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Original article

Effect of Malathion Insecticide Application on Immature Duration of Development of Beans Weevil (*Callosobrochus* ***maculatus***) raised on Insecticide-treated Seeds in Storage

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ABSTRACT

Study was conducted to determine the time of commencement of life stages and duration of time spend in each immature life stages of beans weevil (*Callosobrochus maculatus*) raised on insecticide-treated grains in storage. White cowpea (*Vigna unguiculata*) was obtained from Minna Central market, sowed during 2016 raining season without applying any insecticide/pesticide in order to clear the beans seed from potential chemical processing during storage. The experimental design was completely randomized, involving 7 test, 2 as control (positive and negative controls) and the remaining 5 were treated with varying concentration of malathion insecticide prior to commencement of the study. Results were recorded for two phases of immature life stages with larval and pupal stages having sub-stages of development. The results further revealed that the time of commencement of next life stage from previous once were significantly ($P = 0.05$) faster in days with decrease in insecticide concentrations in storage. In another development, there was significant increase in the duration (days) egg spend before hatching, from the maximum time allowed for oviposition uniformly (5.00 ± 0.00 and 4.00 ± 0.00 days for highest and lowest insecticide application) respectively, as the concentration of insecticide increases. More so, total larval duration (TLD) and total immature duration (TID) also varied significantly as the former (15.00 ± 0.00 and 18.00 ± 0.00 days for TLD and 21.00 ± 0.00 and 24.00 ± 0.00 days for TID) respectively, although there was no significant difference in total pupal duration (TPD) (6.00 ± 0.00 days) among weevils raised in all treatments. This result is an indication that, insecticide in storage could affect egg hatching and larval developmental duration within the beans seed. This information if articulated, can serve as early warning signals/biomarker of grains treated with abused insecticide concentration for consumers and stakeholders of cowpea and the knowledge can be applied to other stored product having similar immature development process.

Keywords: Destructive sampling, Total larval duration, Total pupal duration and Total immature duration,

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INTRODUCTION

Bean seeds are widely consumed in Africa. They provide from (20- 25) % of proteins as well as available minerals. They are significant sources of income for small scale farmers, traders, agribusiness men and exporters (10). The genetic diversity of the bean is the basis for maintaining the means of subsistence and the agro-ecosystem. The world production of cowpea is put at 3.3 million tons of dry seeds, 64% of which are produced in Africa (10). Nigeria is the largest producer and consumer of cowpea grain with approximately 5 million ha under cultivation (13).

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 (11). 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 (6). Cowpea is highly nutritive with (22-24) % protein, (1.3 - 1.5) % fat and (56 – 66) % carbohydrate (3). 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 socioeconomic growth of West Africa in general and Nigeria in particular (12). 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* (2). Another menace to consumers of cowpea and the humanity today is the endangerment of human health due to indiscriminate use of pesticides (11). 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 reported to be responsible for the poisoning and deaths of people in Nigeria (11) and (9).

The study was designed to determine how insecticide concentration in cowpea (*Vigna unguiculata*) seeds affect colonized cowpea weevil (*Callosobruchus maculatus*) in terms of their development within the seed that is not visible by a common end user.

MATERIALS AND METHODS

Source and handling of cowpea (*Vigna unguiculata*) seeds

White cowpea (*V. unguiculata*) was obtained from Minna Central Market and sowed during 2016 rainy season without any chemical/pesticide application. The goal of these activities was to rid the seeds off potential chemical contamination prior to storage. After harvest, the dried cowpea seed samples were kept in freezer for a week. Then, the seeds were dried at an ambient temperature in the laboratory for 7 days to eliminate weevil larvae from the seeds, in order to have non infected seeds for the tests as described by (10). Furthermore, the cowpea seeds were screened by transferring them into clean open transparent glass containers in the laboratory for two weeks to confirm the absence of weevil activities in them.

Culturing of cowpea weevil (*Callosobruchus maculatus*)

Virgin *Callosobruchus 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. These adults were used for the experimentations following modified methods of (12)

Experimental design

White cowpea (*V. unguiculata*) was used as a model for agricultural stored produce for sampling of *C. maculatus.* Complete Randomized Design was used. The experiment involved seven set-ups, with two used as Controls (positive and negative controls). The remaining five set-ups were treated with varying concentrations of the selected insecticide. Recommended concentrations for malathion (8mg/kg), according to (12) and (4) were used to treat the *V. unguiculata* seeds under positive control while the other 5 tests, aside negative control (no chemical treatment) were treated with varying concentrations of insecticide as follows; 2, 4, 6, 10 and 12 mg/kg, respectively. For each set-up, 100g of *V. unguiculata* was used. All the set-ups had five replicates including the Controls, and duration of immature development within the bean seed were monitored through random destructive sampling of 20 bean seed in each replicate after every three days of observation till the first day of adult emergence.

Biological assay of the treatment

The bioassay of the five set-up involving treatments was done in plastic bowls measuring 12cmx12cm in size. Each bowl contained 200g of sterilized cowpea seeds, followed by introduction of insecticide (which was kept closed tight overnight and ventilated thoroughly the following morning) and finally 20 pairs of newly emerged male and female reared adults of *C. maculatus* were introduced into each bowl. The mouth of each bowl was covered with muslin cloth held with rubber bands and kept in the Laboratory at room temperature (12) and (8). After three days, all the adult insects were removed from the bowl and eggs laid by the females were monitored to adult emergence

Duration of Larval, Pupal and Immature development (days)

This is the length of time that separates the egg-laying from adult emergence. Five bowls containing 200g of bean seeds each were used for seven simultaneous tests. 20 pairs of bean weevil, i.e., ten males and ten females (taking into account the sex ratio) aged from zero to one day, were introduce into each bowl and removed after three days of infestations. The bean seeds were monitored through checking and careful dissection 20 bean seeds per replicate, after 3 days simultaneously, till the emergence of the first adult *C. maculatus*. The average duration for development of each immature life stage (i.e., pre- L1, 1st, 2nd , 3rd , and 4th larval and pupal stages as well as, total immature duration) of the insects was calculated on this basis according to modified methods of (12) and (10).

Data analysis

The data collected from this study were analysed using Statistical Package for Social Sciences (SPSS) 23rd version. The data were first pooled and processed using Microsoft excel 2010 and expressed as Mean±SD for each variable. 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

Table 1 showed results on mean duration of egg hatching of Bean weevil (*Callosobruchus maculatus*) in days, raised on bean seed treated with varying concentration of insecticide in storage. There was significant (P = 0.05) increase in the duration of time it took egg to hatch from the allowed duration of oviposition among the various treatment, with grains treated with higher concentration of insecticide recording significantly longer duration of up to five days.

Table 1: Mean Duration of egg hatching of Bean weevil (*Callosobruchus maculatus*) in days

Dichlorvos treatment (mg/kg)	Allowed duration of oviposition (days)	PRE-L1 (Days)
NC (0.0)	3.00	4.00±0.00[a]*
PC (8.0)	3.00	4.00±0.00[a]
T1 (2.0)	3.00	4.00±0.00[a]
T2 (4.0)	3.00	4.00±0.00[a]
T3 (6.0)	3.00	4.00±0.00[a]
T4 (10.0)	3.00	5.00±0.00[b]
T5(12.0)	3.00	5.00±0.00[b]

n = 5
* Means followed by the same super-script alphabet, in the same column, are not significantly different at P ≤ 0.05
NC = Negative control, **PC** = Positive control, **Pre-L1** = newly hatch young larva within the egg before penetrating the seed coat

Mean duration of development (days) of different larval instar within the cowpea seed *(Vigna unguculata*) is presented in table 2. The result revealed that, total larval duration (TLD) also varied significantly (P =0.05). There was significant increase in the duration of time it took first larval stage to reach the forth larval stage within cowpea seed treated with varying concentration of insecticide, with grains treated with higher concentration of insecticide recording significantly longer duration of larval development of up to 18 days and those without insecticide recorded the least of 15 days.

Table 2: Mean duration of development (days) of different larval instar within the cowpea seed *(Vigna unguculata*)

Dichlorvos treatment (mg/kg)	Larval instars L1	L2	L3	L4	TLD (Days)
NC (0.0)	3.19±0.06	3.08±0.11	0.91±0.34	1.02±0.31	15.00±0.00^{a}
PC (8.0)	3.07±0.08	3.11±0.17	0.99±0.27	0.98±0.26	15.00±0.00^{a}
T1 (2.0)	3.28±0.15	2.93±0.06	0.74±0.27	0.86±0.32	15.00±0.00^{a}
T2 (4.0)	2.95±0.05	2.16±1.96	1.72±1.65	0.96±0.25	15.00±0.00^{a}
T3 (6.0)	3.11±0.20	3.04±0.17	0.75±0.17	0.78±0.15	15.00±0.00^{a}
T4 (10.0)	3.22±0.41	2.96±0.19	1.68±0.28	2.10±0.25	18.00±0.00^{b}
T5(12.0)	3.20±0.10	3.04±0.25	1.95±0.19	1.83±0.44	18.00±0.00^{b}

n = 5
* Means followed by the same super-script alphabet, in the same column, are not significantly different at P ≤ 0.05
L1 = 1^{st} instar larva, **L2** = 2^{nd} instar larva, **L3** = 3^{rd} instar larva, **L4** = 4^{th} instar larva
TLD = Total Larval Duration

Table 3 showed result on mean duration of development (days) of different pupal stage within the cowpea seed *(Vigna unguculata*). Total pupal duration (TPD) did not varied significantly (P =0.05) among the cowpea seed treated with different concentration of insecticide, with pupae recorded in all treated took approximately six (6) days to emerge as adult.

Table 3: Mean duration of development (days) of different pupal stage within the cowpea seed *(Vigna unguculata*)

Dichlorvos treatment (mg/kg)	**Pupal stages** **P1**	**P2**	**P3**	**P4**	**TPD** (Days)
NC (0.0)	1.94±0.34	1.83±0.42	0.45±0.21	1.70±0.27	6.00±0.00^{a}
PC (8.0)	2.03±0.35	2.42±0.37	0.40±0.22	1.50±0.00	6.00±0.00^{a}
T1 (2.0)	2.20±0.36	1.91±0.11	0.50±0.18	1.60±0.22	6.00±0.00^{a}
T2 (4.0)	2.29±0.21	1.96±0.12	0.40±0.14	1.70±0.27	6.00±0.00^{a}
T3 (6.0)	1.78±0.44	1.92±0.31	0.36±0.13	1.70±0.27	6.00±0.00^{a}
T4 (10.0)	2.03±0.24	1.95±0.11	0.80±0.67	1.20±0.67	6.00±0.00^{a}
T5(12.0)	2.06±0.18	1.92±0.18	1.10±0.82	0.90±0.82	6.00±0.00^{a}

n = 5
* Means followed by the same super-script alphabet, in the same column, are not significantly different at P ≤ 0.05
P1 = 1^{st} pupal stage, **P2** =2^{nd} pupal stage, **P3** = 3^{rd} pupal stage, **P4** = 4^{th} pupal stage
TPD = Total Pupa Duration

Total larval duration (TLD) and total immature duration (TID) varied significantly (15.00±0.00 to 18.00±0.00 and 21.00±0.00 to 24.00±0.00 days) for cowpea weevils raised on untreated beans seed (NC) and highest concentration treated beans seed respectively, but there was no significant different in the total pupal duration (TPD) among all the treatment. This result shows that chemical insecticide has significantly affected the egg and larval stage duration of the beans weevils development.

Table 4: Total immature duration of development (days) of beans weevil (*C. maculatus*) raised in insecticide treated cowpea seed

Dichlorvos treatment (mg/kg)	TLD (Days)	TPD (Days)	TID (Days)
NC (0.0)	15.00±0.00[a]	6.00±0.00[a]	21.00±0.00[a]
PC (8.0)	15.00±0.00[a]	6.00±0.00[a]	21.00±0.00[a]
T1 (2.0)	15.00±0.00[a]	6.00±0.00[a]	21.00±0.00[a]
T2 (4.0)	15.00±0.00[a]	6.00±0.00[a]	21.00±0.00[a]
T3 (6.0)	15.00±0.00[a]	6.00±0.00[a]	21.00±0.00[a]
T4 (10.0)	18.00±0.00[b]	6.00±0.00[a]	24.00±0.00[b]
T5(12.0)	18.00±0.00[b]	6.00±0.00[a]	24.00±0.00[b]

n = 5 *

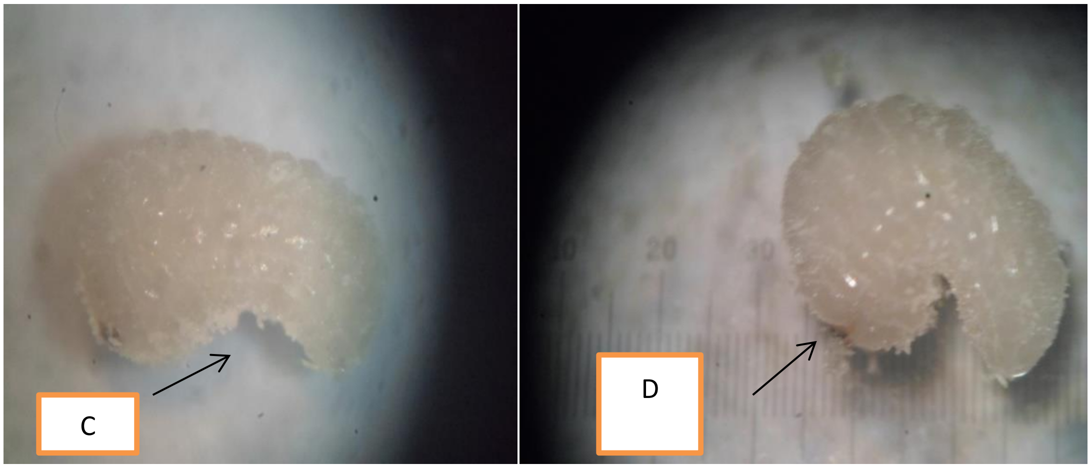
Means followed by the same super-script alphabet, in the same column, are not significantly different at P ≤ 0.05

TLD = Total Larval Duration, TPD = Total Pupa Duration, TID = Total Immature Duration

Microphotographs of different larval instars of *C. maculatus* is presented on plate I. The insect has four larval instar within the beans seed before pupated which was traced through destructive sampling and revealed different sizes of the larval instar

A: 1st larval instar

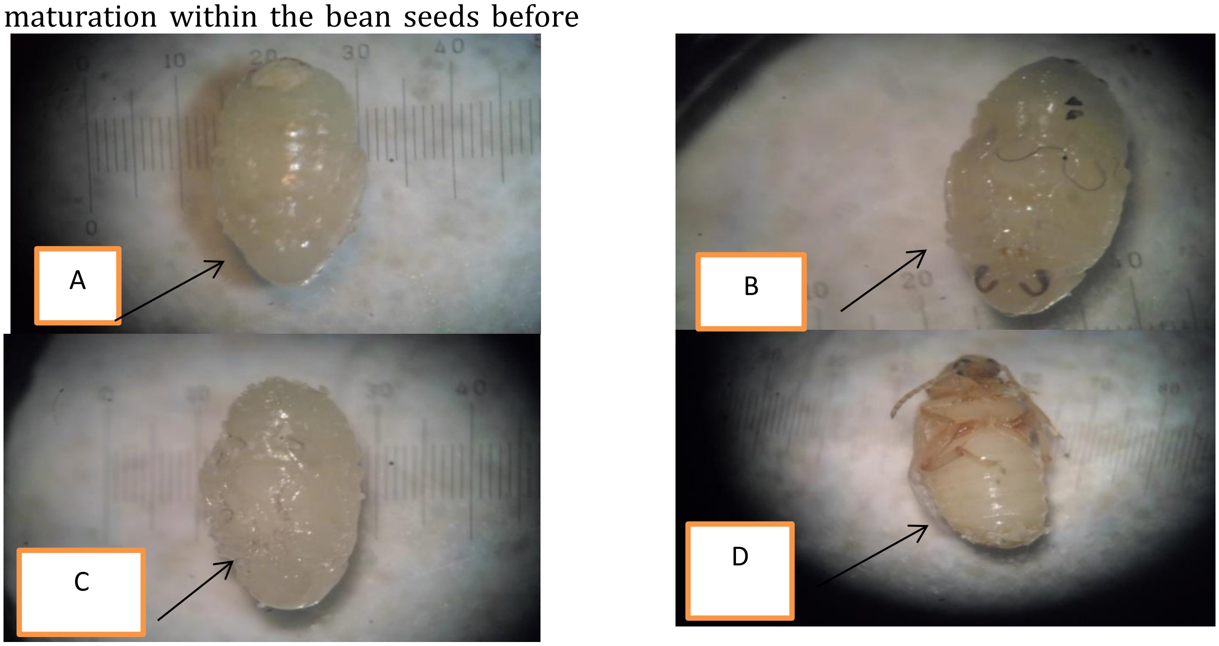
B: 2nd larval instar



C: 3rd Larval instar D: 4th larval instar

Plates I A – D: Microphotographs of different larval instars of *C. maculatus* (X 40 objective) Source: Field source

Microphotographs of pupal stages of *C. maculatus* is presented on plate II. The insect has four pupal stages which are differentiated by the level of pre-adult maturation within the bean seeds before emerging as adult this was traced through destructive sampling and revealed different stages of the pupal development as shown below.



A: 1st pupa stage
B: 2nd pupa stage
C: 3rd pupa stage
D : 4th pupa stage

Plates II A – D: Microphotographs of pupal stages of *C. maculatus* (X 40 objective) Source: Field source

DISCUSSION

There was significant increase in the period of egg hatchability and total immature duration as the insecticide concentration increases in the host environment i.e., cowpea seed. Meaning that chemical insecticide has significantly affected the egg hatching and larval stage duration of the bean's weevil development. This observation could be due to interference of sufficient food absorption from host grain because of anti-feeding effect of some organophosphorus insecticide. This was in conformity with the report of (1), who stated the oviposition and percentage egg hatched were significantly suppressed on seeds treated with higher treatment level of extracts. Leaf extract with hexane at 2ml (10.0% v/w)/20g cowpea seeds was most effective in suppressing oviposition and egg hatched. Though the findings also contradict the work of (5), 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.

On the other hand total larval, pupal immature duration and their respective weight were significantly affected this was in line with the work of (14), who stated that effects of insecticides sub-lethal doses/concentrations upon insect biology may present itself through reduced oviposition, increased development period of immature stages or decreased life span. The sub-lethal effects of the insecticides such as lufenuron, methoxyfenozide, spinosad, endosulfan, novaluron and tebufenozide upon *Anticarsia gemmatalis Hübner* (Lepidoptera: Noctuidae), reducing the pupal weight, adult longevity and fertility (15). In another research conducted by (7), it revealed that insecticide such as hexaflumuron decreased the total number of eggs, oviposition period, pupation and adult emergence of *Plutella xylostella*(Linnaeus) (Lepidoptera: Plutellidae).

Conclusion

Lethal concentration of insecticide significantly affected duration of immature development, with concentration of 10 mg/kg and above increasing the duration of immature development by additional three days. Furthermore the research pprovides monitoring information that will guide on spot assessment of the effect of insecticide contaminated stored cowpea for all users by assessing the internal condition of the immature life stages of *C. maculatus* within the bean seed before it emerge as adult when the destruction is over.

Declarations

We hereby declared that this research work has not been publish elsewhere

Authors Contribution

SIM and OIK conceptualized the study. ICJO and MH designed the study. SIM, NEM and GAY participated in fieldwork and data collection. YI, HHN and GUN performed the data analysis; SIM and OIK interpreted the data. SIM prepared the first draft of the manuscript, reviewed by YI, HHN and GUN. All authors contributed to the development of the final manuscript and approved its submission.

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