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Original article

Effects of Dichlorvos Insecticide and Environmental Conditions on Reproductive Attributes of Cowpea Weevil (*Callosobruchus maculatus*), Infesting Stored Cowpea (*Vigna unguiculata*)

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ABSTRACT

The Influence of insecticide treatment and environmental conditions on reproductive attributes (egg fertility, adult survivorship rate and daily adult emergence) of beans weevil (*Callosobruchus maculatus*) infesting stored grains was investigated. Insecticide-free grains of white cowpea (*Vigna unguiculata*) and test insecticide (Dichlorvos) were obtained from cowpea and agrochemical vendors respectively, in Minna, Nigeria. Nulliparous female individuals of *C. maculatus*, for the experimentation, were obtained from Stored Produce Research Institute, Ilorin. The weevils were exposed to five graded concentrations of the insecticide prior to experimentation, with negative and positive Controls. All experimental set-ups were in 5 replicates. The results revealed significant effects of the insecticide on all reproductive traits investigated. Increase in insecticide concentration significantly ($P < 0.05$) affected egg fertility (76.63±6.98, 35.75±7.67), adult survivorship rate (44.29±5.48, 19.21±5.49 (%)) for Negative Control (NC) and Treated beans (T5) respectively, as well as, daily adult emergence. Correlation between adult emergence and Temperature (r = -0.2706, -0.8222); relative humidity (r = 0.47657, -0.2747) for NC and T5 respectively. The tested insecticide had significant effects on reproductive attributes of the insect species. This information can assist cowpea farmers and stakeholders in pre and post storage planning activities, as well as, monitoring insecticide contamination in stored grains.

Key words: Insecticide, monitoring, insecticide contamination, *Callosobruchus maculatus*, *Vigna unguiculata*, stored grains

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INTRODUCTION

Cowpea is a popular leguminous crop in Africa which is known as 'beans' in Nigeria. The largest production is in the moist and dry Savannas of Sub-Saharan Africa (SSA), where it is intensively grown as an intercrop with other cereal crops like millet, sorghum, maize,[1]. Though, it is grown in other parts of the world, Nigeria remains the largest producer and consumer of cowpea in the world [1].

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 [2]. Damage and losses to stored grains, especially cowpea by insect pests is very severe. About 4 percent of total annual production of cowpea, valued at over 30 million US dollars, is lost annually to the cowpea Bruchid (*Callosobruchus maculatus*) in Nigeria [3]. Severe infestation by this pest can lead to total grain loss in storage. It is a field-to-store pest; adult beetles lay eggs on pods (in the field) or on seeds (in storage). After hatching, the larvae develop within seeds and eat up the cotyledon, thereby, causing extensive damage. Adults emerge from the seeds through characteristic holes made by the larvae [3].

C. maculatus (Coleoptera: Bruchidae) is a major pest of stored legumes crops with infestation starting at pod maturity [4]. It does considerable damage to crops in the field and also causes quantitative loss to stored seeds. The major pulses attacked by *C. maculatus* include Cowpea, Bambara groundnut, Soya beans and Pigeon pea. The damage caused by this pest to leguminous seeds is mostly due to its larvae feeding inside the seed which results in weight loss, decreased germination potential, decrease in nutritional and aesthetic quality of the seeds and diminished market value of the seeds [4].

Dichlorvos is an organophosphorous insecticide that is employed for both agricultural and medical purposes. It is used throughout the world in a variety of formulations and is widely available. Although, it has relatively low toxicity in humans, if taken in excess can be fatal [5]. High levels of insecticide residues arising from improper application and multiple sprays of sub-lethal doses have been reported to be responsible for the poisoning and deaths of people in both rural and urban communities [6].

The study was designed to determine the effects of insecticide treatment commonly used in preserving grains, on reproductive attributes (egg fertility, rates of survivorships and adult emergence as well as, similar influence of immediate micro weather conditions of beans weevil (*C. maculatus*), in order to apply the knowledge gained in stored beans insecticide contamination monitoring process.

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 [7]. 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 Weevils (*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 [7].

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 [8], 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 reproductive traits investigated were monitored in each replicate.

Bioassay of the Set-up Involving 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 [9]. After three days, all the adult insects were removed from the bowl and eggs laid by the females were monitored to adult emergence.

Egg fertility rate

The number of eggs that hatched per bowl was counted till there were no more hatching. The egg fertility rate was calculated following modification of the methods described by [7]:

$$\text{Egg fertility rate} = \frac{\text{Total number of hatched eggs} * 100\%}{\text{Total Number of eggs laid}}$$

Adult survivorship rate

The number of emerged adults per bowl was counted till there were no more emergence. The survivorship rate was calculated following modification of the methods described by [7]:

$$\text{Rate of adult survivorship} = \frac{\text{Total number of emerged adult} * 100\%}{\text{Total Number of eggs laid}}$$

Daily adult emergence

The number of emerged adults per bowl, per day was counted till there were no more emergence. The daily adult emergence was calculated by summing up number of daily emergence per test [10].

Determination of Micro weather Conditions

Temperature and relative humidity of the immediate environment were determined using hygrometer, following the method of [11].

Data Analyses

The data collected from this study were analysed using Statistical Package for Social Sciences (SPSS) 23th 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 of the effects of insecticidal treatment on egg fertility and adult survivorship rate of bean weevils raised on insecticide-treated beans seeds in storage. The two principal parameters (fertility and survivorship rate), under investigation, were significantly (P < 0.05) affected by insecticidal treatment, and such effects were dose-dependent, i.e., such parameters decreased with increase in insecticide concentration.

Table 1: Egg fertility and rate of adult survivorship of beans weevils raised on insecticide treated beans seeds in storage

Insecticide Treatment (mg/kg)	Egg fertility (%)	Adult survivorship (%)
NC (0.0)	$76.63\pm6.98^{f*}$	44.29 ± 5.48^{e}
PC (8.0)	53.36 ± 5.29^{c}	21.39 ± 4.97^{b}
T1 (2.0)	72.32 ± 8.33^{e}	35.52 ± 5.97^{d}
T2 (4.0)	61.15 ± 7.21^{d}	26.82 ± 6.70^{c}
T3 (6.0)	56.45 ± 7.19^{c}	23.91 ± 4.12^{bc}
T4 (10.0)	43.37 ± 3.27^{b}	20.33 ± 2.25^{b}
T5 (12.0)	35.75 ± 7.67^{a}	19.21 ± 5.49^{a}

*. Values followed by 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

KEY: NC = Negative control, PC = Positive control, T1 – T5 = Test concentrations 1 to 5

Temporal distribution of individual daily adult emergence of the weevils, from beans seeds treated with insecticide in storage is presented in Figure 1. There was daily adult emergence for six to seven days irrespective of the insecticide concentration applied but, distinctively, weevils emerging from grains treated with highest concentration of the insecticide emerged only for six days. The temporal distribution of the emergence recorded peak at 4th day (NC, PC, T1, T2, and T3) for recommended doses, 5th day (T4); 6th day (T5) for higher concentration doses.

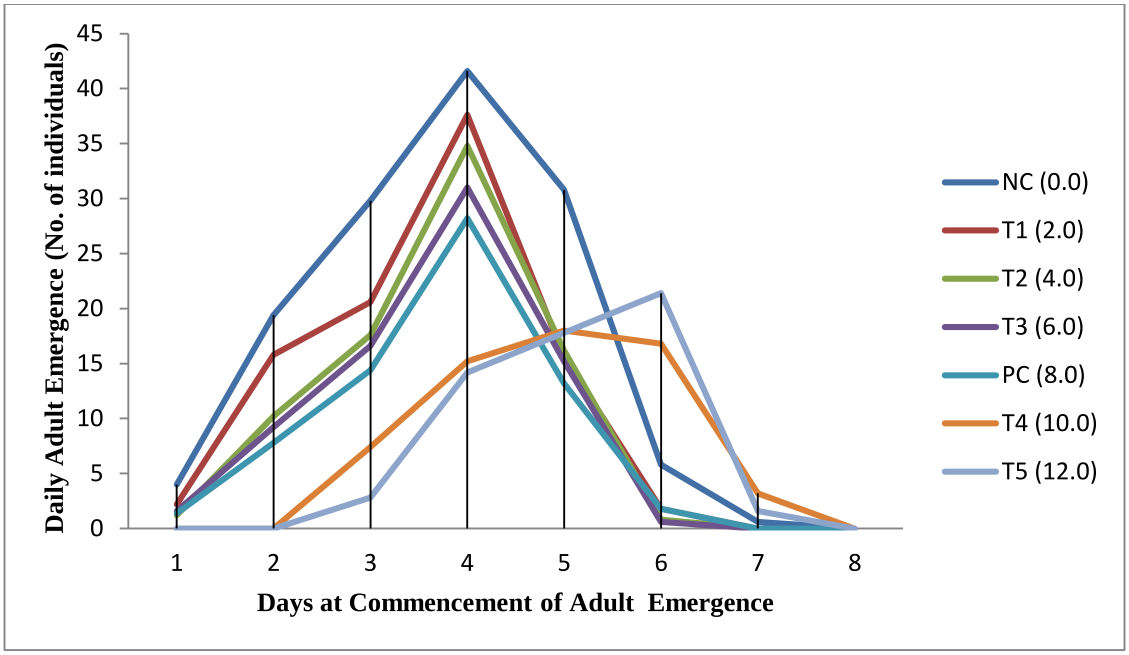

Figure 1: Temporal distribution of daily adult emergence from beans seeds treated with insecticide in storage

KEY: NC = Negative control, PC = Positive control, T1 – T5 = Test concentrations 1 to 5

Micro weather conditions (temperature and relative humidity) of the immediate environment of the insecticide treated beans seed, during *C. maculatus* adult emergence, are presented in table 2. The temperature and relative humidity ranged from 26.0 to 30.0°C and 27.0 to 46.0% respectively. Both parameters recorded were within tolerable ambient condition

Table 2: Micro weather conditions (temperature and relative humidity) of the immediate environment of insecticide treated beans seed, during *C. maculatus* adult emergence

Days of adult emergence progression	Temperature (^{0}C)	Relative humidity (%)
1	28	38
2	29	46
3	28.5	40
4	27	35
5	28	34
6	26	30
7	29	27
8	30	28
Mean±SD (Range)	28.19±1.25 (26.00 – 30.00)	34.75±6.48 (27.00 – 46.00)

Table 3 highlighted results on relationships between adult emergence and immediate micro weather conditions of bean weevils raised on insecticide-treated beans seeds in storage. There was negative correlation between temperature and adult emergence across all treatments, though emergence from grains treated with higher concentrations of insecticide recorded significant ($P < 0.05$) negative correlations. On the other hand, there was positive correlation between relative humidity and adult emergence except emergence from grains treated with higher concentration of insecticide that recorded negative in-significant correlation.

Table 3: Correlation between adult emergence and immediate micro weather conditions of bean weevils raised on insecticide treated beans seeds in storage

Insecticide Treatment (mg/kg)	Temperature vs Adult emergence	R.H. vs Adult emergence
NC (0.0)	-0.2706	0.47657
PC (8.0)	-0.3035	0.3641
T1 (2.0)	-0.2602	0.47624
T2 (4.0)	-0.2982	0.3741
T3 (6.0)	-0.2914	0.3889
T4 (10.0)	-0.7825*	-0.1978
T5 (12.0)	-0.8222*	-0.2747

***** Correlation is significant at the 0.05 level (2-tailed).

KEY: NC = Negative control, PC = Positive control, T1 – T5 = Test concentrations 1 to 5

DISCUSSION

The action of dichlorvos insecticide used, significantly decreased egg fertility and adult survivorship rate. This might be due to inhibitory effect of some synthetic insecticide, as earlier reported by [12], and [13] who worked on insect pests of maize (stalk borer) and recorded significant sublethal effects of insecticide on fertility, fecundity and oviposition among others.

Insecticide application, as observed in this study, significantly influenced the duration of development, and temporal distribution of adult emergence, as well as their density. These effects could be due to insecticidal stress and anti-feeding effect of some insecticide, these was also reported by [14], who stated that hexaflumuron insecticide significantly decreased the adult emergence rate of *Anopheles stephensi* (Diptera: Culicidae).

Results of temperature and relative humidity recorded were within ambient tolerable limits for the insects, which were not more than 30^{o}C and 46%, respectively, this could be because the setup was carried out between months of December and January that coincide with the setting-in of peak dry season in North Central part of Nigeria. The results were however, not in agreement with those reported by [15], who reported slightly higher temperature of 35^{o}C and relative humidity of 65% and 95% to be optimum for the thriving of *Callosobruchus maculatus* and *Callosobruchus subinnotatus,* though, reared on Bambara groundnut not beans seed.

The relationships among adult emergence, temperature and relative humidity is an indication that temperature affects adult emergence, and on the event of insecticide application, such effects could be magnified. While relative humidity influence adult emergence positively, but certain dose of insecticide application brought about negative influence. These insecticidal effects generally could be due to its different chemical additives used during formulation of most synthetic insecticide. This finding is similar to that reported by [16], who stated that temperature, relative humidity and their interaction significantly affect the developmental time of *Chilo partellus*, with temperature being the most important factor. In his study, significant differences were observed in life history processes of *C. partellus* under different relative humidity levels and temperature regimes, suggesting the influence of these factors in determining suitability of the environment for the development of the pest, which, in turn, affect the spread and establishment of the pest.

Conclusion

In conclusion, sub-lethal and lethal effects of insecticide contamination of stored cowpea on colonized cowpea weevils could serve as bio-markers of toxicity, other than killing the insect pest as expected by most farmers and vendors of stored cowpea. However, detailed study on identified traits, by way of articulating noticeable effects and their interpretation could serve as a monitoring tool of early warning signals of insecticide contamination in cowpea, since insects are ubiquitous and most cowpea weevils will always attempt to colonize cowpea seed even when pre-treated with insecticide. Therefore, this research could serve as a baseline study of identifying bio-markers for monitoring insecticide contamination in stored cowpea.

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