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Insecticidal Activity of the Aqueous Leaves Extract of *Andrographis paniculata* as Protectant of Cowpea Seeds from *Callosobruchus maculatus* Infestation

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ABSTRACT

The effect of aqueous leaf extract of *Andrographis paniculata* on cowpea seed treated against cowpea weevil (*Callosobruchus maculatus*) was investigated. One hundred and fifty (150) adult cowpea weevils with three hundred cowpea seeds were used and divided into five treatment groups (0 (control); 0.125; 0.250; 0.375 and 0.500 w/v) with three replicates. The phytochemical analysis revealed that it contains some active ingredients such as terpenoid, flavonoid, glycosides and sugar moiety. All the extracts at different concentration showed antefeedant and repellant effects since all the treated groups gave 100 % mortality at 96 h post treatment. The extract inhibits egg laying and development, prevents seed damage and was statistically different (p>0.05) compared with the control. Survival of adult cowpea weevil was least on highest dosage (50%) of extract treated seeds. The inhibitory potential of the extract shows it was dose- dependent. It is well obvious from our results that plant extract can control cowpea stored pest. Result of this investigation showed that *A. paniculata* possessinsecticidal activity against*C. maculatus*.In conclusion, results obtained from this study confirmed that the aqueous extracts of test plants species has great potential for use as a plant-based biopesticide for effective in controlling cowpea weevil population in stored seeds.The aqueous extracts of*A. paniculata* couldserve as alternatives to synthetic insecticides for use by resource-poor farmers who store small quantities of the seeds for their consumption, sales and planting.

Keywords*:* *Andrographis paniculata*, *Callosobruchus maculatus*, cowpea seed,leaf extract, insecticidal activity

INTRODUCTION

Cowpea is a dicotyledonous, an annual herbaceous plant belonging to the family Fabaceae and is grown extensively in the low lands and mild altitude regions of Africa (particularly in the dry savanna) sometimes as sole crop but more often intercropped with cereals such as sorghum or millet [2]. It was reported by FAO that about 7.56 million tonnes of cowpea were globally produced annually on about 12.75 million hectares of land [12] and Sub-Saharan Africa was reported to account for about 70% of total world production [12]. It was emphasized that all parts of the plant used as food are nutritious providing protein and vitamins, inmature pods and peas are used as vegetables while several snacks and main dishes are prepared from the grains [13]. Although, the crop is destroyed by weevils in storage thereby causing reduction in the market values, nutrients and as a stock source. Also prefer dried cowpea seeds but will attack other beans and peas in storage.Chemical controls using synthetic insecticides had been favourable so far because of their speedy action and easy application [19] but is toxic and adversely affect the environment by contaminating soil, water and air. Botanical pesticides are promising in that they are effective, environment – friendly, easily biodegradable, and also inexpensive [8].

Andrographis paniculata is an annual, herbaceous plant 1-3 feet high, in the family *Acanthaceae*, native to India and Sri Lanka. It is widely cultivated in Southern and Southeastern Asia, where it is used to treat infections and some diseases. It is called Creat in English and is known as the "king of bitters" [15]. Mostly the leaves and roots were used for medicinal purposes. Direct defenses are aimed directly at the attackers, such as herbivores, and include morphological (e.g., trichomes or sticky glands) and chemical (toxic secondary compounds) traits that interfere with colonization, feeding, and development of the herbivore. For example, toxic secondary compounds can act as feeding deterrents or negatively alter the performance of a herbivore through increased mortality, slower growth rates, or reduced fitness [25]. Indirect defenses are aimed at promoting the efficiency of natural enemies, such as predators or parasitic wasps (parasitoids) that kill the herbivores and thus reduce their damage to the plant. In either way the use of plant extract has been alternatives to chemical when considering several factors such as cost, availability, volatility and environmental hazards. [26]reported the availability and nutritional adequacy of the host plant as important factors affecting the distribution and population dynamics of phytophagous insects.Callosobruchus maculatus is a common pest of stored legumes has a cosmopolitan distribution, occurring on every continent except Antarctica[10]. The beetle most likely originated in West Africa and moved around the globe with the trade of legumes and other crops[31]. Despite the subsequent rounds of inbreeding, these populations persist. This ability to withstand a high degree of inbreeding has likely contributed to this species' prevalence as a pest [31]. The beetle tolerates a range of humidity and temperature, making it adaptable in climates worldwide. Its developmental time varies with factors such as humidity, temperature, legume type, crowding, and inbreeding levels in the population[10]. *Vigna unguiculata* (L) Walp., Ife Brown has been reported as susceptible cowpea cultivar to the bean weevil Callosobruchus maculatus (F.) (Coleoptera Bruchidale) [21]. Although, there are many reports dealing with the effects of different host plants on the growth, development and survival of phytophagous insects [14, 26], there is no such study for the extract of *Andrographis paniculata* leaves on the Ife brown seeds (susceptible cultivar) in storage against the bean weevil *Callosobruchus maculatus*.

Therefore, the present study was undertaken to determine the deterrent effect of the plant extract on bean weevil *Callosobruchus maculatus* on *Vigna unguiculata* (L) Walp., susceptible cultivar Ife brown in storage.

MATERIALS AND METHODS

Phytochemical Analysis

The leaves of *Andrographis paniculata* were open air-dried at room temperature after which it was grinded to a powdery form (using pestle and mortar). The aqueous extracts were prepared by soaking 200g of the powdery air-dried leaves of *Andrographis paniculata* in I litre of ethanol at room temperature for 48 h. The extract was filtered after 48 h through a Whatman no 42 (125mm) filtered paper. The filtrate was then concentrated at 40^0C using water bath. The resulting solution was subjected to phytochemical screening using standard procedures described by [33] for saponins [11, 32] for alkaloid, tannin, oxalates, cyanogenic glycosides and flavonoids, terpenes (Salkowski test) and reducing sugar (Fehling's test).

Insect cultures

Parent stock of *Callosobruchus maculatus* was obtained from the Okitipupa market, Okitipupa, Ondo State, Nigeria. The insects were reared in the laboratory on cleaned cowpea seeds at ambient temperature and relative humidity. From this stock, new generation of *C. maculatus* was raised. The cultures were maintained by continually replacing the devoured and infested seeds with fresh, uninfested ones. During the process of replacement, copulating pairs of adult *C. maculatus* were introduced into the containers.

Extraction procedure

Test plants species evaluated for insecticidal activity in *Andrographis paniculata* extract. Extraction of each plant material was carried out in the laboratory by soaking 100g, 200g, 300g and 400g of the plant powder in 800 ml of distilled water for 48 h to give 0.125; 0.25; 0.375 and 0.500 w/v of the solution. The solution was then filtered in order to remove the debris. The resulting filtrate was stored in a plastic container and refrigerated until ready for use. The following parameters were tested for against the prepared extract as follows; Insect mortality, Oviposition and adult emergence, Grain damage and Viability bioassays as described by[16, 20] procedure.

Statistical analysis

Data obtained were converted to percentages. Arcsin transformation was carried out on the percentage value. ANOVA was performed on transformed data and the means separated by DMRT.

RESULTS AND DISCUSSION

The result of qualitative phytochemical studies indicates the presence of flavonoids, terpenoids, glycosides and reducing sugar. The major active principle of the plant extract is terpenoids. The result is presented as shown on Table 1. The incorporation of natural plant products and their analogues into the management of agricultural stored insect pest has been considered as alternatives to synthetic products. This is due to the fact that they are less detrimental to the environment, economical and cheap to source than synthetic chemical insecticides. Synthetic insecticides are noxious to man and livestock and can be pollutants to the environment. They may not be readily available and are un-affordable by the rural farmers. They may also be persistent in the produce [7]. The presence of this active principle might give the bitter taste to the plant and this is evident that the presences of toxic substance serve as a protector to the plants.

Table 1: Phytochemical screening of *A. paniculata* leaf

Compound tested	Tested	Inference
Alkaloids	Dragendorffs reagent	-
Tannins	Ferric chloride test	-
Flavonoids	Shibata's reaction	+
Saponins	Frothing test	-
Oxalates	Anion analysis	-
Cyanogenic glycosides	Hydrogen cyanide	+
Reducing sugar	Fehling's test	+
Terpenoid	Salkowski test	+

+present - absent

The mortality of *C. maculatus* in seeds treated with different concentration of plant aqueous extracts was significantly different from untreated seeds (Table 2). The results obtained from this study showed that aqueous extracts from all the test plant concentrations caused high mortality of adult *C. maculatus*. The cowpea seeds treated with extracts from 37.5% and 50% of the aqueous extractswere the most toxic of all the extracts tested, followed by that of 25% and 12.5% evoking 100% mortality, respectively at 96 h of exposure.

Table 2: Effect of aqueous extracts of test plants species on mortality of adult *C. maculatus.*

Tests (g/ml)	% mortality at hours of post treatment					No of egg laid	% of adult emergence	% of seed damage	% of weight loss	Total no of seeds
	24	48	72	96	120					
0.125	60.00^a	80.00^a	90.33^a	100.00^a	100.00^a	1.67	0.00	0.00	4.13	60
0.250	70.33^a	90.33^a	100.00^a	100.00^a	100.00^a	0.00	0.00	0.00	3.98	60
0.375	90.00^a	100.00^a	100.00^a	100.00^a	100.00^a	0.00	0.00	0.00	4.27	60
0.500	90.67^a	100.00^a	100.00^a	100.00^a	100.00^a	0.00	0.00	0.00	4.08	60
Control	0.00^b	0.00^b	3.33^b	6.67^b	16.67^b	28.33	86.67	78.33	58.89	60
Mean	62.20	74.07	78.33	80.73	82.47	6.07	17.33	15.67	15.07	60
S.E	10.71	12.50	18.07	11.72	5.33	-	-	-	-	-

S.E: Standard error

Means in the same column followed by the same letter are not significantly different at $p \geq 0.05$.

The results from this investigation are similar to the observation of [1] who obtained 97.50% mortality of *C. maculatus* in cowpea seeds treated with acetone extracts from cashew kernels at 0.5% v/w.[9, 20] had also reported the effective protection of cowpea seeds against *C. maculatus* with aqueous extracts from several tropical plant species. Adult mortality increased with length of exposure. All extracts showed weevil mortality ranging from 60.00 to 100%.Aqueous extracts at high concentrationswere most effective against *C. maculatus*, evoking mortality 100% at 48 h of exposure while extracts from B was 72 h and *C* caused 100% weevil mortality by 96 h of exposure. The results agreed with several workers who reported similar reports for other plants part as insecticidal and antifeedant [18]. All the different extracts in this study inhibited the number of eggs laid by *C. maculatus* (Table 2). Oviposition by *C. maculatus* was significantly hindered in extract-treated seeds than untreated seeds. The percentage adult emergence in the untreated seeds was significantly higher than percentage adult emergence in the treated seeds. Aqueous extracts from the test plant were effective because it educed less percentage adult emergence than the control. All the extract provedeffective because it abolished seed damage and weight loss as found in the untreated seeds (Table 2) was evident. In the untreated seeds, 77.78% damage occurred as revealed by emergent holes of the bruchids as a result of the feeding activities of *C. maculatus* larvae on the cowpea seeds. These effects resulted in reduced weight; especially in the control seeds and the feeding activities of *C. maculatus*in particular resulted in holes in the seeds agreed with the reports of [6] who reported 50 % of the pod damaged by *C. maculatus* in store. The weevils found at the sides of the petri dishes indicate that the extract is rich source of bioactive compounds possessing strong repellency effect to the insect during the period. The consistent and significant decrease in the numbers of weevils on the treated confirms the effectiveness of the plant extracts. The fact that no significant difference was observed between the numbers of the weevils on the treated with the plant extracts indicates that the concentrations of the plant extracts were equally effective in the management of these pests. The reduction in pests' numbers was due to the antifeedant properties of the extracts which caused mortality. This has been reported in the case of *A. indica* [4, 30]. In *A. indica* the insecticidal property is due to the triterpernoids, azadirachtin and salanin [29, 28].However, the triterpenoid is responsible for its antifeedant properties [17]. The insecticidal effect of the plants aqueous extracts onC. *maculatus* in the treated cowpea seeds might be as a result of contact toxicity. Since, most insects breathe by means of trachea which usually opens at the surface of the body through spiracles. The extracts that were mixed with the seed might have blocked these spiracles thereby leading to suffocation and death of the insect [24, 3]). It also revealed that the extract of A. paniculata showed contact and systemic effects, as it caused high rates of mortality in all the four concentrations. High concentrations of A. paniculata reduced the reproductive capacity and feeding of *C. maculatus*.Once ingested, their effects are to prevent food utilization by susceptible insects and therefore mortality results from starvation. This explains why relatively high numbers were obtained on the treated seeds even after extract application.

Oviposition by *C. maculatus* was significantly lower in extract-treated cowpea seeds as against oviposition in the untreated seeds. It was also observed that the percentage adult emergence was drastically abolished by 30 days of exposure to the aqueous extracts. The fact that the plant extracts induces inhibition of oviposition by female *C. maculatus* and mortality of the development stages had been reported by a number of authors and fairly well documented [5]. The effect of the extracts on oviposition in the present study could be linked with respiratory impairment which probably affects the process of metabolism and consequently other systems of the body of the bruchids [22]. The plants extracts possibly inhibited locomotion; hence, the weevils were unable to move freely, thereby affecting mating activities and fecundity. The inability of the eggs to stick to the treated cowpea seeds due to the presence of the extracts may also reduce survival after adult emergence.The ability of some plant extracts to protect cowpea seeds from damage by *C. maculatus* over a short-term storage period had been tested with positive results. All the plant extracts at different concentrations considerably reduced seed damage. Some plant extracts have been tested for long time protectant ability on seeds and grains with positive results [23].

CONCLUSION

The use of plant extracts with insecticidal properties has the potential of reducing the effects of insect pests ofagricultural crops. These can be of importance to the resource-poor farmers in many areas of the developing world who store small quantities of the seeds for their consumption, sales and planting. The significant reduction in pests' numbers on the treated seeds was an indication that they can be used as alternatives to chemical insecticides. The aqueous extract of *A. paniculata*can be used for developing natural pest control products that may replace the synthetic bio-pesticides that are currently used against *C. maculatus*.

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