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# Oviposition Deterrent and Egg Hatchability Suppression of *Secamone afzelii* (Schult) K. Schum Leaf Extract on *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)

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**Abstract**

The efficacy of oil extracted from the leaves of *Secamone afzelii* was evaluated in the laboratory against *Callosobruchus maculatus* infesting stored cowpea. Leaf extracts from *S. afzelii* were obtained through the soxhlet extraction method using methanol and hexane as the solvent. Each of the extracts was tested by exposing five pairs of adult beetles to various levels of 0.5, 1.0, 1.5 and 2.0 ml corresponding to 2.5, 5.0, 7.5 and 10.0% v/w concentrations admixed with 20g cowpea in three replications respectively, in a Completely Randomized Design (CRD). Control treatment was set along. The results showed that oviposition and percentage egg hatched were significantly ($P$ <0.05) suppressed on seeds treated with higher treatment level of extracts. Leaf extract with hexane at 2 ml (10.0% v/w)/20g cowpea seeds was most effective in suppressing oviposition and egg hatched. Therefore, *S. afzelii* exhibit promising degree of oviposition deterrent and ovicidal properties and, thus, have a great potential for use as a plant-based biopesticide as an alternative to synthetic insecticides for controlling *C. maculatus* infestation on stored cowpea grains.

**Keywords**: Alternative, Egg hatched, Oviposition deterrent, Ovicidal properties, Biopesticide.

## 1. Introduction

Post-harvest losses of cowpea grains are serious problems in Africa, and as much as 20-50% of grains is lost because of *Callosobruchus maculatus* infestation resulting in weight loss and quality deterioration (Ofuya and Lale, 2001; Lale, 2010; Mailafiya *et al* 2014). Heat, moisture and waste products, produced by the beetles, result into further deterioration and the growth of mould, thus rendering grains unfit for consumption and marketing. Thus, farmers are forced to sell their produce early after the harvest when prices are still low partly because of the anticipated losses in storage (FAO, 1985). The huge post-harvest losses and quality deterioration caused by this insect pest contribute to the inability of achieving food security in developing countries (Rouanet, 1987).

Infestation control of stored grains insect pests has primarily relied upon the use of synthetic chemical insecticides, such as methyl bromide and phosphine. The shortcomings associated with the continuous use of these chemical insecticides, such as high cost of procurement, pest resurgence and resistance, poisonous residue accumulation in foods, risks of user’s contamination, effect on both human and environmental health, have necessitated the need for seeking alternative means of insect infestation control that are non-toxic and eco-friendly (Ileke *et al*., 2014).

*Secamone afzelii* Schult) K. Schum (Family: Apocynaceae) known in major Nigerian languages as arilu, ailu or alu in Yoruba, utunta (Ibo) and Ewuonkwonegie (Bini) (Gill, 1992), is a scandent or creeping woody climber found on fences and trees. It grows to a very long length of about 12m with pinnately compound leaves (Abere and Onwukaeme, 2012; Prota, 2014). *S. afzelii* is used in traditional medicine for stomach problems, diabetes, colic, dysentery, treatment of sexually transmitted infections, purgative for children, sore throat, backache, cough, catarrhal conditions and as galactogogue, purge, also for kidney problems and as a remedy for spinal disease (Abo *et al.,* 2008; Gill, 1992; Watt and Breyer-Brandwijk, 1962; Oliver, 1960).

In literature, there appears to be a dearth of empirical information on the utilization of *S. afzelii* extracts for their insecticidal potential. However, Adesina and Ofuya (2011) and Adesina *et al.* (2012) reported the efficacy of *S. afzelii* leaves and vine powder for the control of *C. maculatus* and *Sitophilus zeamais*. Therefore, the objective of the present study is to evaluate the efficacy of organic solvent extract of *S. afzelii* as protectants for stored cowpea seeds against *C. maculatus* infestation.

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## 2. Materials and Methods

### *2.1. Insect Culture and Experimental Conditions*

*Callosobruchus maculatus* stock, used for the present study, was obtained from an established culture from Entomology Laboratory of Crop, Soil and Pest Management of Federal University of Technology, Akure, Ondo state, Nigeria. The insects were sub-cultured on 200g Sokoto white local cowpea cultivar (a well known susceptible cultivar) in Kilner jar under laboratory conditions (32±0.6°C, 68±3% relative humidity and 12L: 12D photo regime) (Idoko and Adesina, 2013; Idoko and Adesina, 2012; Udo, 2005) for oviposition to produce a steady and sufficient supply of beetles of known age for experimental purpose (Adesina, 2012) in the Entomology Laboratory of Crop, Soil and Pest Management Technology Department, Rufus Giwa Polytechnic, Ondo State, Nigeria (Latitude 5° 12' N and Longitude 5° 36' E).

### *2.2. Collection and Preparation of Plant Materials*

Leaves of *S. afzelii* were obtained from Ipesi Akoko, Ondo State, Nigeria and air-dried under a room temperature for about 2 weeks to avoid possible volatilization of the active ingredients (Adesina, 2012). The dried leaves were milled into powder using a hammer mill (Epidi *et al.*, 2009). Thereafter, the milled leaf powder of *S. afzelii* was taken to the laboratory for extraction using ethanol and n-hexane as organic solvent using soxhlet apparatus.

### *2.3. Soxhlet Extraction*

The ordinary method of extraction was not efficient to yield a good amount of active ingredients of the plant material. The plant material was extracted using the Soxhlet extraction method (Anurag Sharma and Raskesh Gupta, 2009). A known amount (500g) of *S. afzelii* was filled into the Soxhlet apparatus. A cotton plug was used at the place of thimble to stop the entry of the crude material into the siphoning tube. The required solvent (ethanol and n-hexane) was filled up five times more than the total amount of the sample material into the flask of the apparatus. The apparatus was then connected with the water supply to the condenser. The temperature of the heating mantle was maintained at 60-65°C (boiling point of ethanol). The process was carried out for 5 to 6 hours for each sample. The extracts were later concentrated using a rotary evaporator to obtain a concentrated extract, which was stored in specimen bottle for future use.

### *2.4. Sources of Cowpea Seeds*

The freshly harvested seeds of Ife brown cowpea variety, which were used in the bioassay experiments, were obtained from the Teaching, Commercial and Research Farms of Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria, were adequately air-dried to 12% moisture content (Adesina, 2013). The seeds were properly sieved and handpicked, thus ensuring that the only whole and uninfested seeds were used (Olotuah *et al.*, 2007; Adesina *et al.*, 2012). The clean seeds showed no visible signs of beetle eggs, presence of adults or exit holes. These were nevertheless sterilized in an oven at 50°C for 4 hours to kill any immature stage of insect (if any) and were allowed to cool for 1 hour before use (Idoko and Adesina, 2013). This harvested cowpea had no history of postharvest insecticide treatment.

### *2.5. Effect of Extracts on Oviposition and Fecundity*

*S. afzelii*, extracted at four dosages, namely 0.5, 1.0, 1.5 and 2.0 ml (corresponding to 2.5, 5.0 7.0 and 10.0% w/v concentration), was measured into 9.0 cm diameter disposable Petri-dishes containing 20g of disinfected cowpea seeds weighed using a digital weigh balance (model TS 400D) in triplicates using a syringe and each is thoroughly mixed using a glass rod to ensure the uniform mixing of the extract with the grains and is left open to dry. Thereafter, five (5) paired sexed adult insects of 1-2 days old *C. maculatus* were introduced into each Petri-dish containing different dosages of plant extract/ food complex of treated and untreated grains (Udo, 2000). The sex of *C. maculatus* was determined by the pattern of Iloba and Ekrakene (2006). There was also a control treatment that does not involve any addition of extract on the seeds. The Petri dishes were then covered to prevent insects from escaping. The number of eggs laid by the female beetles on the seeds was recorded on the 14th day after the introduction of beetles to seeds; this was used to calculate the percentage of egg hatching according to Abdullahi *et al.* (2011) and the percentage reduction of egg laying (Emman and Abass, 2010), respectively, as follows:

Percentage reduction of eggs laid =

$$\frac{\text{no of eggs laid in control} - \text{no of eggs laid on treated grains}}{\text{no of eggs laid in control}} \times \frac{100}{1}$$

egg hatching (%) =

$$\frac{\text{no of eggs hatched}}{\text{no of eggs in each Petri} - \text{dish}} \times \frac{100}{1}$$

### *2.6. Statistical Analysis*

The experiment was laid out in Completely Randomized Design (CRD) and each treatment was replicated there (3) times. Percentage data were transformed to square root of arcsine to normalize the data before analysis. Data from the 3 replicates of the experiment were pooled together and subjected to one-way Analysis of Variances (ANOVA). Treatment means were separated using Least Significant Differences (LSD) at 5% probability level (Gomez and Gomez, 1994).

## 3. Results

### *3.1. Effect of S. afzelii extracts on oviposition and fecundity by C. maculatus*

The effect of the extracts on oviposition is summarized in Table 1. The results showed that the extracts at all application levels significantly inhibited the female *C. maculatus* from laying eggs on treated cowpea seeds. In spite of the the early death of *C. maculatus* adults, no concentration of the extracts could completely prevent the females from oviposition. The percentage reduction in the number of the laid eggs was inversely proportional to the extracts concentration tested. The laying capacity gradually decreased with the increase in the treatment dose of each extract. The maximum reduction in egg laying was noticed with hexane extract 41.0% reduction on grains treated with 2.0ml dosage rate as against 24.21% recorded in control. The same trend was recorded in ethanol extract when at 2ml, 51.6% eggs reduction was observed on treated cowpea against 30.6% in control treatment. Statistically, there was a significant difference between the tested concentrations compared to control.

**Table 1.** Mean percentage reduction in number of eggs laid by female *C. maculatus*

| Treatments/20g cowpea | percentage reduction in eggs laid | |
|---|---|---|
| (Conc in %) | Methanol | Hexane |
| 0.0ml (0) | 30.62±3.7 | 24.21±1.8 |
| 0.5ml (2.5) | 40.84±2.3 | 28.23±2.2 |
| 1.0ml (5.0) | 47.55±4.4 | 20.77±3.4 |
| 1.5ml (7.5) | 47.89±2.1 | 41.02±4.2 |
| 2.0ml (10.0) | 51.61±3.3 | 52.85±2.3 |
| LSD (5%) | 11.01 | 23.76 |

### 3.2. Effect of S. afzelii Extracts on Egg Hatching

In the present study, the effect of *S. afzelii* extracts on the egg hatching capability of *C. maculatus* revealed that there was a significant reduction. The egg hatching capacity gradually decreased with the increase in the treatment dose level of each leaf extract. The maximum reduction in the hatched eggs was noticed at 2ml (10%) level when only 7.94% and 8.79% eggs found to be laid, hatched on the seeds treated with hexane and methanol extracts, respectively, as against 31.93% and 31.37% hatched eggs in control (Table 2). The results revealed that *S. afzelii* extracts, at different dose level, were very effective against *C. maculatus* egg viability on stored cowpea.

**Table 2.** Mean percentage of egg hatching of *C. maculatus* from treated cowpea seeds

| Treatments/20g cowpea | percentage egg hatching | |
|---|---|---|
| (Conc in %) | Methanol | Hexane |
| 0.0ml (0) | 31.37±4.28 | 31.93±3.3 |
| 0.5ml (2.5) | 19.58±2.1 | 15.78±2.3 |
| 1.0ml (5.0) | 12.86±1.6 | 12.76±1.8 |
| 1.5ml (7.5) | 9.24±0.4 | 10.87±1.2 |
| 2.0ml (10.0) | 8.05±1.2 | 7.94±0.5 |
| LSD (5%) | 8.79 | 4.31 |

## 4. Discussion

The results of the study provide empirical evidence that the insecticidal activity of *S. afzelii* extract with ethanol and hexane can be effective to varying degrees in deterring oviposition and egg viability by *C. maculatus*, and ultimately reduced the percentage seed damage and weight loss due to infestation by the foregoing insect pest. However, no concentration of the plant extracts completely prevented the females from laying eggs on the seeds. This study also showed that the plant extracts significantly reduce the number of eggs laid per female compared to that obtained in the control.

The marked decline in egg laying was perhaps a consequence of the mild suppressing effect exerted by these volatiles on the pulse beetles' mating, which is a decisive factor influencing the subsequent number of eggs laid by the beetles (Engelmann, 1970). The present findings corroborate the observation recorded for oil vapors on *C. maculatus* (Paranagama *et al.*, 2003).

These findings are in accord with Elhang (2000), Kim *et al.* (2003), Ghoswal *et al*. (2004) and Abdullahi (2011) who found that the reduction in egg laying of pulse beetle was significantly high when the seeds were treated with various pesticidal plant extracts, and a similar trend was noticed in the case of some vegetable oils too. The reduction in oviposition in the extract treated seeds may also be caused by an extract film on the seeds which becomes unsuitable for oviposition. The results revealed that various pesticidal plants at a different dose level acted as highest ovipositional deterrents. The ability of the extract to reduce the egg laying capability by the female beetles may be attributed to the presence of flavonoids in the plant (Zabri *et al.*, 2008; Zabri *et al.*, 2009). This confirmed the findings of Righi-Assia *et al.* (2010) that flavonoids significantly reduce the egg laying and fertility in *C. Chinensis.* Results from this study equally suggest that the plant extract might interfere with the normal embryonic development by suppressing hormonal and biochemical processes. A similar physiological inference was observed by Ofuya *et al.* (1992), Jayakumar *et al.* (2003) and Raja and William, (2008). The extract could also involve an ovarian change similar to those that caused chemosterilisant by blocking female egg laying. This supports the findings of Saxena and Rohdendorf (1974) and Schmidt *et al.* (1991a and b). Jadhav and Jadhav (1984) reported that *Jatropha curcas* seed oil applied at 0.2% (v/w) concentration significantly reduced the number of eggs laid by adult *C. maculatus* and prevented eggs hatched 33 days after the treatment. Also, Adebowale and Adedire (2006) observed that the cowpea seeds treated with *Jatropha curcas* seed oil reduced the number of eggs laid by *C. maculatus* and prevented the adult emergence at a concentration between 0.5 and 2% (v/w). While, Ofuya (1990) and Tapondjou *et al.* (2002) suggested that the oviposition inhibition property of botanical powders on adult bruchids (in terms of weakening of adults by powder treatments) made them lay fewer eggs and killed the larvae hatching from eggs laid on grains. Oviposition inhibitors have the advantage of attacking a pest at the start of its life cycle. The insect is deterred from laying its eggs on the cereals/grains, thus preventing the pest population from increasing (Pandey *et al.*, 2011).

The leaf extract used in this study was found to be significantly superior in reducing egg hatching; as the concentrations of the extracts increased, their inhibitory effect on egg hatching also increased. The egg mortality and the failure to hatch on seeds, treated with the extract, were probably attributed to the toxic component of the extracts and also to the physical properties, which caused changes in the surface tension and the oxygen tension within the eggs (Singh *et al.*, 1978). The ovicidal effect of the extracts on the bruchid may also be explained in terms of asphyxiation by blocking the major route of gas exchange between a thin area of the chorion and outside (Credland, 1992), which ultimately reduced the emergence of the insects from the treated seed (Copping and Menn, 2000).

Very few studies on the effect of pesticidal plant on the egg hatching of pulse beetle are available. However, Abdulahi (2011), Abdulahi *et al*. (2011) and Adesina *et al*. (2011) found a significant effect on reduced egg hatchability of *C. maculatus* when laid on seeds treated with plant extracts and powder, respectively, at different doses, which is in agreement with the present study. The current results are in agreement with Ketoh *et al.* (2006) who reported that *Cymbopogon* oil vapor treatment for 24h could be satisfactory for controlling the eggs hatchability of *C. maculatus*. In a related development, Gajmer *et al*. (2002) reported that eggs laid on extract-treated oviposition substrate, exhibited reduced hatching and that marked adverse effects on hatching were noticed when the eggs were dipped in different concentrations of extracts, they also reported that adults, fed on an extract containing sucrose diet, laid significantly fewer eggs with poor hatching.

The extracts coating the seeds may have possible contact effects on the insect during oviposition since eggs are laid on the seed, thus preventing *C. maculatus* eggs to firmly attach to the seed coat (Adebowale and Adedire, 2006) or blocking respiration (Bamayi *et al*., 2007) thus inhibiting or interfering with the normal embryonic development by suppressing hormonal and biochemical processes, which prevent egg hatching. This finding suggests that the *S. afzelii* extracts successfully inhibit egg hatching into the seed and ultimately suppress the F1 progeny emergence.

The present study clearly demonstrates that *S. afzelii* possess anti-oviposition and ovicidal activities that can be employed in the management of *C. maculatus* infestation on stored cowpea. Further studies are needed to explore isolating the bio-active molecule responsible for the insecticidal properties exhibited by the plant.

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