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Full Length Research Paper

Acute Toxicity of Lemon Grass (*Cymbopogon Citratus*) and Wild Basil (*Ocimum Suave*) Applied as Mixed and Individual Powders Against the Cowpea Bruchids, *Callosobruchus Maculatus,* in Cowpea

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A major problem in cowpea seed storage in the tropics is infestation by bruchids, especially the cowpea bruchids, *Callosobruchus maculatus* (F.). Plant mixtures containing lemon grass (*Cymbopogon citratus*) (L) and wild basil (*Ocimum suave*) (W) in the ratio of Lemon grass : Wild basil - 100:0, 80:20, 60:40, 50:50, 40:60, 20:80, 0:100 and 0:0 were used under ambient laboratory conditions with the aim of evaluating the efficacy of these plant powders against the cowpea bruchids. The mixed powders were each applied at 1, 2 and 3 g concentration to 20 g of cowpea seeds and acute toxicity was assessed after up to 96 h exposure. Insect mortality was recorded to estimate the LC_{50}s and LT_{50}s. All mixtures with the exception of 100L: 0W and 80L: 20W) caused significant insect mortality (*p*<0.05) as the concentration increased from 1 - 3 g/20 g cowpea seeds. These results indicate that the plant materials are toxic to the cowpea bruchids especially the mixed proportions 60L: 40W, which was the most effective with the lowest LC_{50} and LT_{50}.

Keywords: *Callosobruchus maculates, Cymbopogon citratus,* infestation, mortality, *Ocimum suave.*

INTRODUCTION

Cowpea (*Vigna unguiculata*) (L.) Walp is a very important and inexpensive source of dietary protein for many countries in the tropics. Cowpea grain is important to the incomes of resource-poor farmer's as well as to the nutritional status and diets of people in West and East Africa, Latin America and in the Caribbean Basin. The seed has high protein content and can be consumed directly as grain, and can also be used to make flour, sprouts and weaning food for young children contributing to reduced malnourishment and stunted growth (Philips and Deheh, 2003). A major problem in storage of cowpea seeds in the tropics is infestation by bruchid beetles, especially *Callosobruchus maculatus* Fabricus (Ofuya, 2003). These post-harvest losses of cowpea are a serious problem and in Africa, as much as 20-50% of grain is lost due to this insect-pest infestation. It has also been estimated that about 40% or 30,000 tons, valued at over 30 million dollars, is lost annually to *C. maculatus* (F) (Caswell and Akibu, 1980). These result in grain weight loss, reduced commercial value and quality deterioration.

Insecticides offer quick and immediate solution to pest problems but their application to crops and farm products often results in residue problems and persistence in the ecosystem. Owing to the problems of synthetic organic chemicals, there is renewed interest on plants as alternative materials for use as stored grain protectants because they have been found to have broad spectrum of insecticidal properties compared to synthetic chemicals, cheap, easily available and can be produced within the farmers' vicinity, thus providing a more sustainable approach to pest control (FAO, 1985). Therefore, many scientists have been conducting studies over the last three decades aimed at identifying botanicals that would replace synthetic organic chemicals but the efficacies of the plant mixtures have been less investigated (Emeasor et al., 2007). This research is aimed at evaluating the acute toxicity of *Cymbopogon citratus*

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Table 1. Mortality of *Callosobruchus maculatus* bruchids exposed for 12-96 h to the toxicity of mixed proportions of *Cymbopogon citratus* (L) and *Ocimum suave (W).*

	Logarithm Conc.	100L : 0W					Percentage mortality	Probit mortality	80L : 20W					Percentage mortality	Probit mortality
		Exposure periods (hours)							Exposure periods (hours)						
		12	24	48	72	96			12	24	48	72	96		
0	0.0000	0	0	0	0	0	0	-	0	0	0	0	0	0	-
1	0.3010	0	0	1	1	0	16.67	4.05	0	0	1	1	0	16.67	4.05
2	0.4771	0	1	2	2	0	41.67	4.80	0	1	2	2	0	41.67	4.80
3		1	0	3	1	0	41.67	4.80	0	0	3	1	0	41.67	4.80
$\bar{x}$		0.25^{a}	0.25^{a}	1.5^{ab}	1.00^{ab}	0.00^{b}			0.25^{a}	0.25^{a}	1.50^{ab}	1.00^{ab}	0.00^{b}		

Means of the same superscript letter do not differ significantly ($p>0.05$) using Duncan's test.

(Lemon grass) and *Ocimum suave* (Wild basil) by ascertaining the Median Lethal Concentration (LC_{50}) and the Median Lethal Time (LT_{50}) of the Leaf powder as they affect *C maculatus*.

MATERIALS AND METHODS

Insect stock culture

Adult bruchids were obtained from already infested cowpea and identified as *Callosobruchus maculatus* by the assistance of the Nigerian Stored Product Research Institute (NSPRI) Sapele, Delta State Nigeria. The *C. maculatus* adults obtained were introduced into undamaged cowpea (*Vigna unguiculata*) L seeds of the Kano white variety 1696 and maintained in large specimen bottles with fine mesh gauze covering the opened end. Adult emergence was checked daily and the newly emerged adults were then used for the experiment.

Experimental cowpea

Undamaged and clean cowpea seeds were purchased from Abraka market, Delta state. Each seed was examined under microscope to make sure they were free of damage and eggs laid. They were then kept by deep freezing for one week and left for 24 h under ambient conditions (Ofuya et al., 2007 with slight modifications).

Preparation of insecticidal plant powder

The two plant species used in our study were identified as *Cymbopogon citratus* and *Ocimum suave* by the Botany Department of Delta state University. The plants were obtained from Issele-Azagba, Aniocha North Local Government Area of Delta State. Fresh leaves from each plant were slowly dried for 3 weeks in an open wooden cabinet (1.0 × 0.5 × 1.0 m) under room temperature before pulverization in a motorized high speed-grinder. The powder was passed through a sieve of 0.1 mm mesh size. The particles were then put in an air tight container to prevent active components from evaporating. This method was adopted by Denloye et al. (2007) with slight modification.

Formulation of insecticidal plant materials into treatment combinations

The powders obtained were mixed in the following ratios (Lemon grass: Wild basil): 100:0, 80:20, 60:40, 50:50, 40:60, 20:80 and 0:100. Each combination was replicated two times. The untreated combination (0:0) served as the control for the experiment. Each combination was admixed with 20 g of experimental cowpea seeds at 1, 2 and 3 g concentrations or rates of application.

Efficacy test

Each formulated treatment combination were mixed with 20 g of cowpea seed of various sizes at different concentration of 1 g, 2 g and 3 g and put into Petri dishes with lid. Three pairs of adult *C. maculatus* (3 males and 3 females) were introduced into each petri dish and kept under laboratory conditions.

Data collection

Acute toxicity tests were carried out for 96 h and mortality bruchids was assessed at 12 h interval to determine the Median Lethal concentration (LC_{50}) and Median Lethal time (LT_{50}) in each treatment.

Statistical analysis

All data collected were subjected to Probit analysis (Finney, 1971), Analysis of Variance (ANOVA) and Multiple Comparism using Duncan's Multiple range test and LSD in SPSS statistical package.

RESULTS

The insecticidal effects (mortality) of the plant mixtures on the insects at 100L: 0W and 80L: 20W showed no significant difference ($p>0.05$) as the concentration increased from 1 - 3 g/20 g cowpea seeds. However, there was significant difference ($p<0.05$) in the mortality of the insects as the time of exposure increased from 12 - 96 h (Table 1). For the plant mixtures 60L: 40W, 50L: 50W, 40L: 60W, 20L: 80W and 0L: 100W, there was a significant difference ($p<0.05$) as the concentration of plant materials increased from 1 - 3 g/20 g cowpea seeds. However, there was no significant difference ($p>0.05$) in the mortality of the insects as the time of exposure increased from 12-96 h (Table 2A, 2B and 2C).

Table 2A. Mortality of *Callosobruchus maculatus* bruchids exposed for 12-96 h to the toxicity of mixed proportions of *Cymbopogon citratus* (L) and *Ocimum suave (W)*.

Conc. (g)	Log. conc.	60L : 40W					$\bar{x}$	Percentage mortality	Probit mortality	50L : 50W					$\bar{x}$	Percentage mortality	Probit mortality
		Exposure periods (hours)								Exposure periods (hours)							
		12	24	48	72	96				12	24	48	72	96			
0	0.0000	0	0	0	0	0	0.00^{a}	0	-	0	0	0	0	0	0.00^{a}	0	-
1	0.3010	0	1	0	1	1	0.60^{ab}	25.00	4.33	0	0	1	2	0	0.60^{ab}	25.00	4.33
2	0.4771	0	1	1	2	1	1.00^{b}	41.67	4.80	0	0	1	2	0	0.60^{ab}	25.00	4.33
3		1	1	3	1	1	1.40^{b}	50.33	5.21	1	1	3	1	1	1.40^{b}	50.33	5.21

Means of the same superscript letter do not differ significantly (p>0.05) using Duncan's test.

Table 2B. Mortality of *Callosobruchus maculatus* bruchids exposed for 12-96 h to the toxicity of mixed proportions of *Cymbopogon citratus* (L) and *Ocimum suave (W)*.

40L : 60W					$\bar{x}$	Percentage mortality	Probit mortality	20L : 80W					$\bar{x}$	Percentage mortality	Probit mortality
Exposure periods (hours)								Exposure periods (hours)							
12	24	48	72	96				12	24	48	72	96			
0	0	0	0	0	0.00^{a}	0	-	0	0	0	0	0	0.00^{a}	0	-
0	0	1	1	1	0.60^{ab}	25.00	4.33	0	0	1	1	1	0.60^{b}	25.00	4.33
0	0	2	1	1	0.80^{ab}	33.33	4.57	1	0	2	1	1	1.00^{b}	41.67	4.80
0	0	1	1	2	0.80^{ab}	33.33	4.57	1	1	1	1	1	1.00^{b}	41.67	4.80

Means of the same superscript letter do not differ significantly (p>0.05) using Duncan's test.

Table 2C. Mortality of *Callosobruchus maculatus* bruchids exposed for 12-96 h to the toxicity of mixed proportions of *Cymbopogon citratus* (L) and *Ocimum suave (W)*

0L : 100W					$\bar{x}$	Percentage mortality	Probit mortality
Exposure periods (hours)							
12	24	48	72	96			
0	0	0	0	0	0.00^{a}	0	-
0	0	0	1	1	0.40^{ab}	25.00	4.33
1	0	2	2	0	0.80^{ab}	33.33	4.57
1	0	2	2	1	1.20^{b}	50.00	5.00

Means of the same superscript letter do not differ significantly (p>0.05) using Duncan's test.

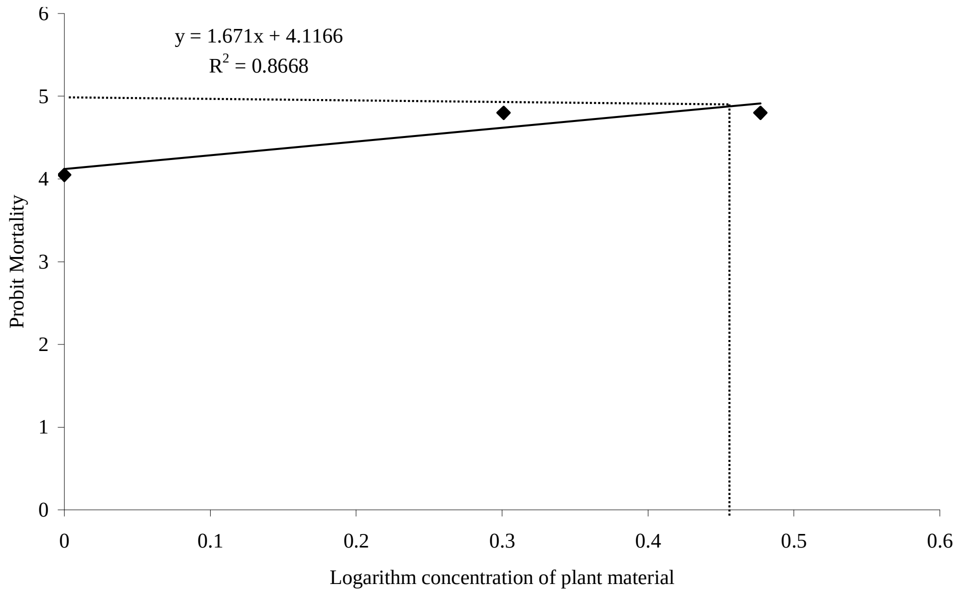

Figure 1. The relationship between probit mortality and logarithm concentration at 100L: 0W to give gram concentration (antilog) at LC_{50}. * L:W represents *Cymbopogon citratus* (Lemon grass): *Ocimum suave* (Wild basil).

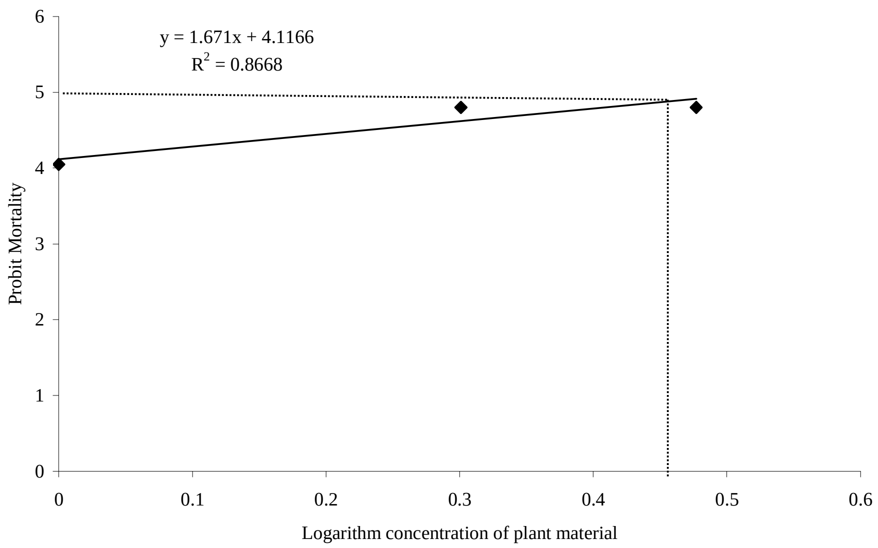

Figure 2. The relationship between probit mortality and logarithm concentration at 80L: 20W to give gram concentration (antilog) at LC_{50}. * L:W represents *Cymbopogon citratus* (Lemon grass): *Ocimum suave* (Wild basil).

The median lethal concentration (LC50) (antilog) for 100L:0W, 80L:20W, 60L:40W, 50L:50W, 40L:60W, 20L:80W, 0L:100W are as follows - 2.80, 2.80, 2.29, 2.69,3.89, 3.90, 3.00 g/20 g cowpea seed (Figures 1, 2, 3, 4, 5, 6 and 7). The median lethal time (LT50) at 3 g for 60:40, 50:50 and 0:100 are 76, 78 and 92 h, respectively (Figures 8, 9 and 10).

DISCUSSION

The plant materials were toxic to the insects as the

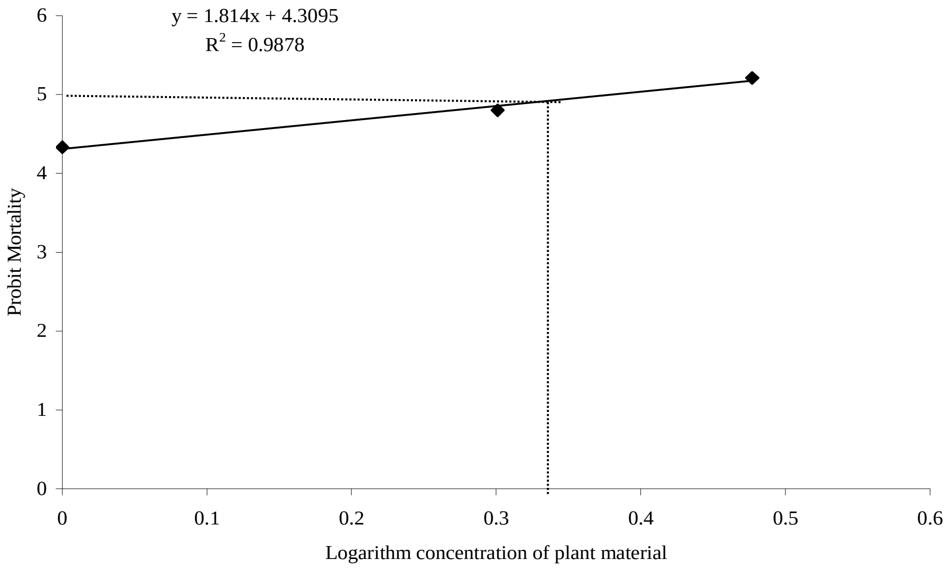

Figure 3. The relationship between probit mortality and logarithm concentration at 60L: 40W to give gram concentration (antilog) at LC_{50}. * L:W represents *Cymbopogon citratus* (Lemon grass): *Ocimum suave* (Wild basil).

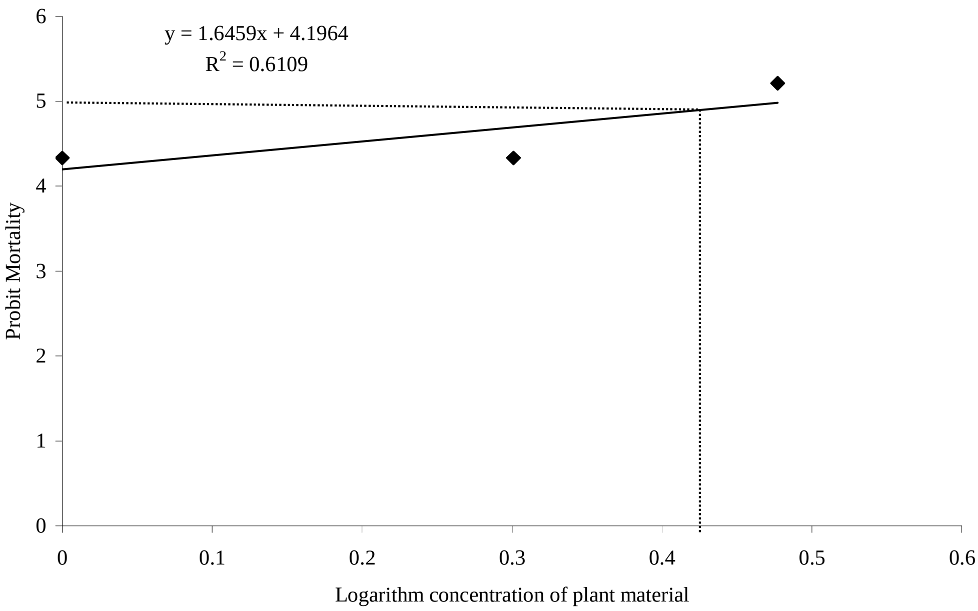

Figure 4. The relationship between probit mortality and logarithm concentration at 50L: 50W to give gram concentration (antilog) at LC_{50}. * L:W represents *Cymbopogon citratus* (Lemon grass): *Ocimum suave* (Wild basil).

concentration increased from 1-3 g/20 g cowpea seeds. The toxicity of the mixtures of plant materials in their different proportions could be as a result of the volatile compounds in the plant materials which acted as fumigants with insecticidal effects on the bruchids. Dike and Mbah (1992) reported that *C. citrates* contains fumigants which produced insecticidal effects on *C. maculatus* and that at 20 g of plant materials (*C. citratus*) per 100 g cowpea seeds protected the cowpea against *C. maculatus* after 8 weeks of post treatment. The main constituent of *C. citratus* is citral. There is a mixture of two stereo isomeric monterpene aldehydes, in lemon

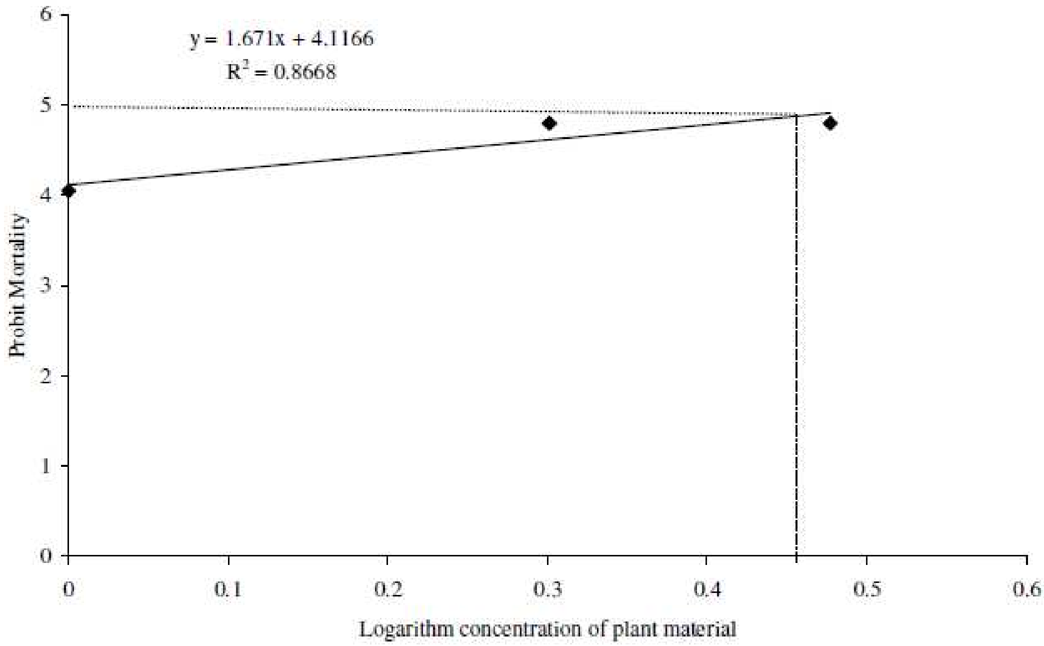

Figure 5. The relationship between probit mortality and logarithm concentration at 40L: 60W to give gram concentration (antilog) at LC_{50}. * L:W represents *Cymbopogon citratus* (Lemon grass): *Ocimum suave* (Wild basil).

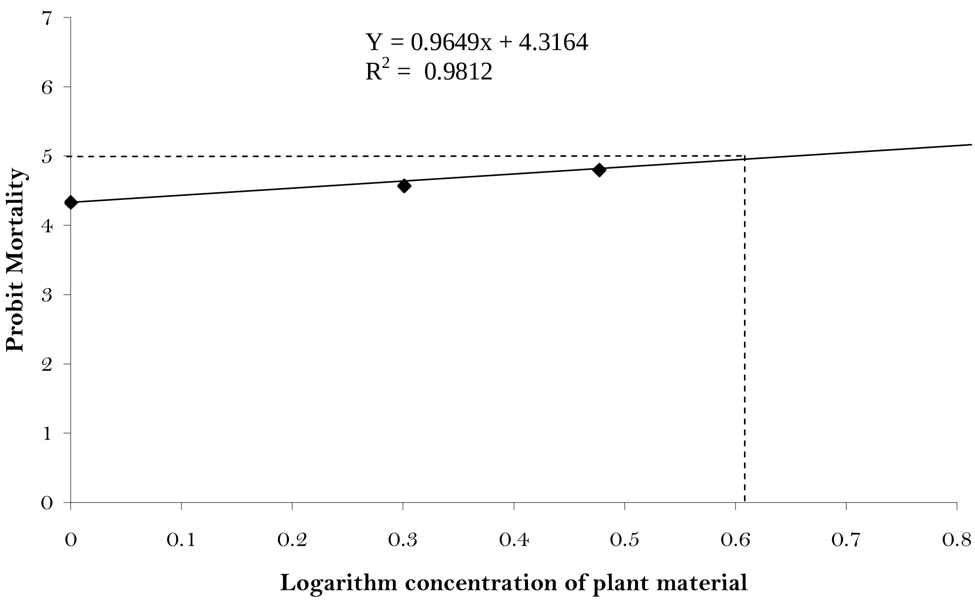

Figure 6. The relationship between probit mortality and logarithm concentration at 20L: 80W to give gram concentration (antilog) at LC_{50}. * L:W represents *Cymbopogon citratus* (Lemon grass): *Ocimum suave* (Wild basil).

grass oil and the trans isomer geranial (40 to 62%) dominates over cis isomer neral (25 to 38%). Ilondu et al. (2004) also observed that *Ocimum suave* (Wild basil) caused mortality of adult *Rhizopertha dominica* in grain cereals at 2 - 8 g plant materials per 50 g cereals (Rice, Maize and Sorghum). This protected the cereals after 90

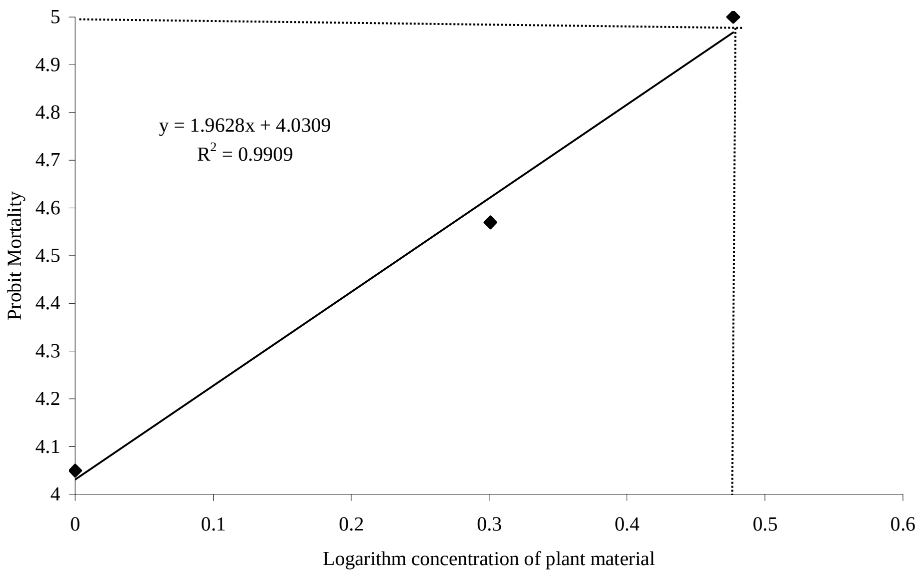

Figure 7. The relationship between probit mortality and logarithm concentration at 0L: 100W to give gram concentration (antilog) at LC_{50} . * L:W represents *Cymbopogon citratus* (Lemon grass): *Ocimum suave* (Wild basil).

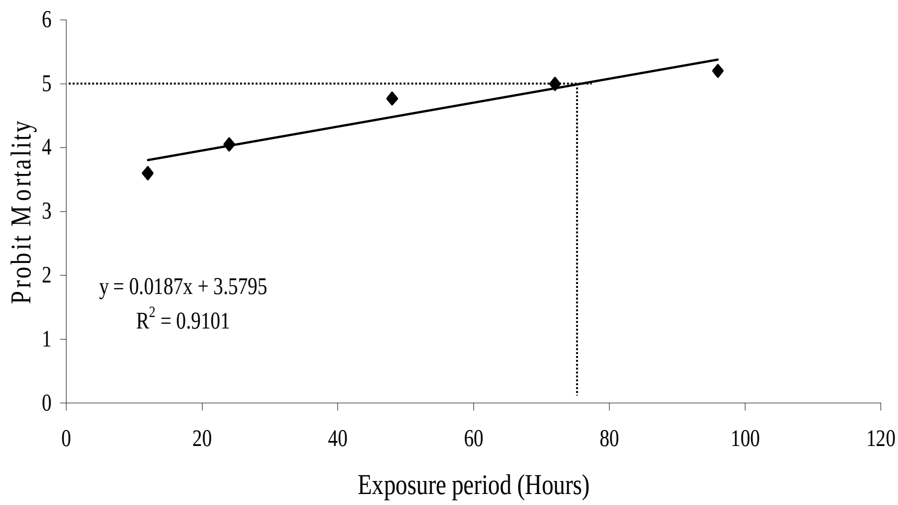

Figure 8. The relationship between probit mortality and exposure period at 60L: 40W to give LT_{50}.

days post treatment. The main constituent of *Ocimum suave* includes eugenol and mono and sesquiter penoids (Hassanli et al., 1990). The toxicity reported in this study may have been due to the toxic effects of the compounds in the plant materials compounds (*Cymbopogon citratus* and *Ocimum suave*).

It was observed that in the different combinations, as the proportion of *Cymbopogon citratus* was decreasing (60-0) and proportion of *Ocimum suave* was increasing (40-100), the mortality generally was significant (p<0.05) as the concentration of the plant material mixture increased from 1-3 g/20 g cowpea seeds (Table 2). The plant combination 60L: 40w was most effective with an LC50 of 2.29 g/20 g cowpea seeds. The low LC50 may be that there was an addictive effect from mixing the two plant materials (*C. citratus* and *O. suave*) at 60L: 40W.

Fayape (1998) observed addictive effects from shoots of *Nicotiana tabacum* with *Piper guineense* powder used against *C. maculatus* in cowpea. The predominant proportion of *C. citrates* in the plant mixture (100L: 0W)

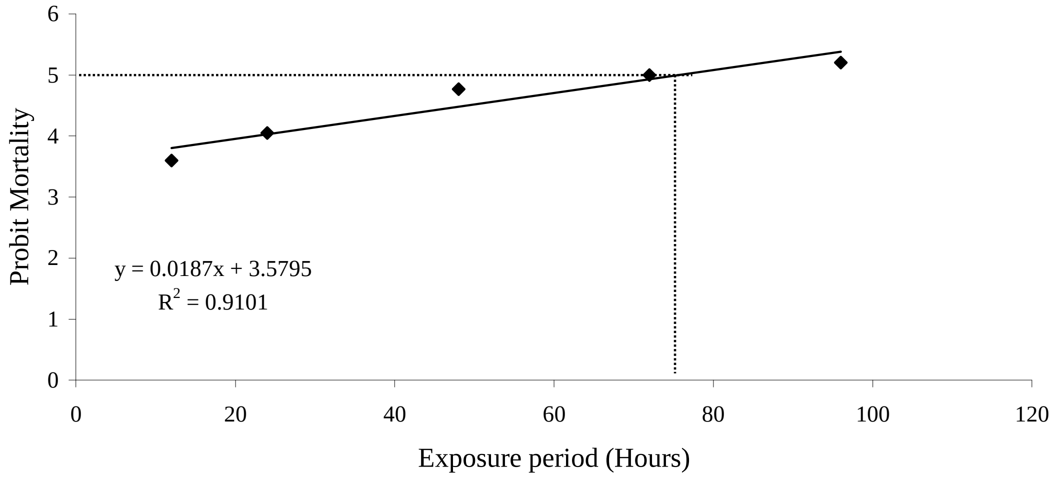

Figure 9. The relationship between probit mortality and exposure period at 50L: 50W to give LT_{50}. It was at 3g concentration that 50% mortality of adult *C. maculatus* was obtained.

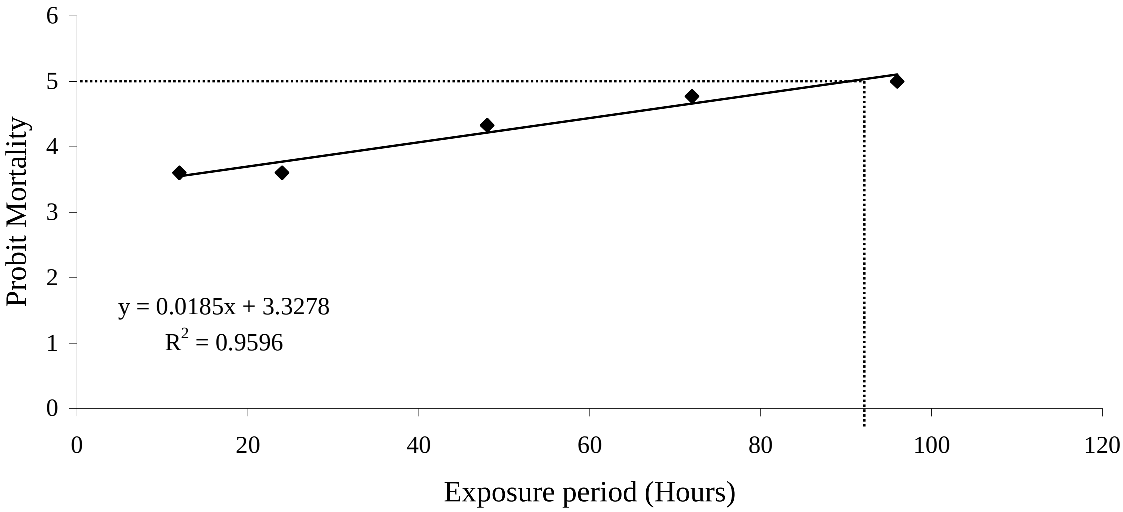

Figure 10. The relationship between probit mortality and exposure period at 0L: 100W to give LT_{50}. It was at 3 g concentration that 50% mortality of adult *C. maculatus* was obtained.

and (80L: 20W) caused no significant difference (p>0.05) in the mortality amongst the concentrations (1 - 3 g/20 g cowpea seeds) of plant materials. However, there was significant difference (p<0.05) in mortality amongst the exposure periods (12 - 96 h) (Table 1). The mortality may have been significant if the concentration (grams) was further increased to 4 g and above. Dike and Mbah (1992) observed that 20 g of *Citratus* /100 g cowpea (4 g of *Citratus* /20 g of cowpea seed) protected the cowpea seeds 8 weeks after post treatment. The plant mixture may be effective over time. It appeared that time for the plant materials (*Cymbopogon citratus* and *Ocimum suave*) to kill 50% of adult *C. maculatus* became obvious only at 3 g (Figures 8, 9 and 10). This indicates that 50% mortality occurred as the concentration of plant material increased (3 g). 60L: 40W had the least LT50 (76 h). This could also mean that there were addictive effects from mixing the two plant materials (*C. citratus* and *O suave*) at 60L: 40W. Similar findings on mortality of *C. maculatus* were obtained by Arannilewa (2002) using extracts of 4 plant materials which included; *Alstonia boonei* (Ahun), *Vernonia amygdalina* (bitter leaf), *Cymbopogon citratus* (lemon grass) and *Gladiolus actinomorphanthus* (Baka) against *C. maculatus*. Mortality was recorded in the adult bruchids. Emeasor et al. (2007) reported that mixing seeds powders of *Piper guineense* and *Thevetia peruviana* in the proportion of 80:20 caused 56.4% mortality of adult bruchids. Dawudo and Ofuya (2000) observed that the mixture of the fruit powders of *Piper guineense* and *Dennettia tripetala* in equal proportion (50:50) significantly caused mortality, reduced oviposition and adult emergence. Echendu (1991) reported that liquid of cashew nuts caused mortality of adult *C. maculatus*. This study has shown that mixed proportions of *C. citratus* (Lemon grass) and *Ocimum suave* (Wild basil) especially at 60L: 40W could be most effective because it has the lowest LC50 (2.29 g/20 g cowpea seeds) and also the shortest LT50 (76 h). However, there is need for more investigation to identify other local plant material mixtures that would be best used in storage of cowpea against the disturbing infestation of *C. maculatus.*

REFERENCES

Arannilewa ST (2002). Assessment of the insecticidal properties of four plant extracts as protectants against the cowpea Bruchid *Callosbruchus maculatus* (F) (Coleoptera: Bruchidae). Ann. Agric. Sci., 3(1): 49-55.

Caswell GH, Akibu S (1980). The use of pirimipho-methyl to control bruchids attacking selected varieties of stored cowpea. Trop. Grain Legume Bull., 17/18:9-11.

Dawudo EO, Ofuya TI (2000). Effects of mixing powders of *Piperguineense* Schum and Thonn and *Dennettia tripetala* Baker on oviposition and adult emergence of *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) infesting cowpea seeds in storage. Appl. Trop. Agric., 5: 156-160.

Denloye AA, Makanjuola WA, Don-Pedro KN, Negbenebor HE (2007). Insecticidal effects of *Tephrosia vogelii* Hook (Leguminosae) leaf powder and extracts on sitophilus zeamais motsch, *Callosobruchus maculatus F.* and *Tribolium castaneun* Herbst. Niger. J. Entomol., 24: 91-97.

Dike MC, Mbah OT (1992). Evaluation of the lemon grass *{Cymbopogon citratus)* products on control of *Callosobruchus maculatus F* (Coleoptera: Bruchidae) on stored cowpea. Niger. J. Plant Prot., 14: 81-91.

Echendu TNC (1991). Ginger, cashew and neem as surface protectants of cowpea against infestation and damage by Callosobruchus maculatus (Fab). Trop. Sci., 31: 209-211.

Emeasor, KC, Emosairue SO, Ogbuji RO (2007). Comparative susceptibility of some cowpea (*Vigna unguiculata* (L) Walp) Accessions, Breeding lines and Winged Bean to the Cowpea Bruchid, *Caollosobruchus maculates* (F) (Coleoptera: Bruchidae). Niger. J. Entomol., 24: 131-136.

FAO (1985). Handling and storage of food grains. Food and Agriculture Organization of the United Nations, Rome. http://www.blackherbals.com/using natural pesticides. htm. Assessed 07/03/2008.

Fayape OE (1998). Effects of some plant materials on oviposition and adult emergence of *C. maculatus* (F) *M. Agrc. Tech. Thesis.* The Federal University of Technology, Akure, Nigeria, 43 pp.

Finney DJ (1971). Statistical method in Biological Assay second edition, London.

Hassanli A, Lwande W, Ole-Sitayo N, Moreka L, Nokoe S, Chapy G (1990). Weevil repellent constituents of *Ocimum suave* leaves and Eugenia. Caryophyllata cloves used as grain protectants in part of E. Africa. Discov. Innov., 2(2): 91-95.

Ilondu EM, Egwunyenga OA, Iloh AC (2004). The effects of wild basil (Oamum *suave)* Wild Labiatae: Lamiaceae) leaf dust as protectant of cereals against *Rhizoperth dominica* Fabricus (Coleoptera: Bostrydae). J. Nat. Acad. Adv. Sci., 3: 129-134.

Ofuya TI (2003). Beans, insects and man. Inaugural lecture series 35, The Federal University of Technology Akure Nigeria, 45 pp.

Ofuya TI, Olotuah OF, Ala desanwa RD (2007). Potential of Dusts of *Eugenia aromatica* Baill. Dry Flower Buds and *Piper guineense* Schum and Thonn Dry fruits formulated with three organic flours for controlling *Callosobruchus maculatus* Fabrieus(Coleoptera: Bruchidae). Nig. J. Bnt., 24: 98-106.

Philips RD, Dedeh SS (2003). Developing nutritional and economic value added food products from cowpea. http://www.economicisp.msu.edu/orsp/ Final Report. PDF. Assessed 07/02/08.