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Effects of Initial Infestation Levels of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) on Cowpea and Use of *Nicotiana tabacum* L. Aqueous Extract as Grain Protectant

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Abstract

This study determines the effects of initial infestation of cowpea seeds (Ife brown variety) with different insect densities (0, 2, 4 and 6 pairs per 50 g seeds) of *Callosobruchus maculatus* (F.) and evaluates the effects of aqueous leaf extract of *Nicotiana tabacum* L. on *C. maculatus* in the laboratory. It was observed that adult beetle population increased significantly ($p<0.05$) with increase in insect density. The increase in population of the beetle and corresponding weight loss of the seeds in different levels of infestation showed that the cowpea variety was susceptible to beetle infestation, emergence and survival of progeny. Significantly more adults emerged on higher infestation compared to lower and no infestation. In Nigeria, *Nicotiana tabacum* L. is a locally available plant, with known insecticidal properties. The plant leaf extract was easily extracted with water and confirmed its effectiveness as a protective agent for stored cowpea seeds. Experiment was conducted to assess the effects of aqueous extracts of *N. tabacum* at 0, 0.1, 0.2 and 0.3 ml/50 g cowpea seeds on *C. maculatus*. Data were recorded and show varying levels of effectiveness against *C. maculatus*. Result shows that seed appearance is dependent on levels of insect population while *N. tabacum* aqueous extract exerts effects on survival of *C. maculatus*. Aqueous leaf extract of *N. tabacum* probably contains some insecticidal properties which might have significantly conferred beetle mortality and reduced beetle emergence leading to a decrease in seed weight loss.

Key words: *Callosobruchus maculatus*, grain protectant, infestation, *Nicotiana tabacum*, , seed damage.

1.0. Introduction

Cowpea, *Vigna unguiculata* (L.) Walpers (Papilionaceae), is popularly grown in northern Nigeria with required climatic and soil conditions suitable for its cultivation. Its nutritional contents have made it an important legume crop in human diet and livestock feeds. All the plant parts used for food are nutritious, providing protein, vitamins and minerals (Emeasor et al., 2007). The crop is however attacked by a number of insect pests both in t he field and storage. Seed beetle, *Callosobruchus maculatus* (F.) is a major field-to-store insect pest of cowpea responsible for a great deal of damage to the seeds making it unfit for human consumption. Musa (2007) estimated the loss caused by *C. maculatus* over a 12-week storage period to be 42.2% in Southern Guinea Savanna of Nigeria. Swella and Mushobozy (2009) examined the storability of ten different legume seeds to infestation by *C. maculatus* over a period of 2 months and recorded mean weight losses ranging from 2.7 to 9.6 g.

Over the past few decades, application of synthetic, broad spectrum pesticides and fumigants to control stored product pest species has been a standard practice (Ukeh, 2009). The insect pest has therefore, been controlled through the use of synthetic insecticides which include organophosphate and chlorinated hydrocarbons. However, these insecticides are not readily available to farmers because of their relatively high cost, pest resistance, lack of application skills, adulteration, toxicity to man and animals (Afun *et al*., 1991; Ajayi and Rahman, 2008; Musa *et al*., 2009). The increasing problems of these insecticides to the environment have given credence to the desire for safer protectants such as botanicals. Biopesticides are biodegradable and less harmful to man and environment (Schmutterer, 1990), a result of which make them popular as potential pesticides with less challenges. The present study was undertaken to investigate insecticidal effects of aqueous extract of *Nicotiana tabaccum* against cowpea beetle, *C. maculatus*. The objective of the study was to determine the effects of different levels of C. maculatus infestation on cowpea and use of aqueous leaf extract of *N. tabacum* as grain protectant.

2.0. Materials and Methods

2.1. Study Area

The study was carried out in the Crop Protection research laboratory of University of Ilorin, Nigeria.

2.2. Source of cowpea seeds

The Ife brown variety of cowpea used for the study was obtained from International Institute for Tropical Agriculture, Ibadan, Nigeria.

2.3. Insect Culture in the laboratory

Callosobruchus maculatus used to establish the culture was obtained from a batch of already infested cowpea seeds kept in the research laboratory of the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria. Fresh and clean cowpea seeds were purchased from a local market, Ilorin, and infested with the beetles in a transparent plastic jar (9 cm diameter) covered with baft cloth to prevent insect escape and entry of insects. The culture was maintained under ambient environment (28 ± 3^0 C and 75±3% RH) of laboratory cupboards.

2.4. Preparation of plant material

Leaves of tobacco, *Nicotiana tabacum*, were plucked from the parent plant on an abandoned area, Sango, Ilorin. The leaves were washed and air-dried slowly in the laboratory for 21 days, before pulverization in a mill. About 100 g of the plant powder were soaked in a closed vessel (30 cm diameter) of 1 litre of water, stirred and left for 7 days. The solution was strained with a 0.1 mm sieve to standardize the particles, solid residue was pressed followed by filtration through Whatman No. 1 filter paper. The filtrate was concentrated in a water-bath set at 40^0 C to vapourize the excess solvent.

2.5. Bioassays

2.5.1. ***Effects of initial C. maculatus infestation on cowpea seeds***

Freshly emerged adults of *C. maculatus* (about 2 days old) from the culture were introduced into 50 g clean cowpea seeds at 2, 4 and 6 insect pairs respectively. The morphology of beetle`s snout, that of the male is rough and coarse compared to that of the female which is smooth and uniform, was used for sex differentiation. Control was also set up with no insect in the experiment. Each mixture of seeds and beetles was covered in their containers with muslin to allow aeration and prevent beetle escape or entry of predators. Data on population of surviving beetles and corresponding seed weight loss were taken at 30, 60 and 90 days after infestation (DAI) while data on seed damaged were taken at 90 DAI.

2.5.2. ***Effects of aqueous leaf extract of N. tabacum on C. maculatus***

Five teneral adults of 3 females and 2 males of *C. maculatus* were introduced to clean and wholesome cowpea seeds mixed with aqueous leaf extract of *N. tabacum* at the rates of 0.0, 0.10, 0.20 and 0.30 ml/50 g seeds. The mixture was thoroughly agitated to achieve uniform coating of the seeds. Each transparent container was covered with muslin. Each treatment, including control (seeds not treated with leaf extract), was replicated four times in a complete randomized design. Parameters quantified commenced with visual counts of dead adult beetle at 24 h intervals and was terminated at 96 h after treatment (HAT). Keen observation on progeny emergence from 23 days after infestation (DAI) was terminated at 35 days after treatment (DAT) while total emergence per treatment was used for computation. Extent of the cowpea seed damaged was determined 90 DAT under small-scale storage condition.

2.6. ***Statistical design***

Data collected were subjected to one-way analysis of variance (ANOVA) and where significant differences existed, means were separated using Least Significant Difference (LSD) at p=0.05 significance level.

3.0. Results and Discussion

Table 1 shows the mean seed weight loss, number of seeds damaged as a result of feeding activities of the *C. maculatus* and mean abundance of the beetle. Seeds exposed to higher number of beetles had more damage and greater weight loss. It was observed that adult beetle population increased with increase in insect density. The increase in population of the beetle in different levels of infestation showed that the cowpea variety was susceptible to beetle infestation, emergence and survival of progeny. Mean population of adults increased significantly ($p<0.05$) with increase in initial levels of *C. maculatus* infestation. All levels of infestation differed significantly ($p<0.05$) in all parameters observed from the control. The seeds in the control were not damaged at all as they were not infested. This study shows that *C. maculatus* readily attack cowpea seeds exposed to infestation. The proximate compositions of cowpea make it possible for the beetle to respond to the seeds. In this study, the seed weight loss, seed damaged and increase in beetle population were consequences of artificial infestation. Grain hardness has not been reported to confer complete immunity on grains from insect attack and infestation (Osipitan and Odebiyi, 2007). However, there is need for further detailed studies on susceptibility of this variety to *C. maculatus* to determine inherent factors responsible for response of the beetle to it. Idoko and Adesina (2012) observed that oviposition and adult emergence by *C. maculatus* differ significantly with insect density.

The effects of rates of treatment of *N. tabacum* leaf extract on mean beetle mortality, progeny emergence of *C. maculatus* and seed weight loss and damaged by the insect are presented in Table 2. There was no adult mortality at 24 hours after treatment (HAT) except with the leaf extract applied at 0.3 ml which caused mean beetle mortality of 0.25. At 48 HAT, all rates of leaf extract caused mean mortality ranging from 1.0 to 1.25. The extract applied at 0.3 ml caused higher mean mortality of 4.50 96 HAT whereas the extract at 0.1 and 0.2 ml/50g cowpea seeds caused mean mortality of 3.50 and 4.25 respectively. Each rate of the leaf extract induced beetle mortality of *C. maculatus* significantly when compared with the control (Table 2). Mortality varied with rates of treatment and exposure period as indicated by significant differences ($p<0.05$) recorded among rates of treatment after a storage period of 96 HAT. Most beetles died in the highest rate of treatment and mortality increased by the day in all treatments except in the control which had no mortality. The observed mortality may be linked to nicotine content of the leaf extract concentration and possibly vigour of the beetle. The contact action of the extract may have inhibited respiration of adult beetle. *Nicotiana tabacum* is reported to possess contact, stomach and respiratory poisoning properties attributed to its active constituent, nicotine (Stoll, 1988). The results confirmed the observation that mortality of insects varied with plant part, rate of application and exposure period (Akinneye and Ashamo, 2009). The present study has shown that aqueous leaf extract of the plant material can also be exploited for use as a grain protectant after careful consideration of nicotine which is a harmful component.

Analysis of variance shows that there were significant differences in the mean number of emergence recorded at different rates of treatment. The higher rates of treatment of 0.3 ml gave the lowest adult emergence which was significantly different compared to control. The highest rate of 3.0 ml was however comparable to other treatments

except control. The toxicity and potency of natural plants have been ascribed to their chemical active ingredients (Adedire and Lajide, 1999; Musa, 2007).

Cowpea seeds treated with aqueous extract of *N. tabacum* suppressed adult emergence. Meanwhile, extract applied at 0.3 ml showed promising effect in suppressing adult emergence. Similar trend which was observed in adult emergence was also recorded for the mean weight loss. The mean weight loss on cowpea seeds increased with increase in initial number of *C. maculatus* population. The leaf extracts applied at 0.2 ml/50 g seeds and 0.3 ml/50 g gave a weight loss of 4.67 g and 2.26 g respectively which were significantly different compared to control (10.96 g) at 60 DAT. The leaf extracts applied at 0.2 ml/50 g seeds and 0.3 ml/50 g gave a weight loss of 5.96 g and 4.36 g respectively which were significantly different compared to control (13.29 g) at 90 DAT. The lowest level of treatment was not significantly (p.0.05) different when compared to control at 60 and 90 DAT. In this study, mean seed weight loss ranged from 2.26 in 0.3 ml to 8.29 in 0.1 ml at 60 DAT. Mean seed weight loss ranged from 4.36 in 0.3 ml to 8.90 in 0.1 ml at 90 DAT. The mean seed weight losses at 0.2 and 0.3 ml during the period of investigation were significantly different ($p<0.05$) compared to control. Aqueous extract applied at 0.3 ml was significantly effective in the control of *C. maculatus* at $p=0.05$ when compared to lower treatments. The performance of the leaf extract of *N. tabacum* showed that when admixed at 0.1, 0.2 and 0.3 ml/50g cowpea seeds reduced adult emergence of *C. maculatus* by 28.85, 46.63 and 68.27% respectively compared to control.

This experiment *N. tabacum* shows may have some insecticidal properties which might have significantly reduced adult emergence from cowpea seeds, leading to a decrease in seed weight loss. Reduction in adult emergence could be ascribed to egg mortality or death of immature stages following the ovicidal and larvicidal properties of *N. tabacum.* Nicotine content may not afford recommendation of the plant for consumption of its toxicity also to humans through bonding to receptors at the nerve synapses (junctures), causing uncontrolled nerve firing and by mimicking acetylcholine at the nerve-muscle junctions in the central nervous system (Ujvary, 1999). Results showed that adult emergence and seed weight loss were insect population dependent and *N. tabacum* can be used for effective management. Danjumma *et al*. (2009) reported that 100% adult mortality of *Sitophilus zeamais* was recorded on maize treated with the powder of *N. tabacum*. Khalif and Al-Farhani (2008) recorded that leaf powder of *N. tabacum* caused 100% mortality of *Tribolium castaneum* after 7 days.

4.0. Conclusion

It has been shown that *N. tabacum* leaf extract has some insecticidal effects against *C. maculatus*. Its inclusion in pest management strategies is therefore suggested for upgrading traditional post-harvest protection practices of seeds required for consumption/animal feeding or next season planting.

Table 1: Influence of different levels of *Callosobruchus maculatus* infestation on cowpea weight loss, seed damaged and insect abundance

No. of insects	Mean wt. loss 30 DAI	Mean wt. loss 60 DAI	Mean wt. loss 90 DAI	Adult beetle abundance 30 DAI	Adult beetle abundance 60 DAI	Adult beetle abundance 90 DAI	Mean seeds damaged 90 DAI
4 beetles	6.52b	17.27b	17.27b	36.75b	137.50ab	109.50ab	72.50b
8 beetles	7.62bc	35.90c	36.68c	106.75c	195.25ab	212.75b	94.50c
12 beetles	8.96c	35.00c	36.68c	119.75c	320.75b	216.50b	111.25d
0 beetle	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
SED	0.64	3.58	3.29	5.52	64.69	37.88	2.73
C.V. (%)	11.0	16.0	16.0	8.0	39.9	28.0	4.0

Values with the same letter(s) in the same column are not significantly different at p=0.05 using Duncan`s multiple range test

Table 2: Protective potential of aqueous leaf extract of *Nicotiana tabacum* against *Callosobruchus maculatus* in stored cowpea

Treatment (ml)	Beetle mortality (HAT)	Beetle mortality (HAT)	Beetle mortality (HAT)	Beetle mortality (HAT)	Mean	Mean grain wt. loss (g)DAT	Mean grain wt. loss (g) DAT	Mean
	24	48	72	96	Adult Emergence	60	90	Seed damaged
0.1	0.00a	1.00a	2.50a	3.50a	37.0ab	8.29ab	8.90ab	24.00b
0.2	0.00a	1.00a	2.50a	4.25a	27.75ab	4.67bc	5.96b	11.00c
0.3	0.25a	1.25a	2.50a	4.50a	16.50b	2.26c	4.36b	2.75d
0.0	0.00b	0.00b	0.00b	0.00b	52.00a	10.96a	13.29a	39.00a
SE±	0.12	0.24	0.38	0.42	8.40	1.62	1.51	2.40

Values in the same column with the same letter(s) are not significantly different at p=0.05 significance level using Duncan`s multiple range test

DAT = days after treatment

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