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Effects of Excess Insecticide Application on Cowpea (*Vigna unguiculata*) Seeds by responses of Colonized Cowpea Weevil (*Callosobruchus maculatus)*

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ABSTRACT: This study was designed to determine the effect of excess insecticide application on stored cowpea (*Vigna unguiculata*) seeds sourced from Lapai market, Niger State, Nigeria using appropriate standard techniques. The effect quantum was using cowpea weevil (*Callosobruchus maculatus*) as Biomarker. Result obtained show that lethal concentration of insecticide significantly ($P < 0.05$) affected duration of immature development, with highest concentration increasing the duration of larval development significantly. Larval and pupal mortality (11.20±1.24 and 67.20±4.65; 8.04±0.44 and 36.20±2.11 for negative control and highest treated concentration respectively) were significantly ($P < 0.05$) affected by increase in insecticide concentration. Mean temperature and relative humidity (28.77±1.2 and 35.88±5.14 respectively) of the immediate stored cowpea environment were within the ambient condition. Protein and Carbohydrate were the two proximate composition that varied significantly as a result of insecticide application. Cowpea weevil have shown a great potential of serving as a potential bio-markers of insecticide contamination of stored cowpea seeds in storage. Further research on similar and other biological effects of abused insecticide on colonizing weevil should be carried out in and outside laboratory condition.

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Adequate seed storage is a significant agricultural problem because of the need to maintain seed viability and vigor, particularly in tropical regions characterized by high humidity (Salihu *et al*., 2018). Cowpea (*V. unguiculata*) is the most important proteinous legume in West Africa and it account for between 60-80% protein intakes of the people in the region (Hongbete *et al*., 2017). Cowpea is highly nutritive with (22- 24) % protein, (1.3 - 1.5) % fat and (56 – 66) % carbohydrate (Carlos, 2004). It also has great potentials and can play a crucial role in contributing to nutritional and food security as well as, poverty reduction, income generation and socio-economic growth of West Africa in general and Nigeria in particular (Salihu *et al*., 2018). The challenge to year-round availability of cowpea is insect pest attack as up to 100% damage to stored cowpea have been reported due to *C. maculatus* (Arannilewa *et al*., 2006). Another menace to consumers of cowpea and the humanity today is the endangerment of human health due to indiscriminate use of pesticides (Otitoju and Onwurah, 2011). And the use of chemical control for seed preservation is the most popular and acceptable practice among the cowpea stake- holders. High levels of insecticide residues arising from improper application doses have

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been reported to be responsible for the poisoning and deaths of people in Nigeria (Obida *et al.,* 2012). FAO, (2000) estimated an annual worldwide of three million cases of acute and severe pesticide poisoning with some 220,000 deaths in the process of eliminating insect pest by chemical means. Although, insects are capable of metabolizing and degrading toxic or detrimental chemicals to survive in a chemically unfriendly environment, insects possess capacity to detoxify toxic chemicals (Bamidele *et al.,* 2017). On the bio-marker development, a concept of biological monitoring can be extended to include sub-organismal measurement, which are often referred to as biomarkers (or biological markers) and also include biochemical responses, as well as, physiological and anatomical alterations in insect and their relatives caused by chemicals (Hanson *et al.,* 2013). Reports have shown that insects can play significant roles in developing a 'biomarker' which is also referred to a biological instruction obtained from the biological elements of the body of an organism that can be used as a guide in monitoring abnormalities in their environs. The study was designed to determine the effects of excess insecticide application on cowpea (*Vigna unguiculata*) seeds through the responses of colonized cowpea weevil (*Callosobruchus maculatus*) as the biomarker.

MATERIALS AND METHOD

Procurement and Handling of Cowpea (V. unguiculata) Seeds: White cowpea (*V. unguiculata*) was obtained from Lapai Market and sowed during 2022 rainy season without any herbicide/pesticide application. The goal of these activities was to get cowpea seeds that is not expose to insecticide contamination prior to storage. After harvest, the dried cowpea seed samples were kept in freezer for seven days at frizzing temperature to eliminate immature and adult stages that may be present from the field before or after harvest. Then, the seeds were air dry at an ambient temperature in the laboratory for 7 days, in order to have non infested seeds for the tests. Furthermore, the cowpea seeds were screened by transferring them into clean open transparent glass containers in the laboratory for 14 days to confirm the absence of weevil activities in them following a modified method of Salihu *et al.* (2018).

Procurement and Culturing of Bean Weevil (C. maculatus): Virgin *C. maculatus* were obtained from the Nigeria Stored Produce Research Institute, Ilorin. The harvested adults of *C. maculatus* were kept in a jar containing 200 g of white cowpea seeds (*V. unguiculata*) and transferred to the laboratory. Forty hours later, the insects were taken out, and the infected seeds were incubated till the emergence of new adult insects; subsequently collected through sieving. Twenty-four hours later, the sieved content of the container was once again sieved in order to obtain newly emerged adults (aged 0-3 days old). These adults were used for the experimentations (Salihu *et al.,* 2018).

Experimental Design: Complete Randomized Design was used. The experiment involved five treatments, with two used as Controls (positive and negative controls), and the remaining three treatment were treated with varying concentrations ranging from low (5mg/kg), moderate (10mg/kg) and high (16mg/kg) considering 8mg/kg of malathion insecticide recommended, for stored grains (Salihu *et al*., 2018). For each treatment, 90g (i.e., 30g per replicate) of *V. unguiculata* was used. All the set-ups had three replicates including the Controls, and developmental traits investigated were monitored in each replicate.

Biological Assay: The biological assay of the treatment involving insecticide was done using a modified method of Salihu *et al.* (2018). Plastic bowls measuring 60cm x 30cm in size were used. Each bowl contained 30g of sterilized cowpea seeds, followed by application of insecticide (which was kept closed tight overnight and ventilated thoroughly the following morning) and finally 10 pairs of newly emerged male and female reared adults, of *C. maculatus* were introduced into each bowl. The bowl was covered with muslin cloth held with rubber bands and kept in the Laboratory at room temperature. Three days infestation period was observed and all adult *C. maculatus* was removed from the bowls and eggs laid by the females were monitored to adult emergence.

Duration of development of C. maculatus: Duration of development was measured as the length of time that separates the egg-laying from series of immature development (larva and pupa), as well as, emergence of the resulting adults. Three bowls with 30 g of cowpea seeds were used for five simultaneous treatment of cowpea seeds. Ten pairs of adult weevils (taking into account the sex ratio) aged from 0 to 3 days, were introduced in each bowl and removed after 3rd day of infestations. The seeds were monitored after each 72 hours to sample immature life stages inside the cowpea seeds through a random destructive sampling (i.e., dissecting five cowpea seeds per replicate) to track and record particular life stage on each sampling day until emergence of the first adult insect. The difference of duration of previous and the next life stage tracked through the sampling process was noted down as duration of development between the two life stages, the average developmental cycle of each life stage of the insects was calculated on this basis as modified from Ouali-N'goran *et al*. (2014).

Immature mortality of exposed C. maculatus: Immature mortality was measured through destructive sampling of the seeds. Five seeds were dissected after every 72 hours to check and record the number of immature mortalities resulting from application of insecticides. Dead immature life stages were recorded and average number of mortalities per treatment was recorded on this basis.

Proximate composition (%) of cowpea seed (Vigna unguiculata): The following proximate composition were analysed; moisture content, ash content, crude protein, crude lipid, crude fibre, and carbohydrate using method describe by Association of Analytical Chemists (AOAC, 2007) and Bamphitlhi *et al.* (2013), for cowpea seed of the various treatments at the Animal Production Department, School of Agriculture and Agricultural Technology, Federal University of Technology Minna, Niger State.

Micro Weather Conditions: Temperature and relative humidity of the immediate environment were determined using hygrometer, following the method of Salihu *et al.* (2018).

Data Analyses: The data collected from this study were. Analysis of variance (ANOVA) test and Duncan Multiple Range Test (DMRT) were done to compare the means among the different treatments, for significance. The results were considered statistically significant at P = 0.05 confidence level.

RESULTS AND DISCUSSION

Effect of insecticide on duration of development of Immature life stages of beans weevil (C. maculatus) raised on treated bean seeds in storage (days): Table shows effect of insecticide on duration of development (in days) of immature life stages of cowpea weevil raised on treated cowpea seeds in storage. There was significant ($P < 0.05$) increase in the duration of time it took the egg to hatch from the allowed duration of oviposition among the various treatment, with cowpea weevils raised on grains treated with highest concentration of insecticide recording significantly longer duration (5.49±0.07 days), with untreated seed taken 3.95±0.19 days. More so, total larval duration (TLD) and total immature duration (TID) also varied significantly as the former (14.17±0.14 to 17.41±0.21 and 21.00±0.00 to 24.00±0.00 days) for cowpea weevils raised on untreated beans seed and highest concentration treated beans seed respectively, but there was no significant ($P > 0.05$) different in the total pupal duration (TPD) among all the treatment. These results showed that excess insecticide has significantly affected the duration of development of the cowpea weevils.

Table 1: Effect of insecticide on duration of development of Immature life stages of beans weevil (*C. maculatus*) raised on treated bean seeds in storage (days)

Insecticide treatment (mg/kg)	Oviposition period	Egg incubation period	TLD	TPD	TID
N Control (0.0)	3.00±0.00	**3.95±0.19^{a}**	**14.27±0.14^{a}**	6.83±0.13^{a}	**21.10±0.02^{a}**
P Control (8.0)	3.00±0.00	**4.16±0.05^{a}**	**15.46±0.16^{ab}**	6.77±0.14^{a}	**22.23±0.03^{ab}**
Low (5.0)	3.00±0.00	**3.99±0.14^{a}**	**14.89±0.43^{a}**	6.55±0.26^{a}	**21.44±0.20^{a}**
Moderate (10.0)	3.00±0.00	**5.46±0.18^{b}**	**17.17±0.31^{b}**	6.90±0.11^{a}	**24.07±0.11^{b}**
High (16.0)	3.00±0.00	**5.49±0.07^{b}**	**17.43±0.21^{b}**	6.59±0.21^{a}	**24.02±0.01^{b}**

**Values with the same superscript alphabets, in a column, are not significantly different at P > 0.05; Values are represented in mean ± standard error of their replicates; KEY: TLD = Total Larval Duration, TPD = Total pupal Duration, TID = Total Immature Duration*

Effect of Insecticide on larva and pupal life stages mortality of C. maculatus (per kg) recorded from insecticide treated beans seeds in storage: Table 2 showed results on immature mortality of *C. maculatus* i.e., larval and pupal mortality recorded from untreated and insecticide treated cowpea seeds in storage. There was significant ($P < 0.05$) increase on larval and pupal mortality with increase in insecticide concentration. Larval and pupal life stage mortality recorded per kg of cowpea seeds ranges from 11.20±1.24 to 67.21±4.65; 8.04±0.44 to 36.20±2.11 respectively for *C. maculatus* retrieved from untreated and highest insecticide treated cowpea seeds respectively.

Table 2: Effect of Insecticide on larva and pupal life stages mortality of *C. maculatus* (per kg) recorded from insecticide treated beans seeds in storage

Insecticide Treatment (mg/kg)	LARVA	PUPA
Negative Control (0.0)	11.20±1.24^{a}	8.04±0.44^{a}
Positive Control (8.0)	34.10±3.44^{c}	13.06±1.37^{b}
Low (5.0)	26.30±2.27^{b}	9.15±1.95^{a}
Moderate (10.0)	49.30±3.97^{d}	29.11±2.56^{c}
High (16.0)	67.21±4.65^{e}	36.20±2.11^{d}

** Values with the same superscript alphabets in a column are not significantly different at P > 0.05; Values are represented in mean ± standard deviation of their replicates*

There was no significant (P > 0.05) difference between pupal mortality recorded from negative control and the lowest insecticide treated concentration (8.04±0.04 and 9.15±1.59) respectively.

Temperature and Relative humidity of the immediate environment of treated bean seeds used for raising C. maculatus: Temperature and relative humidity of the immediate environment of the insecticide treated cowpea seeds, during storage, are presented in Table 3. The temperature and relative humidity ranged from 27.50 to 31.0°C and 28.0 to 59.0% respectively. Both parameters recorded were within tolerable ambient condition.

Proximate composition (%) of bean seeds treated with insecticide and exposed to weevil infestation: Result of proximate composition (%) of cowpea seeds treated with varying concentration of insecticide were presented on figure 1. The moisture, ash, fat and crude fiber content of treated and untreated cowpea seeds did not varied significantly, however crude protein and carbohydrate show variations that were significant.

Table 3: Temperature and Relative humidity of the immediate environment of treated bean seeds used for raising *C. maculatus*

Days	Temperature (C)	Relative humidity (%)
1	31.0	28
3	29.0	30
6	28.5	35
9	28.0	46
12	28.0	40
15	27.5	59
18	28.0	28
21	31.0	29
24	28.0	28
Mean±SD	**28.77±1.29**	**35.88±5.14**
(Range)	**(27.50 – 31.00)**	**(28.00 – 59.00)**

The findings showed a significant increase in the duration of immature development specifically larval duration, which in turn resulted in increase in duration of total immature life stage, development resulting from increased insecticide application of the host environment i.e., cowpea seed. This might be attributed to challenge of acquiring sufficient food from host grain due to anti-feeding effect of some organophosphorus insecticide. This agree with the report of Adesina and Ofuya (2015), who stated that oviposition and percentage egg hatched were significantly reduced on seeds treated with higher treatment with botanical insecticide. Leaf extract with hexane at 2ml (10.0% v/w) / 20g cowpea seeds was most effective in reducing egg oviposition and egg hatching activities of *C. maculatus*. The findings also tally with the work conducted by Mahmoudv *et al.* (2012), who revealed that insecticide such as hexaflumuron decreased the total number of eggs, oviposition period, pupation and adult emergence of *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae). A finding from Harambour (2017), contradict this present study, who reported that the viability of *Chrysoperla externa* eggs treated by immersion with cypermethrin, acetamiprid, azadirachtin and pyriproxyfen were not affected, but neonates hatched from eggs treated with cypermethrin died after 48 hrs.

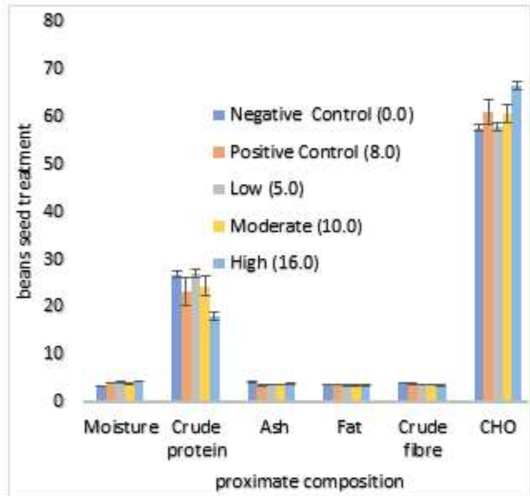

Fig 1: Proximate composition (%) of bean seeds treated with insecticide and exposed to weevil infestation

Larval and pupal mortality within the cowpea seed recorded an increase with increase in insecticide concentration. Larval stages were found to be more susceptible to insecticide treatment than pupae, The increased mortality in larval stage could be because, it is the only feeding stage of the insect species which may led to more accumulation of toxin in the life stage than the pupal stage. This is in agreement with Rodriguez-Saona *et al*. (2016), who reported that effects of insecticides on larval mortality was assessed after seven days of exposure to treated diet or foliage; though the bioassay did not reflect fully the effects of these insecticides on larvae and stronger negative effects on 3rd instar were seen after a longer exposure, which also lead to slow larval death as a result exposed larvae cease to feed. Temperature and relative humidity recorded were within ambient tolerable limits for the insects, though, these results were however, not in agreement with that reported by Mansoor-ul-Hasan *et al.* (2017), who reported slightly higher temperature of 35°C and relative humidity of 65% and 95% to be optimum for the thriving of *C. maculatus* and *C. subinnotatus*, though, reared on Bambara groundnut not cowpea seed. Only crude protein and carbohydrate proximate contents showed significant variation (figure 1).

Others did not show significant variations, the neglected variation recorded from other proximate such as moisture could be due to absorption from the atmosphere. This agree with the work of Masud *et al*. (2014), who worked on management of stored insect pest of cowpea and their effects on nutritional quality of the cowpea using *Gmelina* tree parts (leaves, root, bark and stem extract) in Mokwa, Niger State and reported moisture contents ranging from 13.71 to 15.55% in his study, and attributed the insignificant lower moisture content in the negative control to absorption of the moisture from the atmosphere which would have also been increased by the increased insect population and increased metabolism. On the other side the significant variation recorded from crude protein could be due the activity of proteolytic enzyme and agrees with the reported Masud *et al.* (2014), though they treated the grains with plant extract. and crude protein values in the treated sample were significantly lower than the untreated bean seed (negative control), and attributed this to proteolytic enzyme activities in the treated grains to the activity of proteolytic enzyme activities, as well as, several others due to treatment applied to the grains.

*Conclusion***:** Lethal concentration of insecticide significantly affected duration of immature development, immature mortality with the higher concentration increasing the duration of immature development and immature mortality significantly. The mean temperature and relative humidity are within the ambient condition. Protein and Carbohydrate were the two proximate compositions that varies as a result of insecticide application. The model organism has displayed verge optimization as a bio-marker agent of excess insecticide contamination of stored cowpea seeds.

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