



Toxicity of crude extracts of *Ocimum suave* leaf oil on cowpea weevil (*Callosobruchus maculatus*) (f) (coleoptera; bruchidae) and test of cowpea seeds' viability.

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Abstract

Toxicity of *Ocimum suave* (Wild basil) crude oil extract on *C. maculatus* and test on seeds' viability, was determined in the laboratory using contact toxicity assay method of different concentration 0.02, 0.04, 0.06 and 0.08mg to 20ml of water and 0.00g/20ml of water (control). 20g of cowpea seeds were weighed into separate Petri dishes and 10 adult weevils were inoculated and replicated four times. Each treatment was observed for 96h and recorded after every 12h. The result indicated that the crude oil extract had significant difference ($p < 0.05$) on the mortality of the adult weevil as the concentration increased from 0.02 – 0.08mg/20ml of water and as the period of exposure increased from 24h – 96h. The highest concentration used recorded the highest mortality (85.0%) after 96h of treatment compared to the untreated control (0%). The median lethal concentration (LC_{50}) was Antilog $-2.08 = 0.0083$ mg/20ml of water and lethal time (LT_{50}) was 30.0 hours. There was no significant difference ($p > 0.05$) in the germination of treated cowpea seeds (*Vigna unguiculata* L) compared to the untreated control, which shows that the seeds still remained viable. This result showed that *O. suave* crude oil is effective in the protection of cowpea against cowpea bruchid *Callosobruchus maculatus*.

KEY WORDS: *Ocimum suave* (Wild basil), *Callosobruchus maculatus*, *Vigna unguiculata* (Cowpea), Viability.

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INTRODUCTION

Cowpea *Vigna unguiculata* (L) walp is widely grown in the tropics and subtropics for human consumption as well as animal feeds. Nigeria, Brazil and Niger are among the major producers and account for over 70% of the world's cowpea production (Umar, 2008). Cowpea is also known as blackeye pea, southern pea and crowder pea which is a legume of African origin that is useful as a rational cover-crop and for the production of high quality hay or silage, when mixed with crops such as corn or sorghum, or it can be used for rational grazing (Valenzuela and Smith, 2002). The seed can be consumed directly, can also be used to make flour, sprouts and weaning food for young children thus, reducing manourishment and stunted growth (Philip and Deheh, 2003). The tender green pods and leaves serve as a vegetable while the crop residue serves as fodder for animals (Ntonifor et al., 2010).

A major problem in storage of cowpea seeds in the tropics is infestation by bruchids especially *Callosobruchus maculatus* fabricus (Ofuya, 2003). The cowpea weevil mutplies very fast in storage, giving rise to a new generation every month (Baidoo et al., 2010). The cowpea weevil *Callosobruchus maculatus* (F) belongs to the family Bruchidae members of which are generally associated with the plant family fabaceae, which is capable of causing up to 50% infestation of stored cowpea (Umar, 2008). Farm storage for six months can lead to (a 70% grain infestation and 30% weight loss which renders the grains virtually unfit for consumption (Ntonifor et al., 2010). Cowpea grains losses are a serious problem in Africa and as much as 20 - 50% of grain is lost because of infestation due to this pest. It has also been estimated that about 40% or 30,000 tones valued at over 30 million dollars is lost annually to *C. maculates* (F) (Caswell and Akibu, 1980).

The uses of synthesized chemicals like insecticides and fumigants are of great contribution to fight against insect pests (Owolabi et al., 2009). Owing to the problems of synthetic organic chemical there is renewed interest on plants as alternative materials for use as stored grain protectants because they have been found to have broad spectrum insecticidal properties with reduced persistence compared to the organochlorines and organophosphates, carbamates and pyrethroids. They are also cheap to purchase and have no negative impact on the environment because they

are easily biodegradable (Ojianwuna and Umoru, 2010). The use of serve natural compounds in the protection of grains requires them to be applied in high concentrations, which often exceeds the threshold of acceptable flavour to the consumer (Nazer et al., 2005). The current re-newed interest in reducing interest in reducing environment contamination and global warming have served as an added impetus for the re-evaluation and intensification of environment mentally friendly and cost-effective pest management technologies such as the use of traditional botanical pest control agents (Ntonifor et al., 2010). The objective of this study therefore was to evaluate the Toxicity of *Ocimum suave* (Wild basil) oil extract on *Callosobruchus maculatus* (F) cowpea weevil and the seed's viability in storage.

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* cowpea (*Vigna unguiculata*) L. seed of kano white variety and maintained in large specimen containers with fine mesh gauze covering the opened end, for them to mate and oviposit under laboratory conditions. Adult emergence was checked daily and was observed on the third week which was then used for the experiment(Ojianwuna and Umoru 2010).

Experimental Cowpea

Undamaged and clean cowpea seeds that were used were purchased from Abraka market, Delta State. Each seed was examined under microscope to make sure there were no damages, eggs laid and exit holes on them. They were kept in containers in the laboratory.

Preparation Of Insecticidal Plant Powder

The research plant identified as *Ocimum suave* by Botany Department of Delta State University was used for the experiment. The plant was obtained from Isele-Azagba (Aniocha North) Local Government Area of Delta State in April 2011. Fresh leaves from the plant was slowly dried for 3 weeks in an open wooden cabinet (1.0 x 0.5 x 1.0m) under room temperature before pulverization in the motorized high speed grinder. The powder were put in a air tight container to prevent active components from evaporating. This method was adopted by Ojianwuna and Umoru 2010.



Extraction Of Crude Oil

The *O. suave* plant powder was dissolved in methanol and extracted using a soxhlet extractor. The methanol was then recovered using a rotary evaporator. The extracted oil was then put in a dark air tight container to prevent the active ingredients from escaping then kept in the refrigerator until needed.

Insecticidal Bioassay

Contact toxicity of *O. suave* extracts

Contact toxicity assay was done according to Kim et al., (2003) with some modifications. 20g of cowpea grains in Petri dishes were coated with 0.02, 0.04, 0.06 and 0.08mg plant extracts dissolved in 20ml of water respectively. Ten unsexed insect pairs were then introduced into each Petri dish and exposed to treatments. Controls contained untreated cowpea grains and unsexed insect pairs. The treatment was laid out on a completely randomized design (CRD) with 4 replicates per treatment. Numbers of dead insects were recorded 24, 48, 72 and 96 hours after setup to determine LC₅₀ and LT₅₀.

Germination Test(Seeds' Viability)

The effect of crude oil of *O. suave* on the germinative power of the cowpea grains was assessed using the methodology described by Perez et al., (2001). Groups of 10 seeds were randomly selected from each of the concentrations and placed separately in Petri dishes containing permanently moistened filter paper on the bottom. The experiment was kept at room temperature for 7 days. Subsequently, the percentage of the germination was determined in comparison to the control which was clean cowpea without treatment from

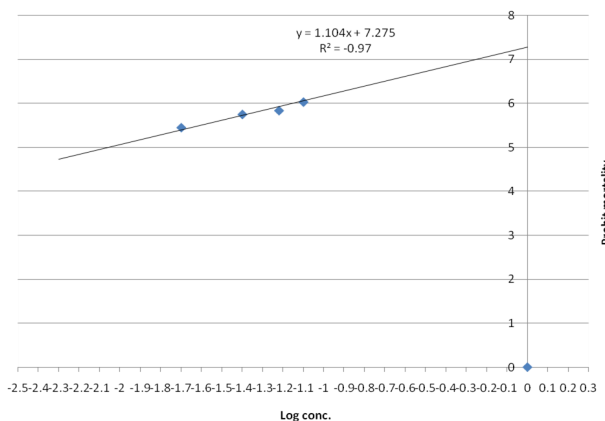
Statistical Analysis

All data collected were subjected to Probit analysis (Finney 1971), Analysis of Variance (ANOVA) and multiple comparisons using Tukey multiple comparison Test and LSD.

RESULT

Acute Toxicity Test

The result of the acute toxicity test carried out in this experiment are shown in **Table 1, 2 and 3**. The insecticidal effects of the crude oil extract (*O. suave*) on the insects shows significant difference on the mortality ($p < 0.05$) as the concentration increased from 0.02 – 0.08mg/20ml of water. The median lethal concentration LC₅₀ was Antilog $-2.08 = 0.0083$ conc. /20ml as shown in **Fig 1**.



$$LC_{50} = -2.08, = 0.0083mg$$

Fig 1: Log Concentration of the oil extract (toxicant) and percentage Probit kill of *C. maculatus*.

However as the period of exposure of the crude oil extract (*O. suave*) on the adult bruchids increased from 24 to 96hrs, there was significant difference ($p < 0.05$) on the mortality of the adult weevil. The survivorships of the insects also significantly ($p < 0.05$) reduced as the exposure period increased from 24hrs to 96hrs as shown in **Fig 3**.

The median lethal time LT₅₀ was 30hours as shown in **Fig 2**. The seed germination test showed that seeds with *O. suave* crude oil extract showed no significant difference ($p > 0.05$) among the treatment when compared to the control as shown in table 3.

DISCUSSION AND CONCLUSION

The aim of this research is to evaluate the Toxicity of *Ocimum suave* (wild basil) crude oil extract against cowpea weevil (*Callosobruchus maculatus*) by ascertaining the LC₅₀, LT₅₀ and the seed's viability.

The results of this present study showed significant difference ($p < 0.05$) level of toxicity of

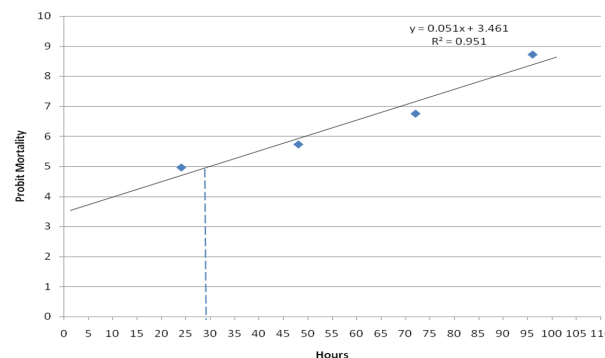


Fig 2: Lethal Time of the oil extract (toxicant) and percentage Probit kill of *C. maculatus*.



Table 1: Mortality, Mean Mortality and Percentage Mortality caused by different concentrations of *O. suave* crude oil extract on Insects for within 24 to 96hrs.

Oil extract conc. (mg)	Hours of Exposure				Total	%	Mean
	24h	48h	72h	96h			
Control	0	0	0	0	0	0	0 ^a
Mean value	0	0	0	0			
0.02	11	9	5	2	27	67.5	1.68 ^{ab}
Mean value	2.75	2.25	1.25	0.5			
0.04	16	6	9	0	31	77.5	1.93 ^b
Mean value	4	1.5	2.25	0			
0.06	15	10	57	2	32	80.0	2.00 ^b
Mean value	3.75	2.5	1.25	0.5			
0.08	18	10	5	1	34	85.0	2.12 ^b
Mean value	4.5	2.5	1.25	0.25			
Mean \bar{X}	3.00 ^a	1.75 ^{ab}	1.20 ^b	0.25 ^b			

Mean of the same superscript letter do not differ significantly (p<0.05) using Tukey multiple comparison test

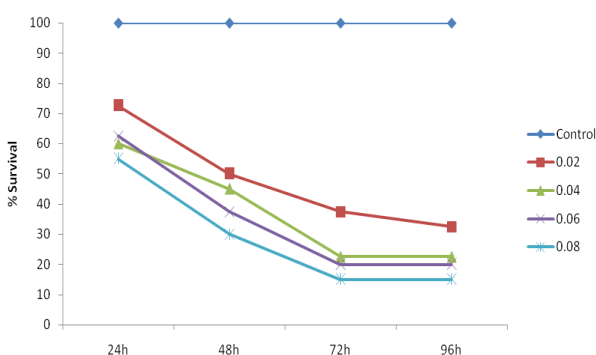


Fig 3: Cumulative survival in relation to exposure periods for *C. maculatus* adults exposed to the toxic effects of *O. suave* in four different concentrations.

the crude oil extract to *C. maculatus* at different concentration and time.

According to the results as the concentration of *O. suave* crude oil increased from 0.02 to 0.08mg/20ml of water, there was significant difference (p<0.05) as the percentage mortality of the weevil increased. The median lethal concentration LC₅₀ was Antilog -2.08 = 0.0083mg/20ml of water. The LC₅₀ even occurred at a low concentration indicating that the plant oil

may still be effective at lower concentration. The toxicity of the *O. suave* crude oil may be due to its constituent which include eugenol and mono and sesquiterpenoids. These notable compounds show insecticidal activities which act as stomach and contact poison (insecticide) to *C. maculatus* (Hassanali et al., 1990).

Ojianwuna and Umoru s(2010) also observed the *Ocimum suave* (Wild basil) powder caused mortality of adult *C. maculatus* in cowpea grain at 1 – 3g plant material per 20g cowpea. This protected the grains after 96h post treatment. The LC₅₀ was (Antilog) 3.0g/20g cowpea seeds, this may be attributed to the fact that enough content of the volatile compounds has reached the respiratory tracts of the tested organism. Ilondu et al., (2004) observed that *Ocimum suave* (wild basil) caused mortality of adult *Rhizopertha dominica* in grain cereals at 2-3g plant materials per 50g cereals (Rice, Maize and Sorghum), which protected the cereals after 90 days post treatment.

The result clearly showed that the material tested (*O. suave*) had distinct effect on survival of *C. maculatus*. The result shows that the median lethal time LT₅₀ was 30 hours. As the treatment of

Table 2: Mortality, % mortality and % probit kill caused by *O. suave* oil extract.

Test container No	No of weevils in each container	Mortality				Conc. of toxicant (mg)	Log conc.	% mortality	% probit kill
		A	B	C	D				
1	10	0	0	0	0	0	0	0	
2	10	10	8	7	2	0.02	-1.70	67.5	5.4538
3	10	8	9	8	6	0.04	-1.40	77.5	5.7554
4	10	7	5	13	7	0.06	-1.22	80.0	5.8416
5	10	7	11	9	7	0.08	-1.10	85.0	6.0364

Mean of the same superscript letter do not differ significantly (p<0.05) using Tukey multiple comparison test

**Table 3: Germination Test.**

Days Conc.	1	2	3	4	5	6	7	%
Control	0	3	3	3	5	7	8	80
0.02	0	4	6	6	6	7	8	80
0.04	0	6	6	6	7	8	8	80
0.06	0	7	8	8	8	9	9	90
0.08	0	8	8	10	10	10	10	100

cowpea with various concentration of the crude oil increased, the survival of the insects decreased, this was similar to the result of Abdullahi and Muhammad (2003) who assessed the toxic potentials of dried leaf powders of *Guinea senegalensis*, *Piliostigma reticulatum* and dried fruit powder of *Piper guineense* on the survival and development of *C. maculatus* during storage. Statistical analysis showed that there was significant difference ($p < 0.05$) in concentration and hours of exposure. This observation was also noted by Law-Ogboma and Egharevba (2006), who used vegetable oils (rubber, seed oil, palm oil and palm kernel oil) against *C. maculatus* show a significant difference ($p < 0.05$) mortality of the adult weevils.

The high percentage (80-100%) seed germination recorded in this study showed that there was no significant difference ($p > 0.05$) compared to the control. This may be that the plant oil has no effect on the seeds viability. This agrees with the work reported by Umar (2008) who reported that the cowpea seeds remained viable which was treated with *Jatropha curcas* powder.

In conclusion, this study has shown that the crude oil extract of *O. suave* was effective in the protection of cowpea (*Vigna unguiculata*) against cowpea bruchid *Callosobruchus maculatus*. The seeds remained viable. Therefore, the use of *O. suave* and other plant materials in post-harvest protection should be encouraged because it is an environmental friendly plant and can easily be propagated.

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