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Bioactivities of Three Botanical Extracts on *Callosobruchus maculatus* (F.) in Stored Cowpea.

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Abstract

This study was carried out to assess the bioactivities of three botanical extracts on *Callosobruchus maculatus* (F.) in stored cowpea and also study the effect of the different treatment at the concentration level of 2.0g; 1.5g; 1.0g; and 0g (control) on the adult mortality within a period of 7days using Completely Randomized Design (CRD). All data were analyzed using Analysis of Variance (ANOVA) procedure using SAS 2003 software package and means were separated using Student Newman Keuls (SNK) test. The result showed that there was no significant difference in the treatments and at their different levels of concentration. The control also showed no significant difference, however, there was increased mortality at periods of application but mortality decreased with time. This indicates that the three treatments (botanicals) used at their various concentration levels are potent for a short period of time.

Keywords: Bioactivity, Botanical Extracts, *Callosobruchus maculatus* (F.) Cowpea.

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Introduction

Cowpea *(Vigna. unguiculata)* is one of several species of the widely cultivated genus "*Vigna*", which is of the family *"FABACEAE"*. *Vigna unguiculata* derived its English name "cowpea" from its use as livestock feed for cattle (cows) in the United States. Cowpea is one of the most important food legume crops grown in the semi-arid tropics of Africa, Asia and Southern Europe. Most importantly, cowpea serves as a major source of protein in human diets, especially in the tropical regions where both the grains and the leaves are used for food. (Singh, 2003). According to the United States Department of Agriculture (USDA), food database, the leaves of the cowpea plant have the highest percentage of calories from among other vegetable foods. Cowpea plant is attacked by pest during almost every stage of its life cycle. During the evolution, plants have developed strategies to maintain favorable growth and also guarantee their survival. Enhancing the protective mechanisms, for example, is one of these strategies that allow them to successfully tolerate/resist insects, phytopathogenic microorganisms, and other unfavorable conditions (Jackson and Tailor, 1996; Malek and Dietrich, 1999; Stotz *et al*., 1999). In recent years, attentions have been focused on the idea of using digestive enzyme inhibitors that affect the growth and development of pest species (Mehrabadi *et al*., 2010, 2011, 2012). Specificity of inhibition is an important issue as the introduced inhibitor must not adversely affect the plant own α- amylases or human amylases and must not change the nutritional value of the crop (Ehsan Borzoui *et al.*, 2013). Transgenic plants expressing serine and systeine proteinase inhibitors have shown resistance to some insect pest species including Lepidoptera and Coleoptera (Falco and Silva-Filho 2003; Alfonso-Rubi *et al*., 2003).

The Coleopteran weevil of the family Chrysomelidae is a major pest that attacks other grain crops, most especially cowpea in storage. It is of the genus *"CALLOSOBRUCHUS*" and the species "*MACULATUS*". It attacks grains and lays its eggs on the pods and immature grains on the field. These eggs metamorphose into larvae and then the adults which emerge by boring holes through the grains leaving a powdered deposit. They also deposit excrements as black spots on grain surface giving it an offensive odour. To avoid huge economic losses from weevil attack the cowpea should be allowed to mature and dry properly on the field before harvest. Threshing should be done immediately after harvest and the grains stored in non-permeable containers, extra heat could also be applied either by direct sunlight or by a dryer. Preservatives like hydrogen cyanide or methyl bromide fumigant may also be used or by traditional storage and preservation by adding little quantity of dry red pepper. Fumigants are mainly used for commercial storage and this could incur cost and environmental and health hazards on the consumer, sometimes, the weevil may become resistant to these chemicals after prolong application. Thus there is an urgent need to develop safe alternatives that have the potential to replace the toxic fumigants, yet are effective, economical and convenient to use (Ayvaz *et al*., 2008) this has necessitated the research on other efficient methods which are less expensive, more eco-friendly and makes consumption of the grains safe. Botanical insecticides are naturally occurring insecticides that are derived from plants (Isman, 2000). This method involves the use of these natural plant materials as treatments against insect attacks which is a more sustainable method of crop protection (Sallam, 1999) than synthetic insecticides which may pose potential risk as it relates to their safe use, (KeAOEta *et al.*, 2000) as host plant resistance and natural plant products offer a potentially safe method for insect pest control. They are safe to the non-target beneficial organisms and human beings (Andow, 2008).

This knowledge was gotten from the consistent use of herb *(Azadirachta indica*) as traditional medicine in humans which had proved to be effective and like-wise in plants; especially in aromatic species of the family "*LAMIACEAE"* which are widely used as botanicals (plant insecticides) for pests control. [Lambert *et al*., (1985), Shaaya *et al*., (1997) and Mortan (1981)]. These aromatic species contain secondary compounds which have no known function in the physiological functions of the plant, but are toxic even in their little quantities to insect pests (Sallam, 1999). Inspite of the fact that essential oils from different plant species have been shown to possess ovicidal, larvicidal, and repellent properties against various insect species and are regarded as environmentally compatible pesticides (Isman 2000; Cetin *et al*., 2004).Yet only 1% of the world insecticide market come from botanical insecticide (Rozman *et al*., 2007). Several botanicals have been discovered for various plants, as control for insect pests, as a result of losses in yield, seed viability, nutritive value of the crops etc. In this research, the bioactivity of three botanicals e.g *Jatropha curcas*, *Delonix regia and Luffa cylindrica* will be tested for the control of cowpea weevil to ascertain the mortality rate of these weevils as a result of these treatments used at different concentration (level).

The objectives of this study is to determine bioactivity of three botanicals for adult cowpea weevils control and to ascertain the most effective dosage of three botanicals for the control of adult cowpea weevils.

Materials and Methods

The experiment was conducted at the Department of Crop Production laboratory, School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology Minna, Niger State. The cowpea variety SAMPEA 6 used was obtained from IAR Zaria. Matured seeds of *Jatropha curcas* plant were obtained from the local farmers, while *Delonix regia* and *Luffa cylindrical* seeds were collected from well matured trees in Gidan kwano campus of the university. Newly cultured adult weevils were collected from already infested cowpea grains. The cowpea grains were screened at the laboratory by selection and heating to ascertain purity. Cowpea grains were weighed and kept in a covered container, the seeds from the three plant botanicals were removed from their pods, washed and oven-dried at a temperature of $110 \pm 2^{O}C$ to moisture content (MC) of 12% and at a room temperature of $28 \pm 2^{o}C$. The dried seeds were ground separately using a mortar and pestle and pulverized using an electric blender. The powdered seed was sieved through a 0.5mm size mesh to obtain uniformity. Each sieved powder was weighed to thirteen and a half grams (13.5g) and kept in three tightly closed glass jars and labeled as treatments; T1, T2 and T3. Three parts of the cowpea, one thousand three hundred and fifty grams

(1350g) was mixed with thirteen and a half grams (13.5g) of each of the powdered seeds at a concentration of 2g, 1.5g and 1g and for T1, T2 and T3 respectively, each treatment was replicated three times.

The fourth part of the cowpea which was one hundred and fifty grams (150g) was used as the control (C). Each of the different concentrations in the four parts of cowpea admixture was weighed at fifty grams (50g). The admixture was shaken manually for five (5) minutes for uniformity of the admixture before placing into a disposable bowl. Twenty (20) unsexed cowpea weevils were introduced to each of the disposable bowls and each bowl was covered with a muslin cloth to prevent the insects from coming out and also to allow for air passage. The four (4) samples were kept under laboratory condition for 7 days and data were collected at an interval of 24 hours. The data obtained per day were used to determine the adult mortality rate of the weevils and the percentage adult mortality. All data collected were analyzed using Analysis of variance (ANOVA) procedure, using SAS 2003 software package. The means were separated at 5% level of significance using Student Newman Keuls (SNK) (SAS Institute, 2003).

Results and Discussion

The result on Table 1. showed that on day 1 to day 7, there was no significant difference between T1, T2, T3 and control, although, there was a simultaneous increase in mortality on day 1, 2, 3 and 4, while on day 5, 6 and 7, there was a simultaneous decrease. The result on Table 2 showed that on day 1, 2 and 3, there was no significant difference between the concentrations. On day 4, there was no significant difference between 2.0g, 1.5g and 1.0g, but there was significant difference between 1.5g, 1.0g and control. On day 5 and day 6, there was no significant difference between concentrations, but there was decrease in mortality at all concentrations. On day 7, there was no significant difference between 2.0g, 1.5g and 1.0g but there was significant difference between 1.0g and control. The result in Table 3 showed that at day 1 to day 7, there was no significant difference between T1, T2, T3 and control. Although there was increase in mortality on day 1, 2, 3 and 4. On day 5, mortality dropped for all treatments but increased on day 6 and day 7. The result on Table 4 showed that, at day 1, 3 and 4, there was significant difference in concentrations 2.0g, 1.5g, 1.0g and control. On day 2, there was no significant difference between concentrations 2.0g, 1.5g and 1.0g, but there was significant difference between 2.0g, 1.5g, 1.0g and control. On day 5, there was no significant difference between 2.0g, 1.5g and 1.0g, also there was no significant difference between 2.0g and control but there was significant difference between 1.5g, 1.0g and control. On day 6, there was no significant difference between 2.0g, 1.5g, and 1.0g but there was significant difference between 2.0g, 1.5g, and 1.0g and the untreated control. There was significant difference between 1.5g and 1.0g, and also between 1.5g and control as well as between 1.0g and control at day 7.

The findings of this experiment showed that the mortality of *Callosobruchus maculatus* was high on the early period of the experiment for all the treatments and at the various concentrations. The decrease in mortality rate was from day 5. This could be as a result of the low potency of the botanicals resulting to the quick expiry of their active ingredients and the limited persistence of these botanicals. This was in agreement with Oparaeke (1997) that most plant species are weak and dependent on ratio and rate of application. The lowest concentration also recorded almost equivalent mortality rate to that of the higher concentration. This corresponds to an earlier finding of Davappa *et al.*, (2010) that *J. curcas* has active ingredient even in low concentration rates, also the discovery of (Ratnadass *et al*., 1997, Valencia *et al.*, 2006, Phowichit *et al.*, 2008), that *Jatropha* leaves exhibit insecticidal activities against some species of *Lepidoptera.* The discovery by Zhu *et al*., 2006, that *M.phalerata* was found on the flowers of cowpea (*V. ungiculata*) and Loofah (*L. cylindrica*) in china, also explains why *L. cylindrica* could be said to be low in potency. The prepared powders tested were effective to some extent in reducing damages caused by *C. maculatus* although not statistically different among the treatments.

Conclusion and Recommendations

Based on the result obtained, it can be concluded that all the botanicals are effective for *C. maculatus* control and be used as insecticides. They however need to be applied at a low rate because efficiency was attainable at low concentrations. Also there is a need for consistency in application in other to boost up the potency of the botanicals and overcome the problem of resistance. Focus should be on effective storage which provide confinement for direct contact between weevil and bio-pesticides and at the same time reduce aerobic activity to almost minimum. The botanicals should be applied at intervals and at low concentrations. Further study should be conducted to assess the defense mechanism of *V. unguiculata*, the bio-mechanism of *C. maculatus* and the shelf life of the experimented botanicals as measures to improve persistence of its active ingredients.

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Tables

Table 1: Effect of Different Treatments on the Mortality of *Callosobruchus maculatus* (Trial one)

MATERIALS	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7
Jatropha curcas	12.08a	15.08a	15.33a	18.25a	6.50a	7.00a	3.92a
Delonix regia	17.50a	16.50a	14.67a	14.83a	7.33a	7.08a	2.58a
Luffa cylindrical	13.33a	14.33a	14.67a	14.92a	8.50a	7.58a	5.75a
CONTROL	14.17a	17.00a	13.50a	15.75a	7.33a	7.67a	6.33a
S.E.±	2.42	2.43	2.06	2.45	1.35	0.96	1.09

Means with the same letter within the same column are not significantly different ($P \leq 0.05$) using Student Newman keuls (SNK)

Table2: Effect of Different Concentration on the Mortality of *Callosobruchus maculatus* (Trial one)

CONCENTRATIO N	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7
CONTROL	10.83a	17.92a	14.83a	22.08a	8.00a	5.58a	1.92b
1.0g	11.25a	14.58a	13.33a	13.33b	7.50a	8.33a	7.50a
1.5g	18.75a	13.75a	13.75a	12.08a	6.25a	6.67a	4.17ab
2.0g	16.25a	16.67a	16.25a	16.25a	7.92a	8.75a	5.00ab
S.E.±	2.62	2.16	1.25	1.96	1.14	0.90	1.23

Means with the same letter within the same column are not significantly different ($P \leq 0.05$) using Student Newman keuls (SNK)

Table 3: Effect of Different Treatments on the Mortality of Callosobruchus maculatus (Trial two)

MATERIAL	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7
Jatropha curcas	8.83a	12.25a	12.92a	14.75a	7.58a	11.17a	9.33a
Delonix regia	15.50a	13.67a	13.00a	11.83a	8.25a	10.67a	7.33a
Luffa cylindrical	12.00a	12.08a	14.08a	11.83a	9.50a	11.33a	11.08a
CONTROL	13.75a	18.58a	19.25a	13.92a	7.75a	10.08a	10.83a
S.E.±	2.27	1.77	1.73	1.92	0.93	1.00	1.03

Means with the same letter within the same column are not significantly different ($P \leq 0.05$) using Student Newman keuls (SNK)

Table4: Effect of Different Concentration on the Mortality of *Callosobruchus maculatus* (Trial two)

CONCENTRATIO N	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7
CONTROL	5.17b	8.83b	10.50a	9.42a	11.00a	16.42a	20.08a
1.0g	11.83a	15.33a	15.58a	14.17a	7.42a	9.52b	8.58b
1.5g	17.92a	15.42a	16.67a	12.92a	6.00b	7.08b	4.17c
2.0g	15.17a	17.00a	16.50a	15.83a	8.67ab	10.25b	5.75bc
S.E.±	2.84	2.72	1.71	1.83	0.94	1.00	1.34

Means with the same letter within the same column are not significantly different ($P \leq 0.05$) using Student Newman keuls (SNK)