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Qualitative Phytochemical screening of *Acalypha fimbriata*, and its methanol extract as protectant against *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored maize

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Abstract
Phytochemical screening of crude methanol extract of *Acalypha fimbriata* was done to check for presence of secondary metabolites. The result indicated that the plant contained alkaloids, tannins, saponins, flavonoids, resins and glycosides. Extracts at different concentrations of (10, 20, 30 and 40 %v/v) with a solvent-treated control (0 %v/v) were also bio-assayed for their insecticidal potential against *Sitophilus zeamais* on stored maize. Five pairs (1 male :1 female) of adult insects were used to infest 50 g TZPB-SR-W maize grains stored for three months. Experiment was laid out in a completely randomised design, in four replicates, in the Entomology Research Laboratory, University of Ibadan, under an ambient conditions of 27 ± 2°C and 65 ± 5% relative humidity. Parameters assessed were insect mortality, oviposition, progeny emergence as well as percentage grain damage and seed viability. Results showed that the plant extract significantly ($P < 0.05$) caused higher mortality, reduced oviposition, inhibited F_1 emergence and with less damage to the grains when compared to those of the control. However, insecticidal activity was concentration dependent, with 40% v/v *A. fimbriata* extract being the most potent. There was no significant difference ($P > 0.05$) in the germination of tested seeds among all treatments including the control.
Keywords: Phytochemical screening, *Acalypha fimbriata*, protectant, *Sitophilus zeamais*

Introduction

Insects are major post–harvest pests of crops both at the farmers' and consumers' level in the tropics (Lale and Ofuya 2001; Adedire, 2003). The heavy post-harvest losses and quality deterioration caused by storage pests are a major problem facing agriculture in developing countries such as Nigeria (Adedire and Ajayi, 1996). The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a primary field to store pest (Adedire, 2001) that starts to infest the ripening maize crop in the field when the grain moisture content is still 50-55%. Its heavy infestation may cause weight losses of as much as 30 - 40% of produce (CABI, 2005). Adult weevils and larvae feed on undamaged grains and reduce them to powdery form (Adedire, 2001).
Plants have contributed greatly in making the surface of the earth habitable for all living things, most especially man. The naturally occurring compounds produced by living organisms have been of interest to human being for centuries. Plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oil, flavonoids, alkaloids, and other chemical compounds which have preventive and curative properties (Oloyede*, et al.,* 2010). Traditional medicines for many cultures are effective because individual organic compounds that occur in the native plant exhibits biological activities that may be used in treating various health problems (Richards, 1999). Organic compounds found in these plants are often incorporated as additives in food and perfume industries; some natural products as those found in flowering plants are highly colored, and these may be used to prepare dyes that color plants and fabrics (Gilbert and Martins, 2006). Increased demand for more sustainable agricultural practices and organic product coupled with renewed awareness of negative effects of conventional pesticides on the environment are serving as impetus to resort to reduced-risk plant-based pesticides (Ntonifor, 2011). Research activities have thus focused on the efficacy of these botanicals, mode of action and appropriate application technologies (Talukder and Howse, 1995; Owusu, 2001; Tapondjou, *et al*., 2002). *Acalypha* is the fourth largest genus of Euphorbiaceae, comprising fast growing, evergreen shrubs, trees and annuals from tropical to subtropical regions particularly in the tropics of Africa, America and Asia. They are found in the tropics of Africa, America and Asia. (Onocha *et. al.,* 2011). Decoction of *Acalypha fimbriata* Schum. & Thonn., is used as laxative whereas its leaves are used in treating asthma, rheumatism, syphilis, ulcers, and also as anthelmintic, antimicrobial and antifungal in Nigeria (Odugbemi, 2008). There is however, a dearth in knowledge on the chemical

composition and insecticidal activity of *A. fimbriata*. As part of a continuous research program on plants, this paper reports the secondary metabolites obtained from the extracts of the whole plant as well as the evaluation of its methanolic extract in the management of *Sitophilus zeamais* in stored maize.

Materials and Methods

Study Location

All experiments were conducted at the Organic Chemistry Laboratory and Entomology Research Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria under ambient temperature of 27 ± 2°C ambient temperature and 65 ± 5% relative humidity.

Source and type of test maize grains

TZPB – SR- W variety of maize purchased from the Seed Storage Section of Institute of Agriculture Research and Training (IAR&T), Moor Plantation, Ibadan was used in this study. Prior to experiments, the grains were disinfested in a deep freezer for one week and later air-dried in the laboratory to prevent mouldiness.

Insect culture

Initial culture of adult *S. zeamais* was raised from infested maize bought from Bodija market, Ibadan. Fifty pairs of adult *S. zeamais*, sexed following the reports of Odeyemi and Daramola (2001), were introduced into 1 litre kilner jar containing 400 g TZPB-SR-W maize grains. The jar was covered with wire mesh lid to allow for aeration and replicated 4 times and samples were observed daily until emergence of F_1 progeny

Sample collection, preparation and extraction

Fresh plants of *Acalypha fimbriata* were collected from botanical garden of University of Ibadan, Ibadan. The plant was identified and authenticated at the Botany Department of the University of Ibadan, Oyo State. The whole plant was air dried for 28 days and ground into fine mesh size by a grinder at wood extraction laboratory, Chemistry Department, University of Ibadan. Four hundred grammes (400 g) of ground *Acalypha fimbriata* was loaded into 5L aspirator bottle for a period of 72 hours using 2.5L methanol for its extraction. The extract was concentrated using a rotary evaporator and dried crude extract was obtained. A stock solution of the crude extract was made and serial dilution was done to obtain concentrations of 10, 20, 30 and 40 %v/v.

Phytochemical screening of extracts

Small portion of methanol crude extract of *Acalypha fimbriata* was screened for different classes of secondary metabolites. The test for alkaloids, saponins, phenols, flavonoids, sterols, tannins carbohydrates and glycosides were done using standard method *(*Trease and Evans, 1989; Ajaiyeoba *et al*., 2003).

Tests for flavonoid

5 ml of 1% hydrochloric acid and extract were shaken with sodium hydroxide; a yellow colour appeared indicating the presence of compound flavonoids.

Tests for tannins

5% ferric chloride ($FeCl_3$) was added to a test extracts. A dirty green precipitate was observed. Presence of tannin was confirmed.

Tests for alkaloids

About 2 ml of test extract was acidified with 1% HCl. Few drops of Dragendorff's reagent were added. An orange brown precipitate was observed which confirmed the presence of alkaloid.

Tests for saponins

About 2 ml of test extracts was shaken vigorously for few minutes, frothing was observed on warming with water, the frothing continues. This indicates the presence of saponins.

Tests for glycosides (Keller-Kiliani Test)

To 2 ml extract, add glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green, this indicates the presence of glycosides.

Tests for sterols (Salkowski's test)

To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4 was added and was shaken very well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence, this confirmed the presence of sterols.

Tests for phenols
Test extract was extracted with ethylacetate and filtered with Whatman filter paper. About 1ml of ferric chloride solution was added to the filtrate. A brown color solution was observed. This confirms the presence of phenols.
Test for anthraquinones
Borntrager's test**:** About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colorations of aqueous layer indicate the presence of anthraquinone.

Bioassays with plant extract

Effects of crude extract of *A. fimbriata* on the survival of *S. zeamais*
Fifty grammes (50 g) of maize grains was weighed into kilner jars. Using a micro-syringe, 0.5ml of the concentrations (10, 20, 30 and 40 % v/v) of the extracts was applied to the grains and shaken to allow for coverage. Grains in the control jar were treated with methanol only. There were five treatments replicated four times. The grains were infested with ten (1 male: 1 female) adult *Sitophilus zeamais* per jar and covered with a lid of fine mesh to allow for aeration. Mortality was recorded at 24, 48, 72 and 96 hours after infestation, with insects considered dead if they did not respond when probed with a camel hairbrush. Dead adults were removed at each assessment, counted and recorded. Data on percentage mortality were corrected using Abbott's (1925) formula:
$P_T = P_O - P_C / 100 - P_C$; where P_T = corrected mortality (%), P_O = observed mortality (%), Pc = control mortality (%).

Effects of plant extract on oviposition of *S. zeamais*
A second experiment was set up to check for the effects of plant extracts on the oviposition of *S. zeamais*. Ten grains were placed in each of 4 petri dishes. 0.5 ml of the concentrations (10, 20, 30 and 40 % v/v) of the extract was applied to the grains and shaken to allow for coverage. Five pairs (1male: 1female) of day-old *S. zeamais* were introduced and weevils allowed to mate and oviposit on the grains for 7 days. Grains were removed and the number of eggs laid determined using the egg-plug staining/detection technique described by Frankenfeld (1948) and modified by Pedersen (1979). With each concentration as treatment, the experiment was arranged in a completely randomized design (CRD) in four replications. Number of eggs laid on maize grains was recorded, analyzed and means separated using the Least Significant Difference (LSD).

Effects of plant extract on emergence of *S. zeamais*, grain weight loss and seed viability

Weevils from the experiment one above were removed at two weeks of oviposition and jars left undisturbed until emergence was complete after 49 days. The grains were later sieved to remove the dust produced from adult feeding and re-weighed at 64 days after infestation by using a Mettler Weighing balance and the percentage loss in weight determined as follows:

$$\text{Percentage (\%) weight loss} = \frac{\text{initial weight - final weight}}{\text{Final weight}} \times 100$$

Germination test was conducted using ten seeds treated with each concentration and the control. The seeds were placed on moist filter paper in plastic petri dishes kept in an incubator at 25°C and the number of germinated seed was counted and recorded and percentage seed viability was calculated as:

$$\text{\% viability} = \frac{\text{Number of germinated seed}}{\text{Number of seed sown}} \times 100$$

Statistical analysis
All percentage data were angular transformed prior to statistical analysis, in order to equalise variances. All data were analysed using analysis of variance (ANOVA) and treatment means that show significant differences ($P < 005$) were separated using the Least Significant Difference (LSD)

Results and Discussions

Phytochemical Screening
The result of the phytochemical screening of methanol extract of whole plant of *A. fimbriata* is shown in table 1. The result indicated a positive test for saponins, tannins, alkaloids, glycosides, flavonoids, resins and sterols and negative for phenols. These phytochemcial compounds are known to play important roles in bioactivity of the plant. Adegboye, *et. al.,* (2008) reported that the bioactivity of plants lies in these

phytochemical compounds and as such produce a definite physiological action on the body. Table 2 shows the mortality responses of adult *S. zeamais* at 24 – 96 hours after exposure to the grains treated with methanol extract of *A. fimbriata* and a solvent-treated control. The different concentrations of the extracts exhibited varying degrees of insecticidal activities on *S. zeamais*. Increase in concentration of the extract significantly (P < 005) increased adult mortality of *S. zeamais* and maintained high toxicity at higher doses. This may be attributed to the fact that extract with higher dosages have higher concentrations of the active compounds than those of lower dosages (Mong *et. al*., 2012). There were significant differences ($p < 0.05$) between all the concentrations and the control in all the days of the trials with the highest percentage mortality of adult insect (85%) at 40 %v/v of the extract at 96 hours post infestation. The effect of the extract on oviposition of maize weevils was evaluated by comparing the total number of eggs laid in the treated and control grains.

Table 1: Phytochemical screening of methanol extract of *A. fimbriata*

Secondary metabolites	Methanol Extract
Alkaloids	+
Tannins	+
Saponins	+
Sterols	+
Flavonoids	+
Resins	+
Phenols	-
Glycosides	+

\- = negative + = positive

Table 2: Effects of methanolic seed extract of *A. fimbriata* on survival of *S. zeamais*

	Percentage mortality (± S.E) at 24 – 96 hours post infestation			
Conc. (%v/v)	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
0 (control)	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
10	$5.00^{b} \pm 5.32$	$32.50^{b} \pm 1.51$	$37.50^{b} \pm 2.83$	$45.00^{b} \pm 1.67$
20	$15.00^{c} \pm 2.34$	$40.00^{bc} \pm 2.41$	$47.50^{b} \pm 2.77$	$62.50^{c} \pm 2.83$
30	$22.50^{c} \pm 1.65$	$47.50^{c} \pm 3.69$	$62.50^{c} \pm 2.83$	$72.50^{d} \pm 1.66$
40	$40.00^{d} \pm 2.40$	$65.00^{d} \pm 2.95$	$77.50^{d} \pm 1.67$	$85.00^{e} \pm 2.34$
$LSD_{(0.05)}$	**8.78**	**7.43**	**6.93**	**5.88**

Means followed by the same letters in a column are not significantly different at 5% level

Lowest number of eggs was laid in the grains treated with 40 %v/v, followed by 30 %v/v. (Table 3). Mean oviposition on grains treated with 30 %v/v (1.75) was significantly different ($p < 0.05$) from that on the control (9.75)

TABLE 3: Effects of methanolic seed extract of *A. fimbriata* on oviposition of *S. zeamais*

Conc. (%v/v)	Oviposition (± S.E)
0 (control)	$9.75^{C} \pm 0.23$
10	$8.00^{C} \pm 0.12$
20	$4.50^{b} \pm 0.23$
30	$4.25^{b} \pm 0.11$
40	$1.75^{a} \pm 0.08$
$LSD_{(0.05)}$	**0.50**

Means followed by the same letters in a column are not significantly different at 5% level

Table 4: Effects of methanol seed extract of *A. fimbriata* on emergence of *S. zeamais*, grain weight loss and seed viability

Conc. (%v/v)	Mean number of emerged adult (± S.E)	Percentage weight loss (± S.E)	Percentage seed germination (± S.E)
0 (control)	25.25^{d} ± 0.38	1.84^{c} ± 0.24	77.50 ± 2.50
10	15.50^{c} ± 0.20	0.96^{b} ± 0.10	80.00 ± 5.77
20	7.25b ± 0.19	0.71^{b} ± 0.12	77.50 ± 4.78
30	2.40^{a}± 0.20	0.28^{a} ± 0.05	75.00 ± 2.89
40	1.75^{a} ± 0.14	0.14^{a} ± 0.02	80.00 ± 4.08
LSD (0.05)	**0.71**	**0.39**	**ns**

Means followed by the same letters in a column are not significantly different at 5% level

Table 4 shows the effect of plant extracts on emergence of *S. zeamais*, grain weight loss and seed viability**.** Application of the different concentrations of extract of *A. fimbriata* on F_1 emergence of *S. zeamais* shows varied effects. Mean F_1 progeny emerged on grains treated with the 40 %v/v was significantly ($p < 0.05$) lower (1.75) than the control (25.25). There was no significant difference ($p > 0.05$) between the treatment dosage at 40 %v/v (1.75) and 30 %v/v (2.40). All the treatments significantly reduced emergence that the control (Table 4). The percentage loss in weight in treated maize grains was significantly ($p < 0.05$) lower when compared to that of the control with no significant difference between treatments with extracts 10 and 20 %v/v and also with 30 and 40 %v/v. The result of weight loss after emergence of the weevils followed the same pattern as the emergence of adult weevils. Table 4 also shows no significant difference ($p < 0.05$) in the percentage viability of seeds treated and in the control**.** All the treated seeds were as viable as the untreated seeds

Conclusions

The findings of the present study indicate that botanical derivatives might be useful as insect control agents for commercial use. All the concentrations of the extract tested were effective to some degree in causing mortality, reducing the oviposition of adult *S. zeamais* and F_1 adult emergence. To minimize the severe damage caused by insect pests, the traditional use of plant products is highly effective against stored product insects. These botanicals are less expensive and safe, and their easy adaptability will give additional advantages leading to acceptances of this technology by farmers. Further work should be done to identify and isolate the active compounds present in this plant.

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