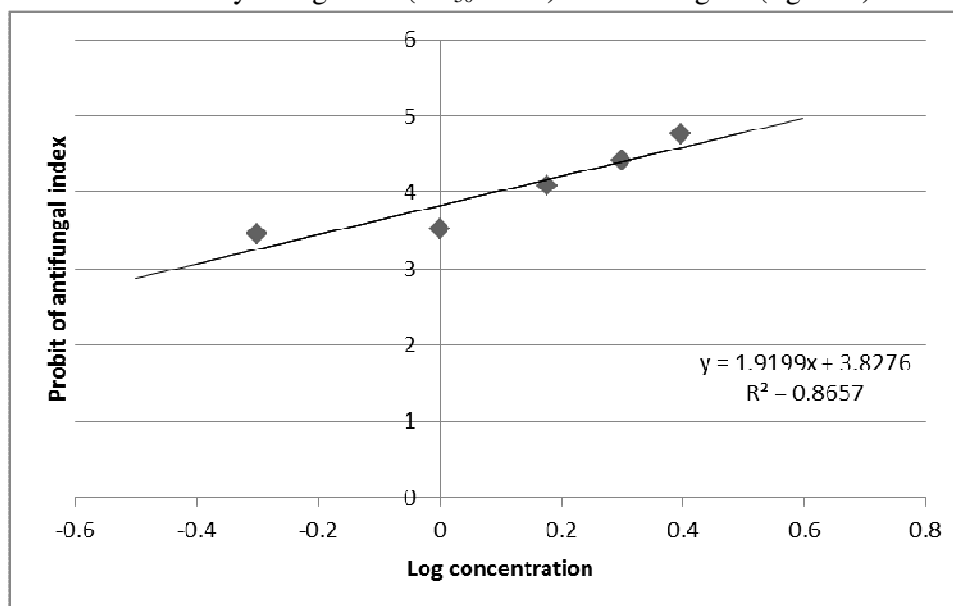


Figure 1: Scatter plot and linear regression of EC₅₀ values for Cuprous oxide

The EC₅₀ value of copper hydroxide against growth of *P. megakarya* was 2.46mg/ml and the antifungal index of 25.40% was recorded at 2.5mg/ml of the same active ingredient (figure 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].

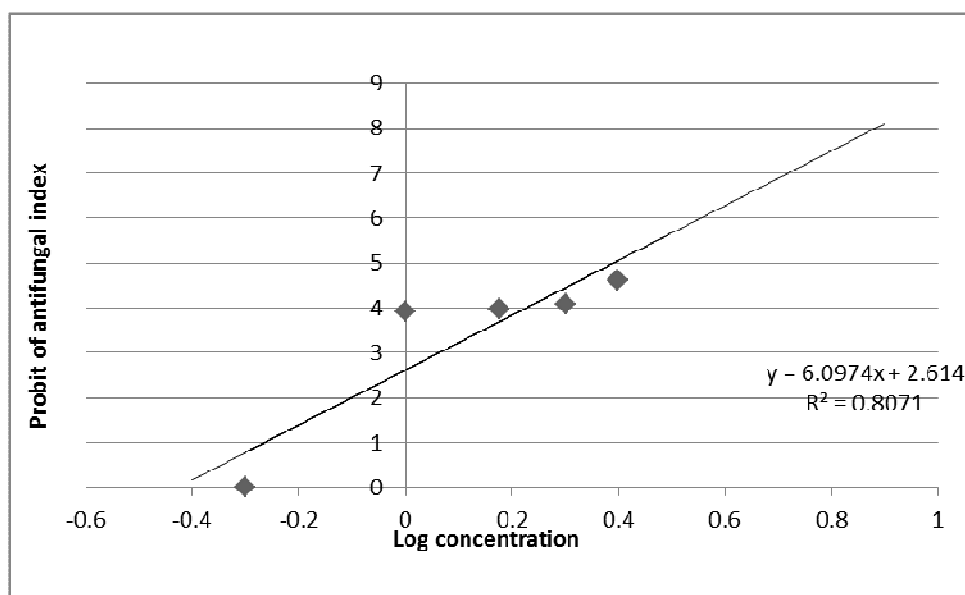


Figure 2: Scatter plot and linear regression of EC₅₀ values for Copper hydroxide

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 0.5mg/ml of copper hydroxide + metalaxyl showed 61.93% mycelial inhibition of *P. megakarya* which was higher than 6.34% and 0% 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 2.5mg/ml of copper hydroxide + metalaxyl and EC₅₀ value of this active ingredient was estimated as 0.18mg/ml (figure 3).

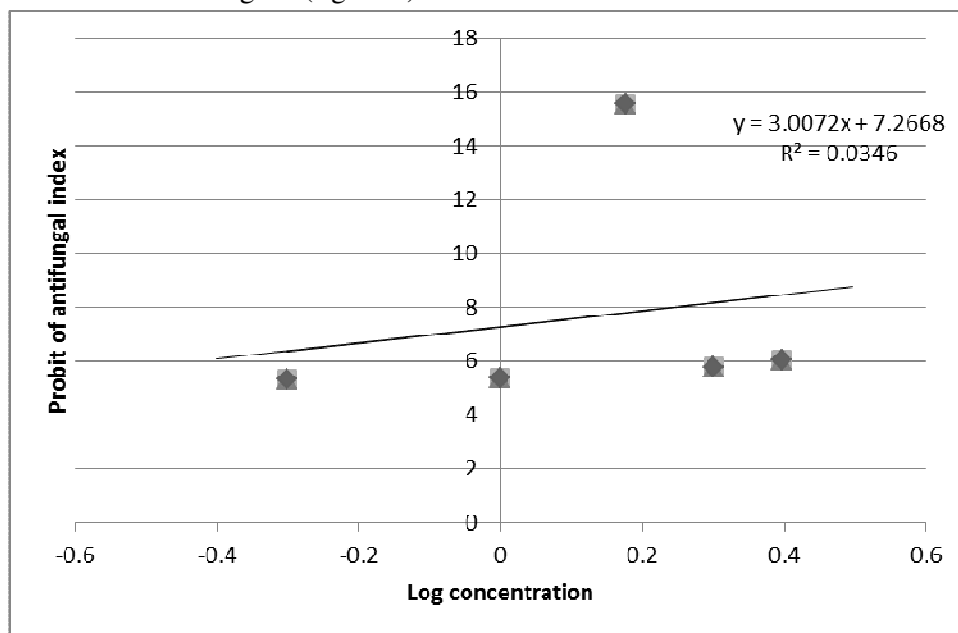


Figure 3: Scatter plot and linear regression of EC₅₀ values for Copper hydroxide + metalaxyl

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 EC₅₀ value though the antifungal index at 2.5mg/ml was higher than copper hydroxide. The toxicity on *P. megakarya* growth increases with decrease in EC₅₀ 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].

Conclusion

Copper is still an irreplaceable metal regarding disease control in cocoa production even with the biological food production gaining importance. The toxicity responses of these fungicides against *P. megakarya* vary with active ingredients; copper hydroxide + metalaxyl showing high fungitoxic effect while others showed less toxicity against this pathogen. The information provided will be valuable in reduction of chemical for safety of the ecosystem, reduce the risk of chemical residue and trials need to be conducted to consolidate these findings under field conditions.

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