Insecticide Repellency of Plant Extracts Against *Callosobruchus maculatus*

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ABSTRACT

Several plant species were selected as potentially safer substitutes for control of cowpea weevil, Callosobruchus maculatus. The objective of the study was to investigate the insecticidal activity effect (that is, avoidance after contact) of aqueous extracts (crude) of Andrographis paniculata, Chromolaena odorata, Datura stramonium, Senna siamea and Vernonia amygdalina (leaves) in comparison with a synthetic insecticide (actellic powder) frequently used in treatment of cowpea in store. The phytochemical studies revealed presence of arrays of secondary metabolites such as alkaloids, tannins, flavonoids, saponins, oxalates, glycosides and reducing sugar in the tested plant. Cowpea weevils of a single strain were subjected to free-choice tests with cowpea seed sprayed with the different aqueous extracts and actellic powder. The behavioral response of C. maculatus to aqueous extracts and actellic powder differed (p<0.05) from the negative control. The results observed that insecticidal activities were demonstrated as Datura stramonium > Senna siamea > Andrographis paniculata> Chromolaena odorata > Vernonia amygdalina with the synthetic insecticide powder and D. stramonium produced the best result. The aqueous extracts of these plants could be employed in developing natural pest control products that may replace the synthetic bio-pesticides that are currently used against C. maculatus.

Keywords: *Aqueous extract, cowpea weevil, insecticidal, phytochemical.*

INTRODUCTION

Insects have evolved a variety of physiological and behavioral responses to various toxins in natural and managed systems. These varied responses can reflect the toxin's mode of action and the extent to which it influences pest behavior. Traditional medicine uses plant extracts without taking into account the toxicity of the plants material. In the last two centuries, there has been serious investigations into the chemical and biological activities of plants and these have yielded compounds for the development of synthetic organic chemistry and theemergence of medicinal chemistry as a route for the discovery of more effective therapeutic agents (Roja andRao, 2000).The plant kingdom contains a huge array of chemical substances; many of these are used by plants for their defense against insect attack. They may act as antifeedants, repellents, growth inhibitors, attractants, and chemosterilants or as insecticides.Organ toxicity is of concern because most herbal medicines

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are often used in their crude form, which contains several constituents. Plants like *Andrographis paniculata Chromolaena odorata, Datura stramonium, Senna siamea* and *Vernonia amygdalina* are used as a medicinal plant in the tropical rainforest of South America and Western Africa. In some countries, Leaves are used for the management of different disease conditions. Their leaves have been reported to be toxic due to secondary metabolites in them. The toxic effects of the leaves were linked to the presence of alkaloid, tannin, oxalate, phytate and barakol. Barakol, a major constituent of the *S. siamea* leaves has been reported to be responsible for the toxic effect of the leaves. Although, toxic compounds are widely distributed in the plant kingdom, it is generally considered that tropical angiosperm contain more complex array of these substances than any the species of plant, cultivars, stage of maturity and post-harvest treatments such as drying, soaking, autoclaving and germination of seed. The cowpea, *Vigna unguiculata* (L) Walp popularly known as beans is a tropical herbaceous annual, belonging to the tribe Phaseoleae and the order *Leguminosales* (Romain 2002).

In the Tropical Africa cowpea is consumed mostly in the form of dry grain. It is a relatively cheap source of protein and provides an adequate complement to a cereal-based diet. The bruchid beetle, *Callosobruchus maculatus* (F), is widespread throughout most of the tropical and subtropical countries, attacking cowpea and other legumes (Hill, 1983). Attack usually starts in the field where it is carried to the store; seeds damaged have reduced weight, low viability and poor marketability (Ofuya and Bamigbola 1991). A female bruchid beetle deposits from 40-80 eggs over a period of 7 days. According to Western (1985) reported about 40% of the total number of eggs is laid in the first day of life. The eggs are ovoid in shape and firmly glued to the testa of the seed. Adenekan, (2002) reported that the colour of the egg is pinkish when freshly laid but turns milky white after eclosion. Several reports on *Callosobruchus maculatus* have been reported on cowpea, *V. unguiculata* and other pulses. It has been reported that *Callosobruchus maculatus* could cause 60-80% damage to cowpea seeds stored for a period of six months (Hill, 1983).

Several plant could be used to protect the seeds against insects' infestation. The naturally occurring phytochemicals are usually biodegradable and non-toxic to plants, warm-blooded animals and the environment. They offer great potential as safer, more effective and economic pesticides with growing awareness of the hazards associated with the use of synthetic organic insecticides, there is a greater need to explore suitable alternative methods of pest control against stored products.Researches are currently being conducted on medicinal plants/extracts to isolate and purify the active fractions for preparation of drugs from natural sources (El-mahmood and Amey, 2007) due to their less toxic effects and affordability (Mohammed *et al.*, 2010).Mostly the leaves and roots of these plants were used for medicinal purposes. Schoonhoven *et al* (2005), stated that 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.In view of the fact that the use of the indigenous plants will be a promising approach to reduce the bruchid population very effectively, an attempt has been made to study the growth, toxicity and ovipositional repellency of cowpea weevil, *C. maculatus* after treating with extracts of some plants such as *Andrographis paniculata*, *Chromolaena odorata*, *Datura stramonium*, *Senna siamea* and *Vernonia amygdalina*.

MATERIALS AND METHODS

Phytochemical Analysis: The leaves of *Andrographis paniculata, Chromolaena odorata, Datura stramonium, Senna siamea* and *Vernonia amygdalina* were open air-dried at room temperature after which each was grinded to a powdery form (using pestle and mortar). The aqueous extracts were prepared by soaking 200g of the powdery air-dried leaves in 1litre 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^{0}C using water bath. The resulting solution was subjected to phytochemical screening using standard procedures described by Wall et al (1952) for saponins; Harbone (1973) and Trease and Evans (1985), 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. The study was carried out in the laboratory under ambient temperature conditions (temperature 32-34^{o}C) and relative humidity 90-95%. Untreated cowpea seeds (white variety) were used for this study was obtained from Okitipupa market, Ondo State. The seeds were cleaned and carefully sorted out, seeds of similar sizes were selected and kept in the laboratory until they were needed for the study.

Extraction procedure: The plant products tested for insecticidal activity were collected from growing stands of *Andrographis paniculata, Chromolaena odorata, Datura stramonium, Senna siamea* and *Vernonia amygdalina* in Okitipupa, Ondo State. The leaves of different plant were separately collected and sun dried before grinding into powdery form. Extraction of each plant material was carried out in the laboratory by soaking 200g of the plant powder in 800 ml of distilled water for 48 hrs. 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 (Obadofin and Fatoba, 2014).

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Experimental set-up/Statistical analysis: The untreated cowpea seeds were collected and placed in petri dishes. 20 seeds were placed in each petri dish arranged in seven groups. There were seven treatments replicated four times each. The treatments were filtrates of the different leaves of *Andrographis paniculata, Chromolaena odorata,* *Datura stramonium, Senna siamea* and *Vernonia amygdalina* .and untreated cowpea seeds, which serve as the control positive (Actellic dust) and negative control experiment. The plant products were administered on each replicate. Ten adults of *Callosobruchus maculatus* were introduced and confined in each petri dish for fourteen days. The petri dishes were arranged in completely randomized design and each group containing five treatments with the control experiment was carefully arranged in the laboratory and observed for several days before data collection commenced. Data were collected on the total number of egg laid, number ecloded and number of adults that emerged from each treatment. The total developmental period in each treatment also noted. The following parameters were tested for against the prepared extracts as follows; Insect mortality, Oviposition and adult emergence, Grain damage and Viability bioassays as described by Magaji *et al* (2012) and Obembe and Kayode (2013) procedure. Data obtained were subjected to statistical analysis using appropriates procedure. ANOVA was performed on transformed data and the means separated by DMRT (Duncan, 1955).

RESULTS AND DISCUSSION

Table 1 shows the phytochemical studies which indicate the presence of arrays of secondary metabolites such as alkaloids, tannins, flavonoids, saponins, oxalates, glycosides and reducing sugar. *Senna siamea* and *Chromolaena odorata* possess all the metabolites and only alkaloids found in *Datura stramonium*. Tanins and oxalates were absent in *Vernonia amygdalina* but flavonoids, glycosides and reducing sugar occurred in *Andrographis paniculata*. The uses of natural plant products and their analogues into the management of agricultural storedinsect pest have been considered useful to synthetic products. This is due to the fact that they are eco-friendly, less harmful to the environment, economical and cheap to source than synthetic chemical insecticides. Syntheticinsecticides posed serious problems to man and livestock and can be pollutants to the environment. They may not be readilyavailable and are un-affordable by the rural farmers. Insects may withstandinsecticide applications either through the evolution of physiological mechanisms allowing them to cope withhigh insecticide levels on or within the body, or throughbehavioral mechanisms minimizing their exposure toinsecticides (Hoy, Head and Hall, 1998; Jallow and Hoy, 2005). The presence of this active principle might give the bitter taste to the plants and this is evident that the presence of toxic substance serve as a protector to the plants.

The plant products significantly reduced the number of eggs laid by *C. maculatus* on the cowpea seed in order as *V. amygdalina< D. stramonium<A. Paniculata<C. odorata< S. siamea* and they are comparable ($p<0.05$). However, fewer eggs were laid by the insect on the seed of positive control followed by *Datura stramonium* treatment, *Senna siamea*treatment, *Andrographis* treatment, *Chromolena* treatment, *V. amygdalina* treatment and more eggs were laid on the negative control compared ($p<0.05$) to the treated plate (Table 2). The weevils exposed to seeds treated with leaves extract of different plant products showed the lowest mean number of egg laid and adult emergence and was significantly different from control.

However, there was no oviposition on cowpea seeds treated with leaves extracts and Actellic dust in all the replicates but eggs were found at the sides of the petri dishes. This was evident that the plant product showed repellency properties and the results was in conformity with other workers who reported phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositor attractants, and these different activities have been observed by many researchers (Silva L., Peres, Silva W. and Macedo, 2009; Ishii, Matsuzawa and Vairappan, 2010; Ntonifor, Forbanka and Mbuh, 2011; Suthisut, Fields and Chandrapatya, 2011; Asmanizar and Idris, 2012). The performance of Actellic in this experiment confirms the assertion of Singh (1989) who reported that synthetic insecticides are relied upon mostly for prevention or reduction of storage wastage. The feeding effect of *C. maculatus* on the treated seed shows significant effect in *V. amygdalina* > *C. odorata* > *A. paniculata* > *S. siamea* > *D. stramonium* and least was observed in positive control and comparable to control ($p<0.05$).

In cowpeas, *A. paniculata*, *Senna siamea* and *D. stramonium* provided the best protection against grain damage while *C. odorata* and *V. amygdalina* show a slow protection against the weevil when all the pesticidal plants were compared. The viability test revealed that all the tested seed were similar ($p>0.05$) despite the infestation of the *C. maculatus* (Table 2). Table 2 shows the ovivoposition of *C. maculatus* in the treated test seed. The different extract reduces ovivoposition as compared to the control ($p<0.05$). Exposure of the treated seeds to the weevils significantly reduced the incidence of infestation in all treatments. Significantly exposure was recorded in the untreated control. All of the plant extracts were found to have significant effect on adult emergence of insect pests attacking stored cowpea grains since there was adult emergence on the negative control but inhibition was found on the treated seeds. The pesticidal plant materials showed varied efficacy as grain protectants; but none of all the treatments levels failed to provide the immediate protection. Farmers normally use the plant as dried wood chips stuck in grain (Mvumi, Fanuel and Albert, 1995). All the extract provides effective because it abolished seed damage as found in the untreated seeds was evident.

In the untreated seeds, damage occurred as revealed by emergent holes of the bruchids as a result of the feeding activities of *C. maculates* larvae on the cowpea seeds. The incidence of adult emergence in the control was an evident of previous result and agreed with other workers who found promising use of indigenous plant products. Aqueous extracts from the test plant were able to reduce the adult emergence than the control. Despite the activities of the insect in creating holes in the tested seed, there were no significant differences ($p>0.05$) in the germination and viability recorded over 92 %. The survival of *C. maculatus* in cowpea incorporated with leaf extracts of *Andrographis paniculata*, *Senna siamea*, *Chromolena odorata*, *Vernonia amygdalina* and *Datura stramonium* at various days after treatment. The results shows that the numbers of the insects reduces as the study progresses and were significantly different ($p<0.05$) but the treated seed were similar ($p>0.05$). 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 (Obadofin and Fatoba, 2014) 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. However, the triterpenoid is responsible for its anti-feedant properties (Mehta, Vaida and Kashyap, 1995). This could have been an issue of the plant attracting insects despite the claimed repellence. Studies by several workers (Venketachalam and Jebasan, 2001; Ishii, Matsuzawa and Vairappan, 2010; Suthisut, Fields and Chandrapaty, 2011) have shown that plant derivatives and other pesticidal plants, known to have repellent properties, attract insects.

The attractant effects found in these plants remain unexplained. The high damage on cowpeas could also be attributed to larvae which typically develop inside the dried cowpeas thus the high initial damage of 13%. Repellence may not work if resident insect pest infestation is already high. The study revealed that, plant materials are effective over a short period of time in cowpeas. Since the plant materials are suitable for short term storage, reapplication at a 16 week interval is recommended subject to availability of plant materials. Residual properties of the plant materials need to be tested so as to get the exact time over which the plant materials will be effective and this will help in avoiding unnecessary applications. Emerging evidence suggest that active ingredients in some pesticidal plants' efficacy vary depending on time of year harvested and geographical location. The mode of action of the plant materials also need further investigation to ensure the plant materials are correctly and effectively used.

The insecticidal effect of the plants aqueous extracts on C. *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. It also revealed that the extract of *A. paniculata* showed contact and systemic effects, as it caused high rates of mortality. High concentrations of *A. paniculata* could reduce 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.

Many synthetic insecticides have been found effective against stored product pests but proved to be hazardous to men and domestic animals. In addition, the risk of developing insect resistance and the high cost-benefit ratio of synthetic insecticide have pushed researchers to find alternative insecticides. They have recently concentrated their efforts on the search for active natural products from plants as alternatives to conventional insecticides. Some of these researchers reported that plant materials and local traditional methods are much safer than chemical insecticides and suggested that their use needed exploitation. In many areas of Africa and Asia locally available plants and materials are being widely used to protect stored products against damage by insect infestations, as alternatives to chemical insecticides.The results of this study therefore indicates that plantleaves which is available all year round in every part of Nigeria could also be used as seed protectant for grain storage.

CONCLUSION

The use of plant extracts with insecticidal properties has the potential of reducing the effects of insect pests of agricultural 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, C. odorata, D. stramonium, Vernonia amygdalina* and *S. siamea,* can be used for developing natural pest control products that may replace the synthetic bio-pesticides that are currently used against *C. maculatus.*

Table 1: Phytochemical screening of the test plants leaves

Compound Tested	Procedure	Inferences				
		A. paniculata	*C. odorata*	*D. stramonium*	*S. siamea*	*V. amygdalina*
Alkaloids	*Dragendorffs reagent*	-	+	+	+	+
Tannins	*Ferric chloride test*	-	+	-	+	-
Flavonoids	*Shibata's reaction*	+	+	-	+	+
Saponins	*Frothing test*	-	+	-	+	+
Oxalates	*Anion analysis*	-	-	-	+	-
Cyanogenics glycosides	*Hydrogen cyanide*	+	+	-	+	+
Reducing sugar	*Fehling's test*	+	+	-	+	+

+ present - absent ***Source:*** Experimental Analysis, 2015

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

TEST	% Mortality at days of post treatments					No of egg laid	% of grains with holes	viability
	5	9	13	17	21			
-ve Control	0^c	0^d	0^c	0^c	0^b	48.50^d	68.75	85
+ve control	66.7^a	92.33^a	100^a	100^a	100^a	8.50^a	13.75	100
A. paniculata	47.50^a	52.50^b	62.50^b	85^a	100^a	17.25^b	27.5	95
C. odorata	55.0^a	75^a	77.50^a	95^a	100^a	30.25^c	32.25	92.50
D. stramonium	32.50^b	52.50^c	67.50^b	70^b	100^a	11.50^a	21.25	100
S. siamea	42.5^b	54.5^c	60.0^b	75^b	100^a	14.75^b	22.50	100
V. amygdalina	28.75^b	47.25^c	57.50^b	67.50^b	100^a	32.25^c	37.50	95
S.E	0.971	1.440	0.888	0.930	0.968	0.771	0.452	

a b, c; means same rows bearing different superscripts differed ($p<0.05$)

Source: Experimental Analysis, 2015

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