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Full Length Research Paper

Estimation of fungicide toxicity for pathogen: *Lasiodiplodia theobromae*

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Abstract

Lasiodiplodia theobromae **attacks plants of major economic interest including cashew. This pathogen cause many problems to farmers resulting into heavy economic loss. Chemical control is currently one of the most effective ways to fight against this pathogen. The efficacy of sixfungicides: Mancozeb 80% WP, Copper-1-oxide 60% + metalaxyl M 6%, Copper hydroxide (57%), Cuprous oxide (86%), Carbendazim 50% WP and Copper-1-oxide 60% +metalaxyl 12% WP were tested, *in-vitro* on the phytopathogenic L. *theobromae* isolated from inflorescence dieback disease of cashew. The fungicides were tested at different concentrations: 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 and 0.1mg/ml of the active ingredients. Toxicity responses were expressed as the effective concentration, which inhibits mycelial growth by 50%, (EC_{50}). Of the six selected fungicides, carbendazim proved to be the most effective with EC_{50} value of the order of 0.068mg/ml followed by mancozeb with 0.21mg/ml and Copper-1-oxide 60% + metalaxyl M 6% with value of 0.67mg/ml. Other fungicides showed lower fungitoxicity with estimated EC_{50} values of 5.14 mg/ml, 6.66 mg/ml and 4.35mg/ml.**

Keywords: *Lasiodiplodia theobromae*, cashew, fungicides, toxicology, active ingredients, mycelial inhibition, EC_{50}

INTRODUCTION

Pesticides are the most effective means of pest eradication all over the world, but their use has reached an alarming rate due to a number of adverse effects on non-target organisms (Yehia *et al*., 2007; Singh, *et al*., 2009; Bhushan, *et al*., 2010). They are used mainly in agriculture to control pest animals (insects, rodents), fungi and weeds (Elkhansa Yahia *et al*, 2015). Parimala and Kaliwal (2005) opined that pesticides are indispensable in modern agriculture by increasing food production by controlling agricultural pests and reducing vector borne diseases. Modern fungicides do not kill fungi; they simply inhibit growth for a period of days or weeks (Rouabhi, 2010). *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl [synonym: *Botryodiplodia theobromae*] is a cosmopolitan and diverse species. As disease agent, pathogen is encountered in its anamorph state, named as *Lasiodiplodia*. This fungus attacks more than 280 species of plants in different parts of the world (Domsch *et al.*, 1980) and the pathogen of inflorescence and twig dieback diseases of cashew in Nigeria (Adeniyi *et al*., 2011). The attempts to test fungicides against *L. theobromae* under laboratory conditions are reported by Ahmad *et al*., (1995), Shelar*et al*., (1997) and Adeniyi and Olufolaji (2014). Recent studies on fungicides efficacy on cashew diseases are very rare but some fungicides are being introduced for commercial cashew production in Nigeria. Among a set of measures aimed at protecting the inflorescences from the disease, primary and leading role has chemical method.

Today there are many different chemical groups of fungicides that are effective, in order to rationalize their use and improvement of plants protection system, studies of the toxicity of a preparation, determine its optimal concentration. The use of chemical fungicides is one of the main tactics being used for the management of plant diseases. Knowledge is scarce about the management of *L. theobromae* by using any of the selected chemical fungicides. The aim of this study is to provide an independent source of information about the activity and toxicity of some fungicides against a major disease of

Table 1: Effective concentrations of active ingredients to inhibit *Lasiodiplodia theobromae* growth

Conc. (mg/ml)	Log Conc.	Mancozeb		Carbendazim		Copper-1-oxide 60% + metalaxyl M 6%		Copper hydroxide (57%),		Copper-1-oxide 60% + metalaxyl 12% WP		Cuprous oxide (86%)	
		%GIH*	Probit	%GIH	Probit	%GIH	Probit	%GIH	Probit	%GIH	Probit	%GIH	Probit
2.5	0.3979	94	6.55	89	6.23	83	5.95	20	4.16	38	4.67	46	4.90
2.0	0.3010	94	6.55	88	6.18	81	5.88	10	3.72	34	4.59	18	4.08
1.5	0.1760	93	6.48	88	6.18	77	5.74	9	3.66	30	4.48	11	3.77
1.0	0.0	79	5.81	89	6.23	59	5.23	0	-	15	3.96	7	3.52
0.5	-0.3010	55	5.13	89	6.23	48	4.95	0	-	14	3.92	6	3.45
0.25	-0.6020	55	5.13	57	5.18	30	4.48	0	-	3	3.12	0	-
0.1	-1.0	40	4.75	54	5.10	3	3.12	0	-	2	2.95	0	-
EC_{50} (mg/ml)		0.21		0.068		0.67		6.66		4.35		5.14	

*Percent Growth Inhibition of mycelial of *L. theobromae*

cashew (inflorescence dieback) caused by *L. theobromae.*

MATERIALS AND METHODS

Observations of severity of inflorescence dieback were carried out at the cashew germ plasm in the Cocoa Research Institute of Nigeria, South Western Nigeria and isolate of *L. theobromae* was collected from diseased inflorescences. Isolation was done by cutting off small pieces (2×2×5mm) of panicles with disease symptom, immersing them in a 1% sodium hypochlorite solution for 1 min(Potocnik, *et al*., 2009) for surface sterilization, dried in-between sterile Whatman No 1 filter paper and placing on potato dextrose agar (PDA). The isolate was maintained on PDA at 5^0C, on a slant in the Plant Pathology Laboratory of Cocoa Research Institute of Nigeria. Six (6) fungicides; Mancozeb 80% WP, Carbendazim 50% WP, Copper-1-oxide 60% + metalaxylM 6%, Copper hydroxide (57%), Copper-1-oxide 60% +metalaxyl 12% WP and Cuprous oxide (86%) at 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 and 0.1mg/ml were assayed to determine their potential toxicity to *L. theobromae*. The *in vitro* evaluation of the fungicides was by food poison method of Nene and Thapliyal (1982). Two milliliters (2ml) of each fungicide concentrations were aseptically introduced into the sterile 85mm diameter Petri dishes, with about 20ml PDA added, vortex with fungicides and allow to solidify. A 5mm disc obtained from a 7-day old culture of *L. theobromae* was inoculated. Each treatment was replicated three and incubated at ambient temperature (28+2^{o}C) and plate without fungicide serve as control. The radial mycelial growth was recorded when the upper surface in control treatment was fully covered (Adeniyi and Olufolaji, 2014). Mycelial growth on the fungicide-amended medium was measured as a percentage against control. Fungicide concentrations inhibiting mycelia growth by 50% (EC_{50}) were determined for *L. theobromae* and data on fungicide concentrations and relative inhibition were processed by probit analysis (Finney1971).

RESULTS

The effect of six fungicides on radial growth of *L. theobromae* was studied to screen out fungicides which are highly effective against this fungus. The average radial growth of *L. theobromae* was significantly affected by different fungicides. Investigated active ingredients showed different toxicity as concerns the inhibition of pathogen of cashew. The effects of the active ingredients of test fungicides on mycelial growth inhibition of *Lasiodiplodia theobromae* vary depending on the active ingredient and concentration of the active ingredient. The results of efficacy ratings of fungicides in the laboratory conditions on mycelial growth of *L. theobromae* are presented in the table 1. The highest percent inhibition was recorded in mancozeb showing 94% at 2.5 and 2.0mg/ml but 40% growth inhibition was recorded in 0.10mg/ml of the same active ingredient.

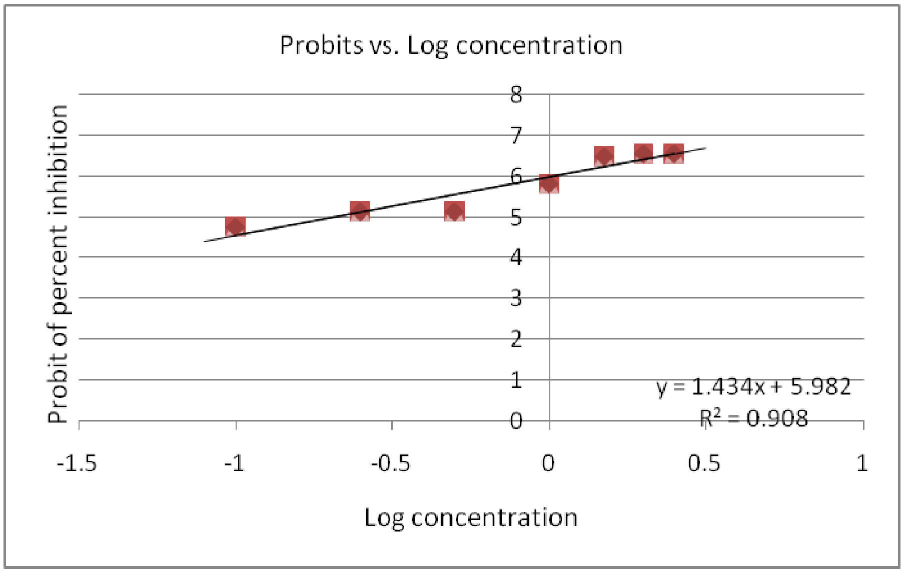

Figure 1: Scatter plot and linear regression of effective concentrations to inhibit mycelial growth by 50% (EC_{50} values) for mancozeb 80% WP

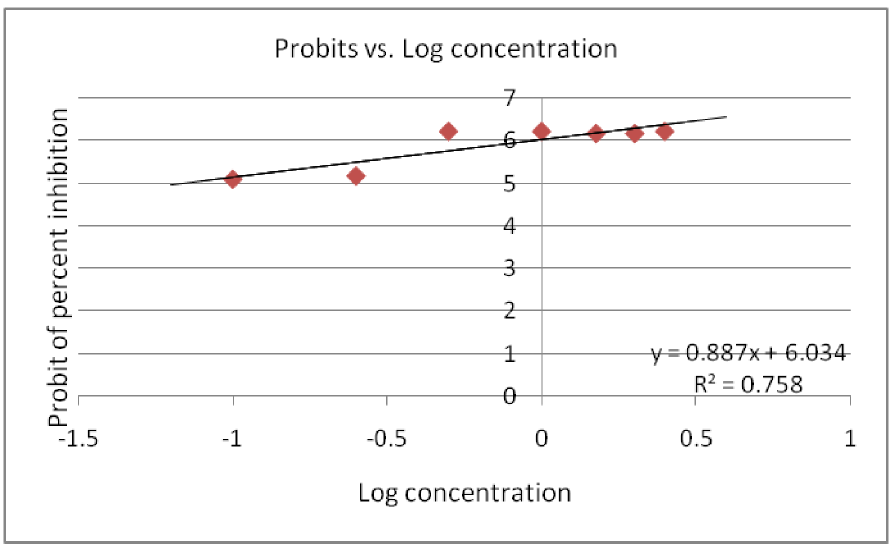

Figure 2: Scatter plot and linear regression of effective concentrations to inhibit mycelia growth by 50% (EC_{50} values) for carbendazim 50% WP

However, the concentration of mancozeb that inhibit 50% growth of *L. theobromae* was 0.21mg/ml (figure 1). Carbendazim on the other hand inhibit 89% mycelial growth of *L. theobromae* at 2.5, 0.5 and 0.25mg/ml and 54% inhibition at 0.1mg/ml of the active ingredient. The concentration of carbendazim that inhibit 50% growth of *L. theobromae* is estimated as 0.068mg/ml (figure 2) and the highest toxicity of all the active ingredients against *L. theobromae* was recorded in carbendazim and EC_{50} estimated as 0.068mg/ml. Copper-1-oxide 60% + metalaxyl M 6% inhibited growth of *L. theobromae* by 83% at 2.5mg/ml, the toxicity of the active ingredient show EC_{50} of 0.67mg/ml (figure 3) against the pathogen of cashew (table 1).

The least toxicity of the assayed active ingredients against mycelial growth of *L. theobromae* was recorded in Copper hydroxide (57%) showing EC_{50} of 6.66mg/ml (figure 4) and 20% growth inhibition of pathogen. The growth inhibition of *L. theobromae* by Copper-1-oxide 60% +metalaxyl 12% WP and cuprous oxide range from 2.35% to 46.67% with highest inhibition recorded in 2.5mg/ml of cuprous oxide and the least in 0.1mg/ml of

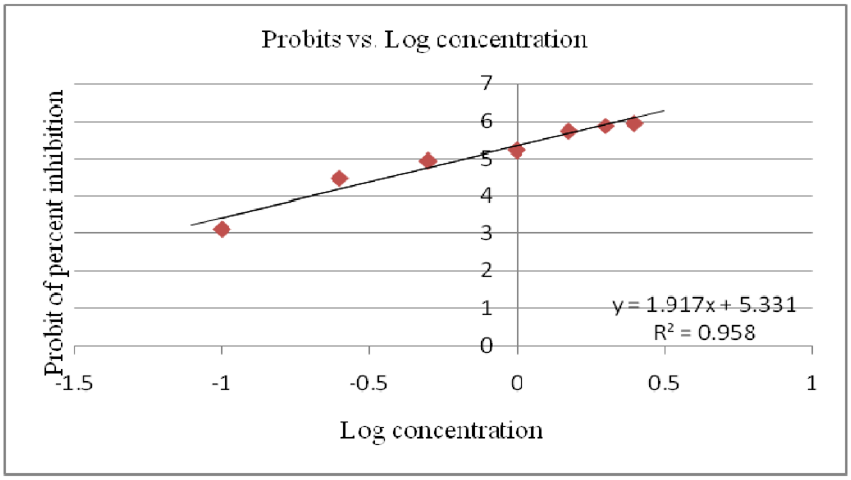

Figure 3: Scatter plot and linear regression of effective concentrations to inhibit mycelial growth by 50% (EC_{50} values) forcopper-1-oxide 60% + metalaxyl M 6%

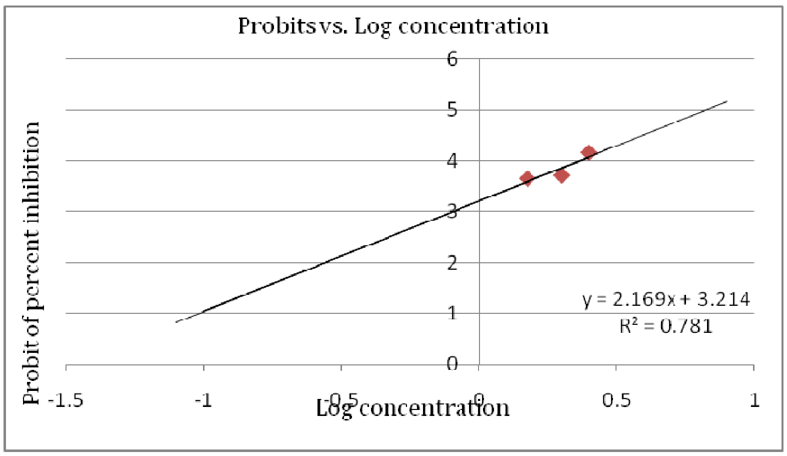

Figure 4: Scatter plot and linear regression of effective concentrations to inhibit mycelial growth by 50% (EC_{50} values) for copper hydroxide (57%)

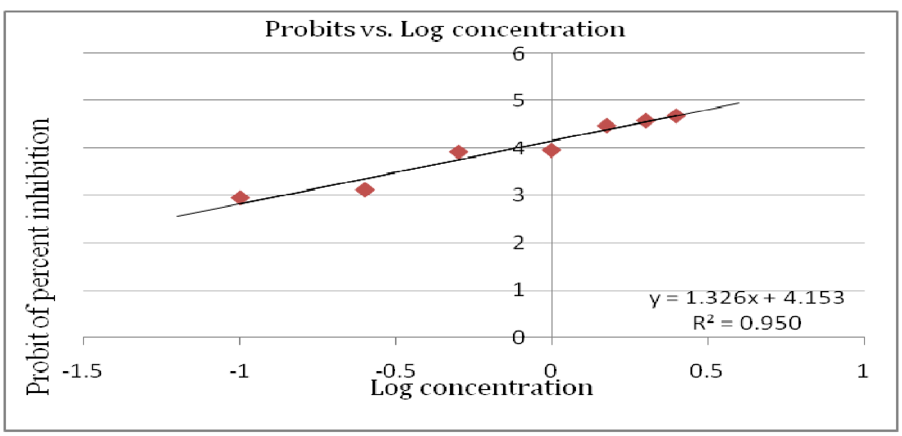

Figure 5: Scatter plot and linear regression of effective concentrations to inhibit mycelial growth by 50% (EC_{50} values) for copper-1-oxide 60% +metalaxyl 12% WP

Copper-1-oxide 60% +metalaxyl 12% WP. The EC_{50} and estimated toxicity of Copper-1-oxide 60% +metalaxyl 12% WP and cuprous oxide are 4.35mg/ml; 5.14mg/ml (figure 5 and 6). The toxicity of these active ingredients

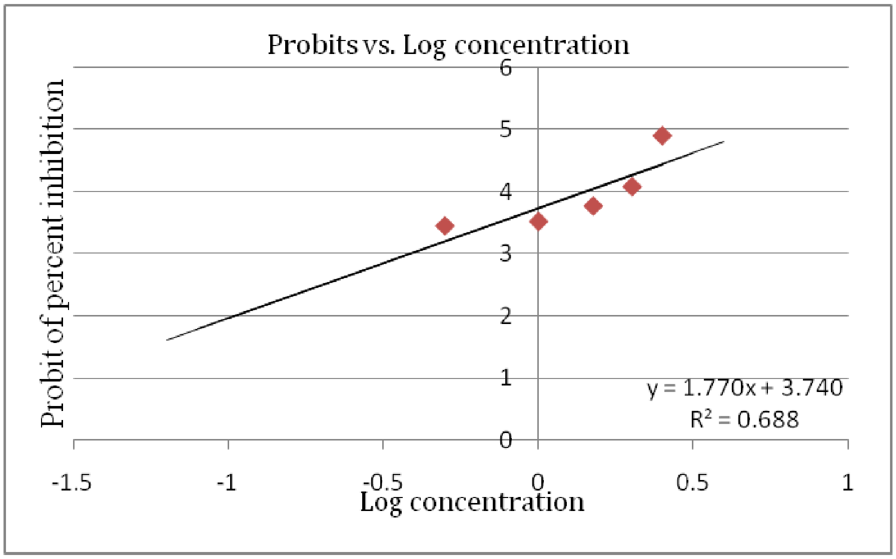

Figure 6: Scatter plot and linear regression of effective concentrations to inhibit mycelial growth by 50% (EC_{50} values) for cuprous oxide (86%)

on pathogen increases with declining EC_{50} value of the active ingredients. The most effective that have greater percent inhibition of *L. theobromae* have low EC_{50} and highly toxic against the pathogen.

DISCUSSION

This research presents *in-vitro* sensitivities of *L. theobromae,* pathogen of inflorescence dieback of cashew against selected fungicides. The primary focus was on pathogen which cause diseases in many crops and specifically on cashew which has industrial and economic value especially the nut and to identify highly effective fungicide to manage this menace and replace the banned fungicides prior used on cashew. Progressive increase of production and application of chemicals fungicides for agriculture as well as for plant protection has converted the problem of environmental pollution into national and international issue (Mathys, 1994). Therefore, more efforts are being directed using selective chemicals as well assessment and usage of their minimum concentration required for disease management (Larson *et al*., 1997). Isolates *L. theobromae* were not sensitive to pyraclostrobin (EC_{50}>100µg/ml) but were sensitive to mancozeb at EC_{50}>50µg/ml (KC and Vallad, 2015). A wide range of effective concentrations to inhibit growth of *L. theobromae* by 50% was obtained for the six fungicides evaluated. In concordance of the fungicides, 50% inhibitory concentrations vary depending on the fungicides. Carbendazim is the most fungitoxic with EC_{50} of 0.068mg/ml of the active ingredient, follow by mancozeb with EC_{50} of 0.21mg/ml out of the six fungicides evaluated. In concordance to the findings in this study, Ye *et al., (*2014) reported that carbendazim was the most aggressive fungicides tested showing the lowest EC_{50} value of 8.04 mg/l against *Ciboria carunculoides.* EC_{50} values of 8.04 mg/l was calculated for carbendazim against *C. carunculoides by* Ye *et al.,* while Li *et al.,* (2009) observed a EC_{50} value of 0.108mg/l carbendazim against *Botryosphaeriaberengriana* both of which are higher to EC_{50} value of 0.068mg/ml reported in this study for carbendazim against *L. theobromae isolate,* however, *Amini and Sidovich* (2010) observed EC_{50} value 0.008 mg/ml of carbendazim against *F. oxysporum*f. sp. *Lycopersici,* Mostert *et al.,* (2000) reported an estimated EC_{50} value of 2.891mg/ml for mancozeb against mycelial growth of *Phomopsisviticola*

Everett and Timudo-Torrevilla (2007) reported different EC_{50} value observed in three copper hydroxide-base fungicides as 2.54, 0.8 and 0.346mg/ml against *Colletotrichumacutatum,* EC_{50} value of 0.647, 0.297 and 0.257 mg/ml against *C. gloeosporioides,* 0.304, 0.228 and 1.321mg/ml against *Botryosphaeriaparva,* 0.153, 0.118 and 0.197mg/ml against *B. dothidea* and 0.105, 0.101 and 0.193mg/ml against *Phomosisspp*. All the values are lower to the EC_{50} value obtains in the copper hydroxide-base fungicide in this study.

CONCLUSION

Toxicity responses vary with different active ingredients tested on mycelial growth of *L. theobromae.* Mancozeb, carbendazim as well as copper-1-oxide 60% + metalaxyl M 6% have high fungitoxic effect on the pathogen while other active ingredients were less with higher EC_{50} values.

Some of the fungicides is currently being tried on cashew diseases and the information provided will be valuable in monitoring of fungicide resistance and in designing effective fungicide application strategies.

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