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Insecticidal Efficacy of Plumeria Species Leaf Extract on Two Economically Important Insects Populations: Mosquito (*Anopheles*) and Bean Weevils (*Callosobruchus Maculatus*)

Aguoru, C. U.[*], Offia, K.L. and Olasan, J. O.

Department of Biological Sciences, University of Agriculture, Makurdi. Nigeria.

ABSTRACT

Insecticidal efficacy of two *Plumeria species (P. rubra and P. obtusa)* foliar extracts was tested on mosquito and beans weevil populations. The extent of efficacy was analysed using linear regression model. The aim was to determine if the plant, as a safer biological control method, could possibly solve the problem of increasing populations of these insects implicated in malaria ailment and food shortages respectively. Completely randomized experimental designs were set up with 4 interaction effects. Each treatment had 5 levels of concentration applied on the test organism and replicated 4 times. Number of dead organisms was recorded at a constant time interval. Percentage mortality was computed at 48hrs. Correlation coefficient R and Coefficient of determination R2 were computed. Equations connecting the dependent variable (mortality) and independent variable (extract concentration) were formulated with corresponding t-values at 95% confidence interval. Residual statistics was also computed. Explanatory power of the extract-mortality model was depicted by analysis of variance. Reliability Statistics of the overall result was tested using the Cronbach's Alpha. As a result, P. obtusa yielded mosquito mortality rate of 86.2% while P. rubra was 88.75%. *P. obtusa and P. rubra* accounted for 90% and 95% weevil mortality respectively. Correlation coefficients were very high (*Plumeria*–mosquito =0.970; *Plumeria*-weevil =0.968) yielding high coefficients of determination R2 (94% and 93.7% respectively). The two linear regression equations thus allows

***Correspondence to Author:**
Dr. C.U. Aguoru
Department of Biological Sciences, University of Agriculture, Makurdi. Nigeria.
Email:celeaguoru@ yahoo.com

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for accurate predictability of the mortality rate of the insects with 95% confidence limit. Mosquito mortality rate= -7.812 + 38.875 x (Extract concentration). Weevil mortality rate=-4.875 + 39.250 x (Extract concentration). The explanatory power of the two models is significantly high ($p<0.05$). The overall result of the study was 98.9% reliable and consistent (Cronbach's Alpha= 0.989). Having confirmed the efficacy of the plant on the two insects using an elaborate statistical approach, the crude extract could be refined and exploited for this purpose. This may find applications in public health, pest and disease management, food preservation and crop protection.

Key words:
Plumeria, foliar extract, efficacy, insecticidal, regression analysis, mosquito, weevils

INTRODUCTION

The Genus *Plumeria* (Family Apocynaceae) consists of different species commonly cultivated for ornamental purposes in Nigeria. Different alluring species and varieties exist as a result of frequent inter and intra specific hybridization respectively (Scot, 2009[16]; Zumbroich *et al*., 2013[21]). This phenomenon mostly accounts for the variation in this Genus as reflected in different floral colours (Aguoru *et al*., 2015a)[1]. *Plumeria* species are easily cultivated by cutting and they are one of the best Genera known for drought tolerance (Dutta, 2007)[8]. These features make them cosmopolitan and successful (Eggenberger and Eggenberger, 2000)[9]. Apart from their ornamental function, ethno-medicinal uses of the plant have been reported (Kardono *et al.,* 1990[13]; Wiart, 2002[20]; Shinde *et al*., 2014[18]; Bobbarala *et al*., 2009[6]). Another important characteristic of the plant is the phenomenon of self-defense mechanism as the leaf is toxic to most insects, herbivores, annelids and other destructive animals (Sallam, 1999[15]; Isman, 2000[11]). This also makes them abundant in any environment.

Environmental scientists have recently criticized the continuous use of chemical insecticides for domestic, industrial and agricultural purposes (Asthana and Asthana, 2012[4]; Aguoru *et al*., 2015b[2]). This is because chemically synthetic biocides are lethal to a wide spectrum of biotic and abiotic components of the environment (Taylor *et al*., 2007[19]; Asthana and Asthana, 2012[4]). This results in biodiversity loss and health related challenges such as carcinogenesis (Aguoru *et al*., 2015b[2]). They have also been implicated in the destruction of the protective ozone layer causing ozone thinning thus exposing the human race to the deleterious effect of UV rays (Taylor *et al*., 2007[19]; Asthana and Asthana, 2012[4]). The 1992 United Nations Earth Summit recommended a drastic reduction in the use of chemical biocides for the biosphere to remain sustainable (CBD, 1992[7]). It is therefore important to seek alternative means of controlling unwanted insects. The use of natural plant product has been suggested being an old practice (Sallam, 1999[15]). Aguoru *et al*. (2015c[3]) scientifically confirmed the efficacy of three species of pepper on insect pests of stored groundnut. According to Taylor *et al*. (2007[19]), plant based insecticides are safe, cheap, effective, degradable and environmentally friendly. Products such as pyrethrum and nicotine have been extracted from Neem, garlic and tobacco, and they are commercially patronized in most advanced countries.

Plumeria species are known for their wide distribution, availability and ease of propagation even in extreme environmental conditions. Hence, they are suitable candidates for scientific investigation (Kalam *et al.* 2013[12]). This research aimed at investigating the insecticidal efficacy of two species of *Plumeria* (*P. rubra* and *P. obtusa*) on *Anopheles* mosquito (the female is a carrier of malaria parasite called *Plasmodium*) and *Callosobruchus maculatus* (a natural pest/weevil of bean grain). Models were designed to determine the efficacy and extent to which each *Plumeria* extract (regressor) affects insect mortality (dependent variable) using equations connecting the two variables. The outcome may be of help on how to combat the negative consequences of these insects.

MATERIALS AND METHODS

Water based extracts were separately prepared from

Table 1a: *Plumeria obtusa*-mosquito interaction (POM)

Concentration (ml)	Number of dead insects per replicate/(20)				Average dead insect @48 hrs	Percentage Mortality
	R1	R2	R3	R4		
0.5	3	5	4	3	3.75	18.75 %
1.0	4	5	3	7	4.75	23.75 %
1.5	10	13	9	16	12.0	60.0 %
2.0	18	14	16	15	15.75	78.75 %
2.5	17	16	18	18	17.25	86.25 %

Table 1b: *Plumeria rubra*-mosquito interaction (PRM)

Concentration (ml)	Number of dead insects per replicate/(20)				Average dead insect@48 hrs	Percentage Mortality
	R1	R2	R3	R4		
0.5	2	1	4	3	2.5	12.5 %
1.0	6	5	6	4	5.25	26.25 %
1.5	9	7	8	6	7.5	37.5 %
2.0	12	17	13	16	14.5	72.5 %
2.5	18	17	17	19	17.75	88.75 %

Table 2a: *Plumeria obtusa*-weevil interaction (POW)

Concentration (ml)	Number of dead insects per replicate/(20)				Average dead insect @48 hrs	Percentage Mortality
	R1	R2	R3	R4		
0.5	6	4	6	4	5	25%
1.0	8	8	6	8	8	40%
1.5	8	10	12	9	9.75	48.75%
2.0	16	14	15	20	16.25	81.25%
2.5	20	18	18	16	18	90%

Table 2b: *Plumeria rubra-weevi*l interaction (PRW)

Concentration (ml)	Number of dead insects per replicate/(20)				Average dead insect @48 hrs	Percentage Mortality
	R1	R2	R3	R4		
0.5	5	8	4	6	5.75	28.7 5%
1.0	9	9	8	6	8	40 %
1.5	10	8	9	10	9.75	48.75 %
2.0	15	15	15	12	14.25	71.25 %
2.5	18	19	20	19	19	95 %

Table 2c: Control experiment (water-mosquito interaction)

Water Concentration (ml)	Number of dead insects per replicate/(20)				Average dead insect @48 hrs	Percentage Mortality
	R1	R2	R3	R4		
0.5	0	1	0	1	0.5	2.5%
1.0	1	0	0	0	0.25	1.25%
1.5	0	2	0	0	0.5	2.5%
2.0	0	1	1	0	0.5	2.5%
2.5	1	0	0	1	0.5	2.5%

Table 3a: Combined effect of *Plumeria* extract on mosquito

Mosquito-Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F
1	**.970[a]**	**.940**	.933	7.76045	.940	125.469	1	8	.000

Coefficient of Determination R^2=94%

Table 3b: Formulation of equation connecting Mortality and Extract-concentration in mosquito

Mosquito Model B	Unstandardized Coefficients		Standardized Coefficients	t	Sig. Lower Bound	95.0% Confidence Interval for B	
	Std. Error	Beta				Upper Bound	
Constant	**-7.812**	5.755		-1.357	.212	-21.084	5.459
Extract	**38.875**	3.471	.970	11.201	.000	30.872	46.878

Linear regression equation for Anopheles is given as: **Mortality= -7.812 + 38.875** X **(Extract Conc.)**

Derived from Y=a+bX, where, Y= Mortality, a= -7.812, b=38.875

Table 3c: Residual Statistics for mosquito mortality rate

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	**11.6250**	**89.3750**	**50.5000**	28.97571	10
Residual	-13.00000	9.50000	.00000	7.31662	10
Std. Predicted Value	-1.342	1.342	.000	1.000	10
Std. Residual	-1.675	1.224	.000	.943	10

a. Dependent Variable: Mortality

leaves of *P. rubra* and *P. obtusa* collected within the University of Agriculture Makurdi, Nigeria. Test organisms (mosquito and bean weevil) collected from stagnant water and infested beans respectively were cultured until sufficient populations emerged. Completely randomized experimental designs were set up in a 2x2 factorial combinations of extracts and test organisms forming 4 interaction effects: *P. obtusa*-mosquito (POM); *P. rubra*-mosquito (PRM); *P. obtusa*-weevil (POW) and *P. rubra*- weevil (PRW) interactions. Each treatment had 5 levels of concentrations: 0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml applied on an experimental unit of 20 insects in a container, replicated 4 times. A control experiment was designed where equal number of insects was also localized and different levels of water concentrations applied instead of plant extracts. After 48hrs post-exposure, number of dead organisms in each container was recorded, though observations were made at intervals of 2hrs post exposure. Average number of all dead organisms per level of extract was calculated. Percentage mortality was also computed.

Data was analysed on the SPSS version 20.0 software. Linear regression model analysis was carried out on the combined effect of both extracts on each test organism. These yielded many statistical parameters such as: Correlation coefficient R, Coefficient of determination R^2, Standard error of estimates, and significant F value. Equations connecting the dependent variable (mortality) and independent variable (extract concentration) were formulated with corresponding t-values at 95% confidence interval. This was done to allow for instant prediction of mortality rate given any concentration of *Plumeria* extract. Residual statistics was also computed. Explanatory power of the extract-mortality model was depicted by analysis of variance. Reliability Statistics of the overall result was tested using the Cronbach's Alpha. Linear regression plots were constructed accordingly.

RESULTS AND DISCUSSION

P. obtusa yielded above LD_{50} mosquito mortality at 1.5ml, recording the highest mortality rate of 86.2% (Table 1a). LD_{50} and above was achieved at 2.0ml of *P. rubra* having the highest mosquito mortality rate of 88.75% (Table 1b). *P. obtusa* killed 90% of weevils (Table 2a) while *P. rubra* killed 95% of the organism, a higher mortality effect than in mosquito (Table 2b). In both cases, LD50 and above was achieved at 2.0ml of the extracts. The control experiment yielded no significant mortality on mosquito (Table 2c). The combined effect of *Plumeria* extract on mosquito shows a high positive correlation of 0.970 with a significant coefficient of determination R^2 of 94% (Table 3a). This is the percentage of dead mosquito explained by the extract only. The remaining 6% unexplained might be due to other factors such as overcrowding and residual effect. The overall linear regression equation connecting mosquito mortality and extract is derived from Table 3b. This is given as: Mortality= -7.812 + 38.875 x (Extract Conc.). By knowing the value of *Plumeria* extract, the mortality rate of mosquito can be significantly predicted with 95% confidence limit, the minimum and maximum predicted mortality rate being 12 and 89 respectively and a mean value of 51% (Table 3c). The model is good to explain the variation in mosquito mortality caused by the extract (as the predictor) with a significant F-value of 125.469 ($p<0.05$) as given in Table 3d.

The combined effect of *Plumeria* extract on beans weevil is shown in Table 4a. They are highly positively correlated (0.968). The coefficient of determination is 93.7%. This is the proportion of death in weevil population significantly explained by the extract only. The linear regression equation connecting both variables as given in Table 4b is: Mortality= -4.875 + 39.250 x (Extract Conc.). With 95% confidence, the minimum and maximum predicted mortality values will be 15% and 93% respectively (Table 4c). This model also significantly explains the variation in weevil mortality caused by the extract used with a significant F-value of 118.862 ($p<0.05$) (Table 4d). The overall result obtained in this study is 98.9% reliable and consistent from the Cronbach's Alpha value (Table 5). Regression plots (Fig.1 and Fig.2) have clearly revealed a positive linear relationship between both variables measured, as increase in one would be accompanied by an increase in the other variable.

This elaborate analysis has statistically confirmed the insecticidal efficacy of *Plumeria* extracts on both mosquito and bean weevil studied. This agrees with the work of Sallam (1999[15]) and Isman (2000[11]) that the plant is toxic to insects and herbivores as a self-defense mechanism. The mode of action of this extract is still unclear, possibly by exerting genotoxic and larvicidal effect, though cytotoxic effect of the bark of the plant has been reported (Kardono *et al*., 1990[13]). Kalam *et al.* (2013[12]) reported the presence of high quantity of saponin in *Plumeria* leaf.

Table 3d: Model explanatory power in mosquito mortality using ANOVA

Mosquito Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	7556.328	1	7556.328	**125.469**	**.000**[b]
Residual	481.797	8	60.225		
Total	8038.125	9			

a. Dependent Variable: Mortality
b. Predictors: (Constant), Extract

Table 4a: Combined effect of *Plumeria* extract on beans weevil

WeevilModel	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	**.968**[a]	**.937**	.929	8.05014	.937	118.862	1	8	**.000**

Coefficient of Determination R^2=93.7%

Table 4b: Formulation of equation connecting Mortality and Extract-concentration in bean weevil

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
B		Std. Error	Beta			Lower Bound	Upper Bound	
1	(Constant)	**-4.875**	5.970		-.817	.438	-18.642	8.892
	Extract	**39.250**	3.600	.968	10.902	**.000**	30.948	47.552

Linear regression equation for bean weevil is given as: **Mortality= -4.875 + 39.250** X **(Extract Conc.)**

Derived from Y=a+bX, where, Y= Mortality, a=-4.875, b=39.250.

Table 4c: Residual Statistics for beans weevil mortality rate

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	**14.7500**	**93.2500**	**54.0000**	29.25522	10
Residual	-14.75000	10.25000	.00000	7.58974	10
Std. Predicted Value	-1.342	1.342	.000	1.000	10
Std. Residual	-1.832	1.273	.000	.943	10
a. Dependent Variable: Mortality					

Table 4d: Model explanatory power in weevil mortality using ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7702.813	1	7702.813	**118.862**	**.000[b]**
	Residual	518.438	8	64.805		
	Total	8221.250	9			

a. Dependent Variable: Mortality

b. Predictors: (Constant), Extract

Table 5: Cronbach's Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	No of Items
.989	.991	4

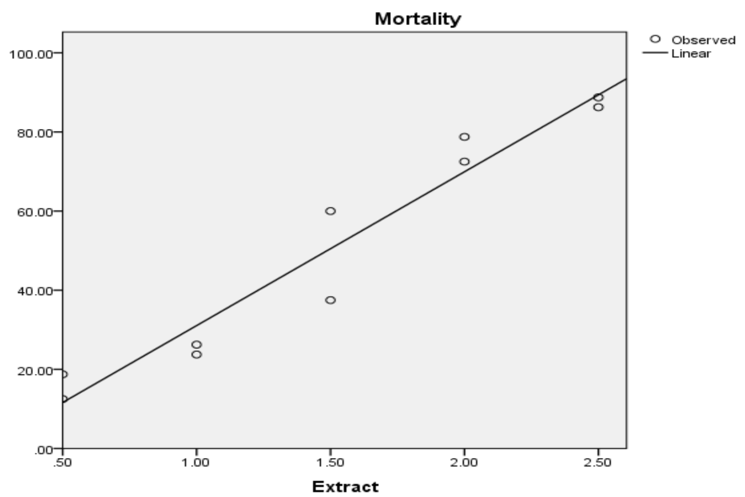

Figure 1: Linear regression line in mosquito

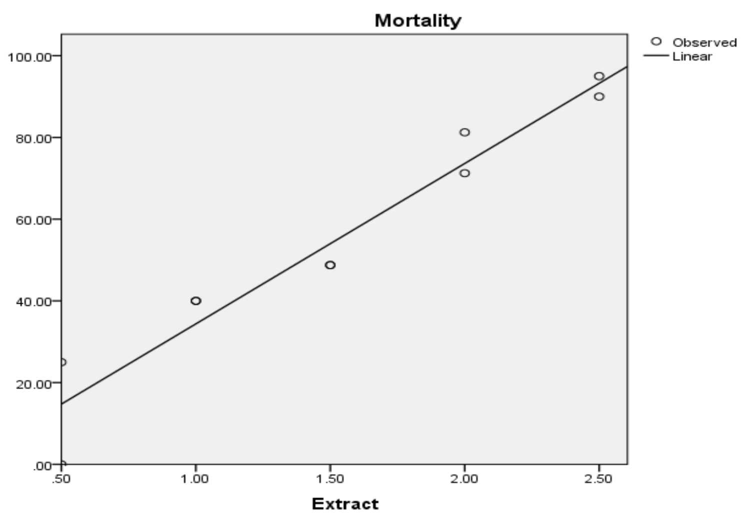

Figure 2: Linear regression line in weevil

This active ingredient is not only therapeutic in curing many life threatening ailments in man, but also effective against pathogens (Morisaki *et al*., 1995[14]; Fleischer, 2003[10]; Shaida *et al.,* 2008[17]). The insecticidal effect of this plant may therefore be due to the high saponin content. According to Kalam *et al.* (2013[12]), factors to be considered if such a plant is to be explored for this purpose includes: longevity, availability, ease of propagation, high toxicity, and ease of extraction, physiological tolerance and other economic uses. *Plumeria* plant, having met all the above requirements should therefore be commercially utilized in combating mosquito proliferation as the carrier of *Plasmodium* (malaria parasite) as well as food bio-deterioration caused by weevil. This may also find application in pest and disease management for crop protection (Isman, 2000[11]). The dependence on use of chemical insecticide known to be harmful to man and the environment will automatically reduce when properly utilized (Aguoru *et al*., 2015b[2]; Batta *et al*., 2013[5]). However, more elaborate phytochemical and toxicological studies need to be carried out on this plant that may provide vital information on its active principles, persistence and specificity on diverse organisms.

CONCLUSION

Having confirmed the efficacy of the plant on the two insects using an elaborate statistical approach, the crude extract could be refined and exploited for this purpose. This may find applications in public health, pest and disease management, food preservation and crop protection.

CONFLICT OF INTERESTS

We the authors declare that we have no conflict of interests.

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