ISSN 2276-7029
Volume 12, Number 1, 2022
Pages 14-18

Insecticidal and Anti-infestation Efficacy of *Sesamum indicum* L. Leaf Powder against *Callosobruchus maculatus*

Negbenebor,[1] H.E., Mohammed,[2] A.A. and *Nura,[2] S
[1]Department of Biological Science, Baze University, Abuja, Nigeria.
[2]Department of Biology, Ahmadu Bello University, Zaria, Nigeria
* Correspondence: nsalisu@abu.edu.ng; DOI: https://doi.org/10.52417/njls.v12i1.287

Abstract

Callosobruchus maculatus is the major insect pests in Nigeria that causes significant loss by damaging stored cowpea seeds thereby reducing their market value and nutritional quality. A study was conducted to investigate the insecticidal potency of sesame (Sesamum indicum L.) leaf powders in protecting cowpea seeds against cowpea bruchids' infestation. The adult insects were introduced into plastic containers containing cowpea seeds treated at four different concentrations of sesame leaf powder (2.0, 4.0, 6.0, 8.0g each per 20g of cowpea, respectively) while Ampligo insecticide treated seeds and untreated seeds were used as positive and negative controls, respectively. The experiment was laid out in a Completely Randomized Design with three replications and left for a period of three months. Data obtained were analysed using Analysis of Variance, with Duncan's New Multiple Range Test to separate significant means. The result obtained revealed significant difference ($P \leq 0.05$) in the efficacy of the powders in protecting cowpea seeds against the pest. The result showed that 8.0 g of the powders exerts more than 50% mortality to the test insects and inhibit oviposition to as high as 91.18%. The powders offered comparable protection of treated cowpea seeds for one to three months with respect to the negative control against C. maculatus. The effect is dose dependent, increases with increase in dose. The insecticidal potency of the powders could be attributed to the active phytochemicals present therein. Therefore, 8.0 g of sesame powder per 20 g of cowpea is recommended as effective biopesticide against stored cowpea bruchids' infestation.

Keywords: Callosobruchus maculatus, Cowpea, Insecticides, Sesame Leaf Powder

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp) is an important pulse crop produced and consumed largely by subsistence farmers in the semi-arid and sub-humid regions of Africa (DeBoer, 2003). It is an important crop grown for its high nutritious seeds and foliage. The seeds and foliage are good sources of protein (23-35%), carbohydrate (60-68%), minerals (iron and calcium), vitamins and carotene (Adedire *et al.*, 2011) and are used in preparing several dishes for man and livestock (Bressani, 1985). It is relatively cheap and supplements the protein requirements of many families in Africa (Nta *et al.*, 2013) where meat and other sources of animal protein are very expensive. Nigeria is the largest producer and consumer of cowpea in the world (Lowenberg-Deboer and Ibro, 2008; Akah *et al.*, 2021) producing 3.6 million tons in 2019 (Osipitan *et al.*, 2021). However, despite the significance of cowpea to Nigeria's economy it is one of the grains that suffer postharvest losses the most, especially from insect pests attack, both in the field and during storage (Ilesanmi and Gungula, 2010). One of such insect pests is the cowpea weevil, *Callosobruchus maculatus*.

Callosobruchus maculatus F., (Coleoptera: Bruchidae) is a serious pest of stored cowpea seeds in sub-Saharan Africa (AlMoajel and AlFuhaid, 2003). Postharvest losses of cowpea 3-4 months in storage caused by *C. maculatus* infestation have been reported as high as 50% in Africa (FAO, 2011). The damage of this magnitude is incredibly high and demonstrates the destructive nature of the pest which can threaten food security at both household and national levels. Weevil infestation cause weight loss, quality deterioration, resulting in overall unacceptability in markets and impaired germinability of grains (Keita *et al.*, 2001). Infested grains are rendered unfit for consumption and sale. Consequently, farmers are compelled to sell their products early after harvest when prices are still low, partly because of anticipated losses of the grain in storage. Postharvest losses due to *C. maculatus* are well documented (Emeasor, 2005; Udo and Epidi, 2009; Gusmão *et al.*, 2013). The insect pest population increases rapidly during period of seeds storage and results in total destruction within a short period of 3-4 months (Rahman and Talukder, 2006).

Synthetic insecticides employed in the control of cowpea beetle are proven to be effective, very expensive and unavailable at critical periods and they sometimes constitute health hazards to consumers (Lale, 2002). Due to the accumulation of residues of chemicals in grains, the selection of resistant insect population and other side effects, alternative approaches in Integrated Pest Management (IPM) have been considered. The need to control cowpea weevil using synthetic chemicals that impacts negative consequences onto the environment and other non-target population including man, besides conferring resistance and other clinical symptoms in man and other animals should be adopted. These problems led to a search for more effective method of controlling cowpea weevils that is safer and eco-friendly. One alternative control method is the use of plant extracts (Trevisan *et al.*, 2006), which favours natural enemies, necessary for the biological balance (Gallo *et al.*, 2002). This study therefore aimed at assessing the efficacy of sesame leaf powders as protectants against cowpea weevil's infestation on stored cowpea grains.

Materials and Methods

Plant samples collection and plant materials preparation

Untreated cowpea seeds (1000g) were obtained from Institute for Agricultural Research (IAR), Samaru, Zaria. The sample collected was disinfected at -4^{o}C for two weeks (Kossou *et al.*, 1992). The seeds were then air dried in a screenhouse to prevent possible re-infestation by insects. Fresh sesame leaves were collected from the Biological Garden, Ahmadu Bello University (ABU), Zaria. The leaves were transported to the Herbarium section of the Department of Botany, ABU, Zaria for authentication (ABU01583). The collected leaves were air dried at room temperature and ground with pestle and mortar to fine powder.

Phytochemical screening
Standard phytochemical tests were carried out on the plant samples to determine the presence of anthraquinones, tannins, saponins, flavonoids, alkaloids, cardiac glycosides, terpenoids, glucosides, and steroids (Sofowora, 1993). Fifty grams of the powdered sample was used for phytochemical screening.

Culture of test insects
Callosobruchus maculatus adults were obtained from Institute for Agricultural Research (IAR), Samaru Zaria and maintained on disinfested cowpea seeds at the laboratory of the Department of Biology, ABU, Zaria. Fifty unsexed 7–14-day old adults of the test insects (*C. maculatus*) were introduced into 500 g of disinfested cowpea seeds in 1L kilner jars. All adult insects were left for seven days to allow for oviposition, after which they were removed. The seeds were then left undisturbed until adults were observed to emerge. At each peak of emergence, the adults were removed and used to set up new cultures. Series of fresh cultures were made from these to ensure regular supply of adult insects of known ages for use in subsequent experiments

Mortality, oviposition and progeny development deterrent tests
Twenty grams of cowpea seeds was introduced into 750ml plastic containers. Varying volumes of sesame leaves powder (2.0, 4.0, 6.0 and 8.0g as well as the controls) were separately introduced into plastic containers containing 20g of disinfested seeds and shaken vigorously after every 30 minutes for 2 h to ensure uniform distribution of each powder over the grain surface. The treated seeds were allowed to stand for 2hrs before the introduction of the weevils. Thirty pairs of unsexed adults starved for 24hrs were introduced into each plastic container. The plastic containers were covered with muslin cloth sandwiched between two wire mesh. The experiment was arranged in a Completely Randomized Design with three replications including the untreated seeds (0.0 g/20g) as negative control and Ampligo (a synthetic insecticide) treated seeds as positive control. The number of dead insects was counted after 2, 4, 6 and 7 days to estimate weevil mortality. Insects were recorded dead when there is no response to prodding of the abdomen with a sharp pin.

Weevil mortality was assessed as: $\frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$

After 10 days, number of eggs laid on treated seeds and control seeds were recorded and the percentage of oviposition deterrence was calculated using Singh and Jakhmola (2011) formula:

$$\% \text{ Oviposition deterrence} = \frac{Cs - Ts}{Cs} \times 100$$

Where: Cs = Number of eggs laid on control seeds.
Ts = Number of eggs laid on treated seeds.

The experimental set up was let inside the containers for another 30 days. The number of adults that emerged from each container per replicate were removed, counted and recorded each day for a period of 60 days.

Insect damage to seeds
Monthly insect damage in each treatment and control was determined as described by Odeyemi and Daramola (2000)

$$\text{Percentage weight loss} = \frac{(Wu \times Nd) - (Wd \times Nu)}{Wu\,(Nd + Nu)} \times 100$$

Where: Wu =weight of undamaged grains
Nu = Number of undamaged grains
Wd = weight of damaged grains
Nd =Number of damaged grains

Statistical analyses
Data obtained from the study are expressed as means and percentages where applicable. Statistical differences between means of doses were analysed using Analysis of variance (ANOVA) with Duncan's New Multiple Range Test used to separate the means that were significant at $P \leq 0.05$ and not significant at $P > 0.05$. The ANOVA statistical analyses were conducted using SAS (2012) version 9.0.

Results
The result for phytochemical screening of the sesame aqueous leaf extracts is shown in Table 1. The result showed the presence of eight (8) active phytochemicals. The phytochemicals were: Carbohydrates, flavonoids, tannins, alkaloids, cardiac glycosides, phlobatannins and proteins.

Table 1: Phytochemical constituents of sesame leaf Powder

S/N	Phytochemical	Test used	
1	Carbohydrates	Molisch's Test	+
2	Flavonoids	Shinoda's test	+
3	Tannins	Ferric Chloride test	+
4	Alkaloids	Dragendoff's Reagent	+
5	Saponins	Frothing test	+
6	Cardiac glycosides	Salkowski's test	+
7	Phlobatannins	Lead acetate	+
8	Protein	Biurettest	+
9	Anthraquinones	Bontragers test	-

KEY: += Present -= Absent

The result for the effect of various doses of sesame powder in inducing mortality to *C. maculatus* is presented in Table 2. The result revealed significant difference ($P \leq 0.05$) in the effect of the doses on the mortality of *C. maculatus*. The powder induced a total mean mortality of 0.95-11.53 mortality which represents 3.2-38.43%. The result showed high weevils' mortality of 64.0% on the 7th day of exposure to 8.0 g/20 g of the powder. This value is 10.7% lower than the effect of the positive control. The effect is dose dependent increases with increase in dose.
More so, the effect of sesame powders in suppressing oviposition and progeny development of *C. maculatus* is presented in Table 3. The result indicated significant difference ($P \leq 0.05$) in the effects of the powder in suppressing oviposition and progeny development. The highest percentage of progeny development deterrence was found under the effects of 8.0 g of sesame leaf powder (91.18%) which is slightly lower than the value obtained under the effect of the positive control. It also reduced the mean number of eggs laid by the pests to as low as 8 eggs while completely suppressing the larvae formation and adult emergence. The effect is dose dependent, increases with increase in dose.

Table 2: Mortality of *Callosobruchus maculatus* adults during exposure to treated seeds

Days	0.00 g/20g	2.0 g/20g	4.0 g/20g	6.0 g/20g	8.0 g/20g	Ampligo
2	0.0[b] (0.0%)	0.0[b](0.0%)	0.0[b] (0.0%)	0.0[b] (0.0%)	2.9[a] (9.7%)	3.1[a] (10.3%)
4	0.0[d] (0.0%)	0.0[d] (0.0%)	0.0 [d] (0.0%)	2.7[c] (9.0%)	8.6[b] (28.7)	9.3[a] (31.0%)
6	0.0[f] (0.0%)	1.2[e] (4.0%)	3.8[d] (12.7%)	6.6[c] (22.0%)	15.4[b] (51.3%)	18.7[a] (62.3%)
7	0.0[f] (0.0%)	2.6[e] (8.7%)	5.4[d] (18.0%)	8.7[c] (29.0%)	19.2[b] (64.0%)	22.4[a] (74.7%)
Mean	0.0[f] (0.0%)	0.9[e] (3.2%)	2.3[d] (7.7%)	4.5[c] (15.0%)	11.5[b] (38.4%)	13.4[a] (44.6%)

N.B: Value(s) with the same superscript(s) across a row are NOT significantly different (P=0.05)

Table 3: Effect of sesame leaf powder on Oviposition and progeny development of Cowpea weevil

Dose (g/20g)	Mean No. of eggs laid	Larvae Development	Percentage adult emergence (%)	Oviposition deterrence (%)
0.00	92.22[a]*	89.12[a]	96.64	0.00
2.0	63.35[b]	42.14[b]	66.52	31.31
4.0	27.38[c]	12.12[c]	44.27	70.31
6.0	20.16[d]	7.01[d]	34.77	78.14
8.0	8.13[e]	0.00[e]	0.00	91.18
Ampligo	3.02[e]	0.00[e]	0.00	96.73

N.B: *Values down a column with the same superscripts are not significantly different (P=0.05)

The effect of sesame powder in protecting stored cowpea seeds from *C. maculatus* for a period of three months is shown in Figure 1. The result showed no damage to the seeds in the 6.0 g/20 g and 8.0 g/20g treated seeds for the first month. No damage was recorded for 8.0 g/20 g treated seeds in the second month. The least value for the weevils' infestation among the treatments was recorded in 8.0 g/20 g treated seeds (0.26%) after the 3rd month of storage.

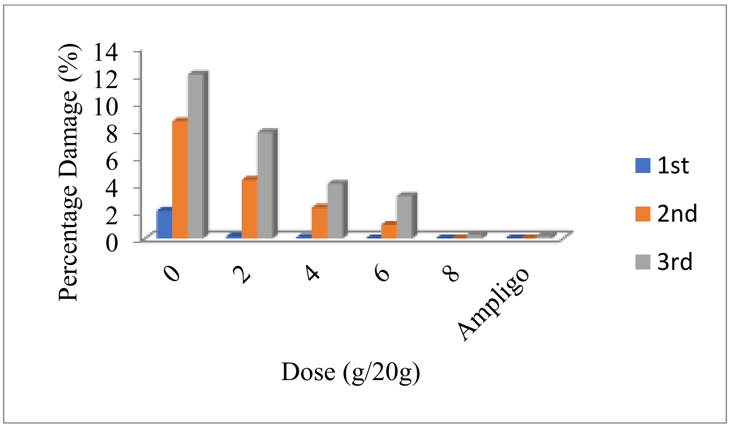

Figure 1: Percentage damage of cowpea seeds treated with Sesame powder by *C. maculatus*

Discussion

The application of botanical insecticides to protect stored cowpea seeds from infestation by *C. maculatus* has been proven to be vital element in insect pests' management strategies. Nathan *et al.* (2007) reported that, botanicals serve as alternatives to commercially used synthetic insecticides and many of them have often been used against a number of species of stored product insect pests. This finding agrees with that of Ilesanmi *et al.* (2020) who reported high efficacy of sesame leaf ethanolic extracts against *C. maculatus*. The present study reported highest percentage mortality induced by higher doses of the plants powders. This is in conformity with the findings of Alvi *et al.* (2018) who reported high mortality induced by the leaf and seed extracts of *Rhazya stricta* on *Rhyzopertha dominica* and *Trogoderma granarium* under laboratory conditions. The powders showed certain degrees of toxicity on the insect by inhibiting more than 50% of the eggs laid from emerging into larvae and suppressed progeny development to adults. This may probably be attributed to the toxicity and lethality exerted on the insects, thereby interfering with physiological processes of eggs development. This finding is in conformity with the report of Osawe *et al.* (2007) which opined that extracts of *Alstonia boonei* leaves adversely affected the survival and growth of *Sesamia calamistis*. Upadhyay and Jaiswal (2007) also reported that botanical insecticides significantly suppressed the progeny development of *Tribolium castaneum*. Similar finding was also reported by Ogendo *et al.* (2004) who reported more than 50% induced mortality by *Tephrosia vogelii* extracts on *Sitophilus zeamais*. Similarly, Ugwu *et al.* (2021) reported the insecticidal potency of *Piper guineense*, *Aframomum melegueta*, *Zingiber officinale* extracts against pupariating larvae of oriental fruit fly (*Bactrocera dorsalis*). Furthermore, botanical extracts have been reported by Amuji *et al.* (2012) to exhibit appreciable magnitude of toxicity to insects, inducing mortality. Ousman *et al.* (2007) reported that *Piper nigrum* leaf oil was toxic to stored insect pests and concluded that the extract be used as a substitute for synthetic insecticides by small scale farmers.

Cheruvan and Ragesh (2018) reported insecticidal effects of cassava leaf extracts against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* (L.) under laboratory conditions whilst Ileke and Emmanuel (2018) reported high bioefficacy of *Alstonia boonei* leaf extract against the cowpea beetle (*Callosobruchus maculatus*) infestation of stored cowpea seeds. The powders obtained from sesame leaf possess insecticidal potency for the protection of stored cowpea seeds probably due to the active phytochemicals constituents present in the powder. This conforms to the findings of Mbailao *et al.* (2006) who attributed toxicity of plant extracts on insect pests to the active constituents present in the extracts. The differences in protection capacity of the different doses of sesame leaf powder against *C. maculatus* can probably be related to the proportion of the active chemicals in each dose. This is in agreement with the work of Ito and Ighere (2017) who reported differences in the insecticidal activities of different doses of five plant extracts against *C. maculatus*. The suppression of oviposition and progeny development of *C. maculatus* on treated cowpea seeds can be attributed to the toxicity and lethality conferred on the insects thereby interfering with physiological processes of eggs development. Similarly, Negbenebor *et al.* (2020) reported the insecticidal potency of sesame leaf extracts against *Clavigralla tomentosicollis*. More so, dried leaves of *Tephrosia vogelii* were reported by Koona and Dorn (2005) to be effective in protecting stored legume seeds against bruchids infestation and reduced damage by *Callosobruchus maculatus*. *C. chinensis* and *Acanthosceli desobstectus* on the treated seeds by 7.10% compared with 99.80% recorded on grains in the control experiment.

Conclusion

The outcome of the study revealed that, sesame leaf powder applied at the rate of 8.0 g per 20 g of cowpea seeds protects the seeds against *C. maculatus* by inducing mortality (65%) to the insects, suppressing 91.18% oviposition and completely suppressed larvae and adult emergence. The effect of the powders on the mortality of *C. maculatus* was dose dependent, increasing with increase in dosage rates.

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