Tolerance Activities of *Callasobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) Against *Secamone afzelii* (schult) K. Schum Leaf extracts

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ABSTRACT

A laboratory experiment was conducted to investigate the efficacy of methanol and n-hexane leaf extracts of *Secamone afzelii* against the development and tolerance of the cowpea weevil, *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae) on stored cowpea, *Vigna unguiculata* (W) seeds. The study was laid out in Completely Randomized Design (CRD) with five pairs of adult beetles exposed to 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 under ambient laboratory conditions. Control was also set up with no admixture of extracts. The results revealed that the extracts of the plant exerted significant adult mortality; caused considerable reduction in the number of adult weevil emergence, which ultimately significantly suppressed percentage seed punctured and weight loss. Percentage treatment efficacy showed that the effectiveness of the plant extracts was concentration dependent and seed treated with n-hexane extract recorded the highest bioactivity against *C. maculatus* compared to ethanol extracts treated seeds. *S. afzelii* extracts exhibited commensurable pest tolerance activities compared to untreated seeds. It can be used to prevent attack of cowpea by *C. maculatus*. It might be a good alternative to synthetic insecticides for the protection of cowpea seeds .However, further study is needed to isolate and characterized the bioactive compounds responsible for the plant insecticidal activities.

Keywords: Efficacy, Pest tolerance, Plant extracts, Punctured seed, Insecticides.

INTRODUCTION

Cowpea (*Vigna unguiculata*) remains a major component part of Nigeria dietary. It is an important protein source and essential adjuncts to a predominantly cereal-based diet. They enhance the biological value of the protein consumed by the teeming population of Nigerians. Post-harvest losses of cowpea grains are serious problems in Africa., As much as 20 – 50% of grains is lost because of *Callosobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae), infestation resulting in weight loss and nutritional quality deterioration (Adesina and Ofuya, 2015; Upadhyay and Ahmad, 2011).

The protection of agricultural products in storage against insect attack is essential for the safe and steady supply of food. Currently control of *C. maculatus* infestation relies heavily upon the use of gaseous fumigants and residual chemical insecticides. When properly used, synthetic insecticides might continue to play an important role in reducing storage losses due to insect pest activities

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(Mean, 1983; Redlinger *et al*., 1988).

However, these insecticides have serious drawbacks such as development of genetic resistance in the treated pests, toxic residue problem, health hazards to warm-blooded animals, risk of environmental contamination and increasing costs of application (Butt and Goettel, 2000; Baskaran *et al* 2010; Hassan *et al* 2013). Moreover, the poor storage facilities of traditional farmers in the developing countries are unsuitable for effective conventional chemical control, as most storage types are open to re-infestation by insect pests.

Owing to growing awareness of the hazards associated with the use of synthetic insecticides, search for lower risk eco-friendly alternative has resulted in renewed interest the use of plant extracts for reducing insect infestation (Rahman and Talukder, 2006; Keita *et al* 2001; Keita *et al* 2000). The use of plant products has assumed significance as an important component of insect pest management because of their economic viability and eco-friendly nature. They hold promise as alternatives to chemical insecticides to reduce pesticide load in the environment. The botanical insecticides are generally regarded more pest-specific, relatively harmless to non-target organisms including humans, biodegradable and harmless to the environment than chemical pesticides. Furthermore, unlike conventional insecticides that are based on a single active ingredient, many plant-derived insecticides comprise an array of chemical compounds, which act concertedly on both behavioural and physiological processes. Thus the chances of pests developing resistance to such substances are less likely.

Secamone afzelii (Schult) K. Schum (Family: Apocynaceae) is a climbing or scrambling shrub with pinnately compound leaves (Abere and Onwukaeme, 2012) commonly found in the secondary forest of West Africa (Mensah *et al*, 2006). *S. afzelii* is used in traditional folk medicine for the treatment of various aliments (Gill, 1992; Watt and Breyer–Brandwijk, 1962; Oliver, 1960; Odugbemi, 2008). There is paucity of information on the utilization of *S. afzelii* in the management of storage insect pest, although several workers (Adesina and Ofuya 2011, 2015; Adesina *et al* 2012) reported insecticidal activities of the plant. Therefore, the present study investigated the tolerance activity of *S. afzelii* as a botanical insecticide for a sustainable pest control strategy of *C. maculatus* infesting stored cowpea grains.

MATERIALS AND METHODS

Insect culture and Experimental condition

Initial insects (*C. maculatus*) stock used for the study was obtained from established culture obtained from Entomology Laboratory, Department of Crop, Soil and Pest Management Technology, Rufus Giwa Polytechnic, Ondo State, Nigeria (Latitude 5° 12' N and Longitude 5° 36' E). The insects were sub-cultured on 200g Sokoto white local cowpea cultivar (a well known susceptible cultivar) (Adesina and Ofuya, 2015) in Kilner jar under laboratory conditions of 32±0.64°C, 68±3% relative humidity and 12 hrs photoperiod 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 purposes (Adesina, 2012).

Collection and Preparation of Plant Materials

S. afzelii leaves from the whole plant, plucked at flowering stage were obtained from Ipesi Akoko, Akoko South East Local Government Area of Ondo State, Nigeria. Samples were taxonomically identified and voucher specimens deposited in Department of Forestry and Wood Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. The leaves were thoroughly washed and air-dried under a room temperature for a period of 2 weeks to avoid possible volatilization of the active ingredients (Adesina, 2012). The dried leaves were milled into powder using hammer mill (Epidi *et al*, 2009) and was kept in polythene bags at room temperature and properly sealed to prevent quality loss (Chayengia *et al*. 2010) till subjected to extraction. Hundred grams of milled leaf powder was mixed with 350ml of methanol and n-hexane solvent and kept for 48 hours while stirring from time to time. After 48 hours each of the resulting crude extract was filtered using Filterman® (125mm) filter papers. The filtered extract was then concentrated using a vacuum rotary evaporator at 65^{0}C until the extract was reduced approximately to 60ml and was considered as the stock solution. A series (0.5ml, 1ml, 1.5ml, and 2ml) of this stock solution was dissolved in 10ml of the appropriate solvent and four dilutions (2.5, 5, 7.5 and 10%) each were made. The dilution was stored in specimen bottle for future use.

Cowpea source

Freshly harvested seeds of Ife brown cowpea variety used as bioassay 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 only whole and healthy 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 sterilized in an oven at 60°C for 4 hours to kill any immature stage of an insect and allowed to cool for 1 hour before use (Idoko and Adesina, 2013). Only visually healthy cowpeas were uses for the experiments. The harvested cowpea had no history of postharvest insecticide treatment.

Insecticidal and Pest Tolerance Activity

S. afzelii extracts at four concentrations namely: 0.5ml, 1.0ml, 1.5ml, 2.0ml (corresponding to 2.5, 5.0 7.0 and 10.0% w/v concentration) were measured into 9.0 cm diameter disposable Petri-dishes containing 20g of disinfected cowpea seeds weighed using digital weigh balance (model TS 400D) in triplicates using a syringe. The seeds were thoroughly agitated using glass rod to ensure uniform mixing of extracts with the grains and left open for some minutes to allow for dryness. Thereafter, five paired sexed adult insects of 2 - 3 days old *C. maculatus* were introduced into each Petri-dish containing different dosages of plant extracts/food complex of treated and untreated grains (Udo, 2005). The sex of *C. maculatus* was determined by the pattern of Iloba *et al* (2007). There was also a control treatment involving no addition of extracts on the seeds. The Petri dishes were then covered to prevent insects from escaping. Each treatment was replicated three times and left on the laboratory benches for daily observation. Mortality was monitored and recorded after 24 and 48 h from the exposure. Knocked-down adults were regarded as alive if they showed continued movement of their appendages by the touch of "safety pin". Percentage insect mortality was corrected using the Abbott's formula (Abbott, 1925).

The number of adult insect emerging was counted as from 30 to 35 days after infestation (DAI) to avoid overlapping of generations. This was used to determine the percentage adult emergence and percentage reduction (El-Lakwah *et al*., 1992).

Percentage adult emergence =

$$\frac{\text{mean no of emerged adult}}{\text{mean no of egg laid}} \times \frac{100}{1}$$

Percentage Reduction=

$$\frac{\text{mean no of emerged in control} - \text{mean no of emerged in treatment}}{\text{mean no of emerged adult in control}} \times \frac{100}{1}$$

At 30 days after infestation observation period, the extent of weevil damage was assessed by counting exit-holes as a measure of damage to the grains, also undamaged seeds were counted and recorded. This was used to determine percentage pest tolerance **(**Olakojo *et al*, 2007) and percentage grain puncture, respectively.

Percentage pest tolerance=

$$\frac{\text{No of undamaged seed} - \text{No of damage seed}}{\text{No of undamaged seed}} \times \frac{100}{1}$$

Percentage grain puncture =

$$\frac{\text{Number of seeds with exit hole per treatment}}{\text{Total number of seeds per treatment}} \times \frac{100}{1}$$

At the end of the experiment, the grains were sieved and the final weight of each of the treatment was taken and used to calculate the percentage weight loss.

Percentage weight loss =

$$\frac{\text{initial weight of grain} - \text{final weight of grain}}{\text{initial weight of grain}} \times \frac{100}{1}$$

Percentage efficacy of the plant extracts was computed through Abbot's formula (Abbot, 1925)

Statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) and each treatment was replicated there times. Data from the 3 replicates of the experiment were pooled together and was subjected to Analysis of Variances (ANOVA). There were no mortality in control treatments; so, there was no need to correct the mortality data (Ziaee, 2014). Data observed in counts, were subjected to square root transformation and percentages were arc sine transformed before analysis to normalize the data. Treatment means were separated using Least Significant Differences (LSD) at 5% probability level (Gomez and Gomez, 1994).

RESULTS

The results of different concentration of *S. afzelii* with methanol and n-hexane extracts are presented in Table (1). Many of the extracts concentrations resulted in adult beetle mortality. Maximum mortality was observed in sets treated with 2ml/20g of cowpea, while minimum mortality was found to occur in sets treated with 0.5ml/20g cowpea. A significantly high mortality was noted in the sets treated with 2ml compared to control, 0.5ml and 1.0ml concentrations. All test insects were found alive showing zero percent mortality in the control sample throughout the period of the experiment. Perusal of present data also revealed that n-hexane extract of *S. afzelii* was significantly more toxic to test insects than methanol extract of the same plant 24 h after infestation, whereas at 48h after infestation there was non-significant difference among n-hexane concentrations. In general, there was a significant increase in cumulative insect mortality with an increase in the concentration as well as exposure time of the treatments.

Table (1). Mean mortality of adult *Callosobruchus maculatus* after treatment and exposure to extracts of *S. afzelii* under laboratory conditions.

Treatments/20g cowpea (Conc in %)	Hours after infestation 24 h		48 h	
	Methanol	n-Hexane	Methanol	n-Hexane
0.0 (0%)	0	0	0	0
0.5 (2.5%)	23.71±2.34[a]	29.08±2.94[a]	40.05±2.53[a]	55.26±2.61[a]
1.0 (5.0%)	26.08±1.05[a]	24.74±2.70$_a$	48.12±1.46[a]	58.71±2.08[a]
1.5 (7.5%)	27.49±2.24[a]	34.16±2.46[ab]	52.41±2.41[b]	59.59±2.76[a]
2.0 (10.0%)	30.60±2.10[ab]	42.91±1.68[b]	55.25±2.75[b]	65.19±2.48[a]
LSD (5%)	4.58	7.33	9.65	11.26

Each value is a mean ± standard error of three replicates and means within column followed by the same letters (s) are not significantly different at (P> 0.05) using Least Significant Difference.

The data shown in Table (2) revealed that the effect of extracts on percentage adult emergence reduction of pulse beetle shows a significant reduction among the treatments and their respective concentrations compared to control treatment. The maximum reduction in the adult emergence was observed in n-hexane extract (74.75%) compared to untreated seed (31.93%) and; ethanol extract maximum reduction (73.57%) at 2ml/20g of cowpea and minimum percentage adult emergence reduction in control dish (31.37%). Statistically, there was a significant difference between 2ml treated seeds compared to untreated seed, and non-significant difference between 2ml and seed treated with 1ml and 1.5ml ethanol n-hexane extract, respectively. However, the reduction in adult emergence increased with the increase in concentration rates of each extracts.

Table (2). Mean percentages adult emergence reduction of *Callosobruchus maculatus* from treated cowpea seeds with *S. afzelii* extracts under laboratory conditions

Treatments/20g cowpea (Conc in %)	percentage of adult emergence reduction Methanol	n-Hexane
0.0ml (0%)	31.37±2.08[a]	31.93±1.48[a]
0.5ml (2.5%)	38.28±1.56[a]	45.49±2.19[b]
1.0ml (5.0%)	57.84±2.57[ab]	60.25±2.12[c]
1.5ml (7.5%)	71.18±2.93[b]	65.59±2,64[c]
2.0ml (10.0%)	73.57±2.33[b]	74.75±2.06[bc]
LSD (5%)	21.48	11.65

Each value is a mean ± standard error of three replicates and means within column followed by the same letters (s) are not significantly different at (P> 0.05) using Least Significant Difference.

Percentage weight loss and seed puncture data is presented in Table 3. The weight loss rate in cowpea seeds 6 weeks post treatment shows decreased rate with increasing extract concentration. Overall percentage weight loss was lower in value from seed treated with 2ml extracts than that of untreated control. Seeds treated with n-hexane extract gave appreciable reduction in weight loss compared with ethanol. All the plant extracts were more effective at higher application rates than the lower rates. All the plant extracts treatments were also significantly different (P<0.05) from control in terms of weight loss. With regards to percentage seed punctured, untreated seeds significantly suffered greater damage compared to treated seeds. Non-significant difference was observed in cowpea grains treated with 0.5ml – 2.0ml of plant extracts with close similar value.

Table (3). Mean percentage weight loss and seed punctured by *C. maculatus*

Treatments/20g cowpea (Conc in %)	% weight loss		% seed punctured	
	Methanol	n-Hexane	Methanol	n-Hexane
0.0 (0%)	28.26±1.54[a]	26.60±2.04[a]	8.08±1.64[a]	8.54±0.92[a]
0.5 (2.5%)	16.87±1.79[b]	13.77±1.49[b]	6.78±0.96[b]	5.88±1.54[b]
1.0 (5.0%)	9.17±1.46[c]	8.98±1.66[c]	5.56±0.82[bc]	5.03±0.44[b]
1.5 (7.5%)	6.57±1.04[cd]	5.17±0.32[d]	4.89±0.85[bc]	5.16±1.22[b]
2.0 (10.0%)	4.84±1.05[cd]	3.81±61.1[d]	4.39±0.33[bc]	5.06±0.77[b]
LSD (5%)	6.12	2.34	1.51	1.17

Each value is a mean ± standard error of three replicates and means within column followed by the same letters (s) are not significantly different at (P> 0.05) using Least Significant Difference.

Regardless of extracting solvents and the concentration levels, all plant extracts significantly (P <0.05) affected *C. maculatus* tolerance to the treated seeds due to infestation (Table 4). Results showed that percentage pest tolerance was concentration dependent. Cowpea grains treated with 2.0ml showed high tolerance level across extracts. Untreated seeds had the least tolerance level.

Table (4). Mean percentage pest tolerance by *C. maculatus* on cowpea grains treated with *S. afzelii* extracts

Treatments/20g cowpea (Conc in %)	Percentage pest tolerance Methanol	n-Hexane
0.0ml (0%)	30.62±2.62[a]	24.21±1.88[a]
0.5ml (2.5%)	40.84±2.46[b]	28.23±2.66[a]
1.0ml (5.0%)	47.55±2.12[c]	20.77±2.04[a]
1.5ml (7.5%)	47.89±1.92[c]	41.02±2.33[b]
2.0ml (10.0%)	51.61±2.88[d]	52.85±2.70[b]
LSD (5%)	5.67	19.39

Each value is a mean ± standard error of three replicates and means within column followed by the same letters (s) are not significantly different at (P> 0.05) using Least Significant Difference.

Effect *S. afzelii* efficacy on treated cowpea seeds

Toxicity of the extracts was found to be concentration dependent. The percentage efficacy of the extract was found to be low at the lowest concentration for the two extract evaluated and performed better at higher concentration level (Table 5). Cowpea seed exposed to 2.0ml concentration significantly suppressed *C. maculatus* infestation and seed damage compared to untreated cowpea seeds.

Table (5). Effect *S. afzelii* efficacy on treated cowpea seeds

Treatments/20g cowpea (Conc in %)	Percentage efficacy of extracts Methanol	n-Hexane
0.5ml (2.5)	10.79±1.64[a]	28.11±1.82a
1.0ml (5.0)	33.01±2.05[b]	46.99±2.46a
1.5ml (7.5)	43.22±2.41[bc]	50.06±2.32[ab]
2.0ml (10.0)	52.21±1.78[c]	56.27±2.68[ab]
LSD (5%)	17.86	19.86

Each value is a mean ± standard error of three replicates and means within column followed by the same letters (s) are not significantly different at (P> 0.05) using Least Significant Difference.

DISCUSSION

Prior to the development and commercial success of synthetic insecticides beginning in the 1940s, botanical insecticides were major weapons in the farmer's arsenal against crop pests (Isman, 2008). In many villages of Africa, farmers often mix plant materials with stored grains against pest infestation (Akinkurolere, 2007).

The mortality reported in this study was observed to have a direct relationship with the concentrations of the extracts and the exposure time of the insect to the treatment. It indicateed that higher concentration and longer time exposure periods were needed to achieve appreciable management of *C. maculatus*. Furthermore, Shaaya *et al* (1997) suggested that higher concentration and longer exposure were needed to achieve appreciable level of mortality, which concurred with the result obtained in this study. The overall effects of the plant extracts corroborated an earlier study conducted by Srivastava and Mann (2002) using plant *Peganum harmala* against *C. chinensis* which showed that ether extract at higher concentration of 10% was most effective in causing adult mortality. This result was in accordance with those of Ewete *et al.,* (1996) who reported that the effectiveness of a substance for insecticidal purpose was related to its capacity to cause a strong significant mortality rate in a population of pest. *S. afzelii* extract might have been very potent because of the odour it produced, which might have exerted a toxic effect by disrupting normal respiratory activities of the insects, thereby resulting to asphyxiation and subsequent death (Adedire and Ajayi, 1996). 2008). Generally, the adult mortality might be attributed to contact toxicity or to the induction of some unknown physiological changes as opined by Mathur *et al*. (1985).

The reduction in F_1 progeny could have resulted from adult mortality, ovicidal (egg mortality) and larvicidal (larval mortality) properties of plants' oil. This could have arisen from the plant extracts which form a film covering the surface of the pulse which caused a reduction in respiration and gas exchange between the seeds and their environment, thereby interfering with normal respiration. The toxicity of compounds penetrating the seed or the accumulation of the toxic metabolites also killed insects. This assertion confirmed the findings of several workers (Gupta and Srivastava, 2008; Araya and Emana, 2009; Adesina and Ofuya, 2011; Adesina *et al*. 2012).

The extracts coating the seeds might have possible contact effects on the insect during oviposition since eggs were 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 larval penetration into the seeds and possibly interfering with the normal development of the adults *C. maculatus* by suppressing hormonal and biochemical process, which prevented adult emergence and weight loss. Also oil coating on grains could prevent penetration of oxygen to the developing stages (Adebowale and Adedire, 2006) which consequently affected the survival of the immature stages (Larvae and pupae) of *C. maculatus*. The adult emergence that was recorded could also be due to low eggs hatchability. This finding suggests that the *S. afzelii* extracts successful inhibited larval penetration into the seed and ultimately suppressed F1 progeny emergence.

The reduction in seed damage by the beetles was concentration dependent and could be attributed to higher adult beetle mortality and low adult beetle emergence in treated grains. This confirmed the findings of Adesina and Ofuya, (2011). In addition to that, Adesina *et al* (2012) reported that the lower the percentage adult emergence from treated grains, the lower the percentage seed damage or adult exit hole and weight loss. Dick and Credland (1984, 1986) reported that the number of adults of *C. maculatus* that emerged from infested cowpea seeds depended amongst other factors, on the number of eggs initially present. Successful infestation was determined by number of eggs hatched, as well as the number of first instars larvae that were able to penetrate the cotyledons, and interfering with any of these processes, leading to a reduction in the population of the bruchid and the degree of seed damage (Lale and Mustapha, 2000a). It has been reported that the larvae which hatched from the eggs of *Callosobruchus* species must penetrate the seeds to survive (FAO, 1999).

The result obtained from this study suggested that reduction of infestation and seed damage in pulse seeds treated with *S. afzelii* extracts were achieved mainly through reduced egg hatch and increased mortality. This supported the findings of several authors (Lale and Abdulrahman, 1999; Lale and Mustapha, (2000b)**.**

The reduction in adult exit hole and percentage weight loss on treated grains as observed in the study might be due to the high mortality rate of the adult beetles and inability of the eggs to hatch; thereby reducing metabolic activities of insects. The ability of *S. afzelii* to reduce weight loss could also be ascribed to the odour produced by the extracts which might have exerted a toxic effect by disrupting normal respiratory activity of the beetles (Adedire and Ajayi, 1996; Adesina *et al* 2011). This aligned with Adedire and Ajayi (1996) who reported that essential oils of plant origin were highly lipohilic and therefore had the ability to penetrate the cuticle of insects. This might be another reason for the potency of the extracts. By this method the plant material apart from its odour, might have acted as a contact poison.

The cause for a considerable protection of cowpea seeds against the attack by *C. maculatus* in the current investigation could be due to the presence of different phytochemicals which interfere with the insect biological activities. Some workets (Zabri *et al,* 2008 and Zabri *et al* (2009), reported that phytochemical evaluation of the tested plant with methanolic extract, revealed the presence of alkaloids, tannins or tannoids, cardiac glycosides and saponins, quinones, flavonoids, coumarines, sterols and polyterpenes which were to possess some insecticidal properties.

Flavonoids have been considered as one of the plant's defensive systems against phytophagous insects (Masanori *et a*l, 2000). Wakako *et al,* (2012) reported that flavonoids and their related compounds have indicated that these compounds possessed antifeedant activities against the subterranean termite *Coptotermes formosanus* Saponins have been reported to exhibit clear insecticidal properties in several pest insects; exerted a repellent/deterrent activity, bearded digestive problems, provoked insect moulting defects or caused cellular toxicity effects. (De Geyter, *et al* 2007; Chaieb, 2010). Righi-Assia *et al.* (2010) reported that flavonoids significantly reduced egg laying and fertility in *C. Chinensis.*

CONCLUSION

The plant *S. afzelii* due to its higher tolerance for holding lower pest incidence under storage condition might be utilized to provide reasonable protection to cowpea seeds against *C. maculatus* infestation. Further research work is needed to be carried out to isolate and characterize the active compounds in the *S. afzelii* extracts; also to reveal the complexity of physiological activity exerted by toxic compounds on insect and to evaluate their mammalian toxicity.

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أنشطة تحمل *Callasobruchus maculatus* (F.) (غمدية الأجنحة: Chrysomelidae) ضد لمستخلصات أوراق *Secamone afzelii* (SCHULT) K. Schum

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ملخص

تم إجراء تجربة في المختبر لدراسة كفاءة المنثول، n – هكسان المستخلص من *Secamone afzelii* ضد تطور وتحمل خنفساء اللوبيا (.F) *Callasobruchus maculatus* غمدية الأجنحة: Chrysomelidae على بذور اللوبيا المخزنة Vigna umguiculata (w). تم تصميم التجربة العشوائي الكامل (CRD) مع تعريض خمسة أزواج من الخنافس الكاملة لتركيزات 2.0 ,1.5 ,1.0 ,0.5 مل، تماثل تركيز 10.0 ,7.5 ,5.0 ,2.5% حجم / وزن مخلوطة مع 20غم من بذور اللوبيا، بثلاث مكررات تحت ظروف المختبر، معاملة الشاهد كانت بدون إضافة المستخلصات. من النتائج تبيّن أن مستخلصات النبات أظهرت نسبة موت معنوية للحشرة الكاملة، وتسببت في تخفيض ملحوظ في أعداد فقس البالغات، مما نتج عنه تخفيض معنوي في أعداد البذور المثقوبة وخسارة في الوزن. أظهرت النسبة المئوية للمستخلص النباتي أن الكفاءة اعتمدت على التركيز، وأن البذور n – هكسان سجّلت أعلى نشاط بيولوجي ضد حشرة *Callasobruchus maculatus* مقارنة مع البذور المعاملة بالإيثانول. مستخلصات *Secamone afzelii* أظهرت قوة تحمل الحشرة مقارنة بالشاهد. من الممكن أن يتم استعمالها لمنع مهاجمة بذور اللوبيا بحشرة *Callasobruchus maculatus*. ومن الممكن أن تكون بديلاً جيداً للمبيدات العضوية المصنعة لحماية بذور اللوبيا. على العموم، الموضوع بحاجة إلى دراسة أعمق لعزل وتعريف المواد البيولوجية المسئولة عن النشاط الإبادي للنبات.

الكلمات الدالة: كفاءة، تحمل الآفة، مستخلصات النبات، بذور مثقوبة، مبيدات.

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