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Koomson CK

Department of Integrated
Science Education, University of
Education, Winneba, P.O. Box
25, Winneba, Central Region,
Ghana

Opong EK

Department of Chemistry
Education, University of
Education, Winneba, P.O. Box
25, Winneba, Central Region,
Ghana

Entomotoxicant potential of Christmas bush, *Alchornea cordifolia* (Schum. & Thonn.) leaves powder in the control of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) infesting stored maize

Koomson CK and Opong EK

Abstract

The efficacy of leaf powder of *Alchornea cordifolia* against the maize storage pest, *Sitophilus zeamais* on stored maize was investigated in the Integrated Science Education Department laboratory of the University of Education, Winneba, Central Region, Ghana, at a temperature of 30±2°C and 75±5% relative humidity. *A. cordifolia* leaf powders were added to 20.0 g of grains at the following rates of 3.0g, 4.0g, and 5.0g to assess contact toxicity, damage assessment, progeny production, repellency and seed germination ability. Results showed that the plant material was toxic to the insect ($P<0.05$). The leaf powder of *A. cordifolia* applied at 5.0g concentration greatly caused the highest mortality of 93% after 21 days. It also repelled almost 90% of the maize weevils and significantly inhibited adult emergence up to about 97%. The 5.0g concentration of the leaf powder further offered a protection of nearly 98% to the maize seeds against damage by the weevils compared to other concentrations ($P<0.05$). The leaf powders at all the concentrations also had no effect on germination. The result revealed that *A. cordifolia* powder can be used as an entomotoxicant against *S. zeamais* and its incorporation into traditional storage pest management and integrated pest management is strongly recommended in developing countries.

Keywords: Weevil perforation index, *Sitophilus zeamais*, entomotoxicant, *Alchornea cordifolia*

1. Introduction

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) is the most widespread and major destructive insect pest of stored maize throughout the world [12]. It is an internal feeder causing considerable loss to cereals by affecting the quantity as well as the quality of the grains [12]. The insect damage to stored grains is known to cause major economic losses to warehouse keepers, the milling industry and small scale farmers throughout the world [2]. This problem is greatest in developing countries where modern storage technologies are lacking. FAO production figures in 2014 showed that 2,543 million metric tons of major grains were produced worldwide [13] and using the often quoted conservative minimum overall food grain loss figures of 10% [7], the total loss to mankind of these cereal grains to be 254.3 million tones. Several attempts to develop insecticide-based techniques for protecting grains in small traditional farm stores have only been partially successful because of problems such as high cost of synthetic insecticides and erratic supply due to foreign exchange constraints [20]. Moreover, the abuse of synthetic chemicals has led to serious problems, including the development of insecticides resistant strains to insecticides, toxic residues on stored produce, health hazards to grain handlers, food poisoning, environmental pollution [9, 29, 31]. These problems have stimulated research into plants with insecticidal properties grown locally that are readily available, effective, affordable, less poisonous and less detrimental to the environment [27, 15], reported that when mixed with stored grains, leaf, bark, root powder or oil extracts of plants reduce oviposition rate and suppress adult emergence of stored product pests. The tropical region is well endowed with a wide array of floristic species with defensive chemicals and quite a number of them have been used traditionally in protecting against grain beetles attack and many of which are yet to be identified [2]. The use of plant products in crop protection may help to prevent the environment and the consumer from the effect of synthetic pesticides [26]. One of such plants is *Alchornea cordifolia* (Schum. & Thonn.).

Correspondence

Koomson CK

Department of Integrated
Science Education, University of
Education, Winneba, P.O. Box
25, Winneba, Central Region,
Ghana

It is a straggling, laxly branched, evergreen dioecious shrub or small tree growing up to 8 meters tall [8]. *Alchornea cordifolia* is an important medicinal plant in African traditional medicine and much pharmacological research has been carried out into its antibacterial, antifungal and antiprotozoal properties, as well as its anti-inflammatory activities, with significant positive results [3]. The leaves or leafy stems are also believed to be abortifacient, antispasmodic, blood purifier, diuretic, emetic (in large doses), emmenagogue, oxytocic, purgative, sedative and tonic [3]. The crushed fresh leaves or powdered dry leaves are applied externally as a cicatrizing to wounds, to relieve pain, e.g. backache and headache, to fractures to improve healing and to treat eye infections and numerous skin afflictions including venereal diseases, leprosy, sores, abscesses, yaws and filariasis [3]. Therefore, there is need to find out whether this plant has some insecticidal properties in addition to its numerous medicinal properties. This present study reports on the results of laboratory investigations on the bioactivity of *Alchornea cordifolia* against *S. zeamais* in stored maize in the laboratory.

2. Materials and Methods

The research was carried out in the Integrated Science Education Department laboratory of the University of Education, Winneba, Central Region, Ghana. Temperature in the laboratory was 30±2 °C and relative humidity was 70±5%. The study was carried out from August to September 2017.

2.1 Insect Culture

Initial stock used for the experiment was obtained from maize seeds that were bought from the Mandela market at Agona Swedru in the Central Region of Ghana. The maize seeds were put in different jars covered with net and adult *S. zeamais* were introduced into the jars. The jars were kept at room temperature in the Integrated Science Education Department laboratory of the University of Education, Winneba for the insects to breed and multiply under favourable laboratory conditions (temperature of 30±2 °C, and relative humidity of 75±5%) The moisture content of maize grain was adjusted to 12 to 13% [25]. Three weeks after oviposition, the parent weevils were sieved out and the grains were kept in the laboratory for adult emergence. The emerging generation of same age insects was re-cultured at temperature of 30±2 °C, and relative humidity of 70±5%. The F1 generation was used for the experiment.

2.2 Collection and preparation of plant materials

Alchornea cordifolia plants were collected from the Gomoa Otapirow area of the Central Region of Ghana. Leaves were separated from the plant, rinsed in clean water to remove sand and other impurities, air dried at room temperature in the laboratory for 15 days, after which, pulverised into very fine powder using an electric blender. The powders were further sieved to pass through 1mm² perforations. The powders were packed in plastic containers with tight lids to ensure that the active ingredients are not lost and stored in the laboratory prior to use.

2.3 Source of maize substrate

The uninfested maize (local variety) used for the experiment were procured from the Mandela market at Agona Swedru in the Central Region of Ghana. These were properly handpicked and sieved. Thus, ensuring that only whole and infestation-free seeds were used. Nevertheless, the maize seeds, with the exception of those to be used for the viability

tests were then sterilized in the electric oven for an hour at 60 °C. The seeds were then cooled at room temperature. Twenty gram each of the uninfested maize seeds were weighed separately and kept at room temperature. The experiment was carried out in triplicate for each treatment.

2.4 Effect of contact toxicity of *Alchornea cordifolia* leaves powders on adult mortality, oviposition and progeny development of *Sitophilus zeamais*

a. Contact toxicity of *A. cordifolia* leaf powder

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Twenty pairs of *S. zeamais* were introduced into the a clean sterilized 250ml plastic containers containing 20.0g of uninfested sterilized maize seeds at 0.0, 3.0, 4.0, and 5.0g% (w/w) of *A. cordifolia* leaf powder, while in the control treatment there was no plant material added. The *A. cordifolia* leaf powder was weighed and added to the maize grain in each jar and shaken well for uniform coating. The jars were covered with muslin cloth and secured with rubber bands as a ventilated lid. The treated grains in the jars were kept for about 21 days and mortality rate assessments were performed regularly every 1, 7, 14 and 21 days after exposure of *A. cordifolia* leaf powder. Adults were considered dead when probed with sharp objects and there were no responses [20]. Percentage adult mortality was corrected using [1] formula, thus:

$$P_T = \frac{P_o - P_c}{100 - P_o} \times \frac{100}{1}$$

Where P_T = Corrected mortality (%)

P_o = Observed mortality (%)

P_c = Control mortality (%)

b. Determination of effect of powder on progeny production

The experimental set up was kept in the laboratory for further 30 days for the emergence of the first filial (F1) generation. The containers were sieved out and newly emerged adult *S. zeamais* were counted and recorded. The percentage adult emergence was calculated using the method of [22].

$$\% \text{ Adult emergence} = \frac{\text{Total number of adult emergence} \times 100}{\text{Total number of eggs laid} \times 1}$$

c. Damage assessment

Percentage weight loss of the maize seeds was determined by re-weighing after 35 days and the % loss in weight was determined using the method of [20] as follows:

$$\% \text{ Weight loss} = \frac{\text{Change in weight} \times 100}{\text{Initial weight} \times 1}$$

After re-weighing, the numbers of damaged maize seeds were evaluated by counting wholesome seeds and seeds with weevil emergent holes. Percentage seed damaged was calculated using the method of [20] as follows:

$$\% \text{ Seed damaged} = \frac{\text{Number of seeds damaged} \times 100}{\text{Total number of seeds} \times 1}$$

Weevil Perforation Index (WPI) used by [10], quoted by [13] was adopted for the analysis of damage. WPI was defined as follows:

$$\text{WPI} = \frac{\% \text{ treated maize seeds perforated}}{\% \text{ control maize seeds perforated}} \times \frac{100}{1}$$

WPI value exceeding 50 was regarded as an enhancement of infestation by the weevil or negative protectability of the plant material tested.

d. Repellency test

The repellency effect of the plant powder against maize weevil was assayed using the method of preferential zone on a filter paper described by [17] with some minor modifications. A petri dish was lined with a Whatman filter paper (No. 10). The paper was divided into 3 equal zones along the diameter of the petri dish using a line drawn with an HB pencil [17]. 10 unsexed adult insects were starved for 24hrs in a clean glass jar. 30.0g of sterilized maize seeds were placed at the center of the two extreme zones of the petri dish. Plant powders (0.0, 3.0, 4.0, and 5.0g) were placed at one heap of grain at one of the extreme zones in the petri dish. 10 starved adult maize weevils were placed at the center of the central zone of the divide and the number of insects moving into the two extreme zones was recorded after 10mins [17]. The experiment was conducted in triplicate for each dose of the plant powders in the CRD. The process was repeated for maize weevil using maize. Percent repellency was calculated using the formula proposed by [4];

$$\text{PR} = \frac{\text{NC} - \text{NT}}{\text{NC} + \text{NT}} \times \frac{100}{1}$$

Where: NC – number of insects in the controlled zone (no plant powder)

NT – number of insects in the treated zone (plant powders available)

PR – percent repellency. The PR was ranked in six different classes as described by

[17] as shown below:

Percent Repellency (PR) classes ranked by [17]

Class	PR proportion (%)	Description
O	PR < 0.01	Not repellent
I	0.1 < PR ≤ 20	Fair repellent
II	20.1 ≤ PR ≤ 40	Moderate repellent
III	40.1 ≤ PR ≤ 60	Good repellent
IV	60.1 ≤ PR ≤ 80	Very repellent
V	80.1 ≤ PR ≤ 100.0	Perfect repellent

Source; [17]

Percent repellency less than one was considered zero [19]. Data from repellency test was analyzed using chi square test to assess the repellency activity of the various powder doses of

Table 1: Percentage mortality of adult *S. zeamais* treated with *A. cordifolia* leaf powders at rate 2.0g/20.0g of maize seeds

Dose (g) of <i>A. cordifolia</i> leaf powder	Mean % Mortality + SE on Days after treatment			
	1	7	21	21
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
3.0	10.00 ± 2.01 ^b	22.50 ± 3.50 ^b	50.00 ± 5.79 ^b	65.00 ± 2.50 ^b
4.0	15.00 ± 2.89 ^{bc}	35.00 ± 2.89 ^{bc}	60.00 ± 4.08 ^{bc}	87.00 ± 2.50 ^{cd}
5.0	25.00 ± 2.89 ^c	50.00 ± 5.79 ^d	85.00 ± 4.89 ^d	93.00 ± 3.05 ^b

Each value is a mean ± standard error of four replicate means within column followed by the same letters (s) are not significantly different at ($P > 0.05$) using New Duncan's Multiple Range Test

3.2 Protection ability of the *A. cordifolia* leaf powder on

A. cordifolia leaf and the susceptibility of the weevils. PR₅₀ was calculated using [11] method based on the probit regression of mortality as a function of the logarithm of plant powder doses. All analysis was done using SPSS (version 16.0).

e. Seed germination

The effect of treatments and their interactions on seed germination and viability was examined after 21 days of the grain storage period. Seed germination was tested using 50 randomly picked seeds from undamaged grains after separation of damaged and undamaged grains in each jar according to the methods described in [13]. The 50 grain sub-samples were germinated on moistene filter paper (Whatman No. 1) in Petri dishes arranged in a RCBD with four replicates. The experiment was maintained under laboratory conditions. The number of germinated seedlings from each Petri dish was counted and recorded after 7 days. The percent germination was computed according to the methods of [32] as follows:

$$\text{Viability index (\%)} = \frac{\text{NG} \times 100}{\text{TG}}$$

Where NG = number of seeds that germinated, TG = total number of test seeds

2.5 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and treatment means were separated using the new Duncan's multiple Range Test. The ANOVA was performed with SPSS 16.0 software. While egg counts, damaged and undamaged seeds were subjected to square root transformation and percentages were arcsine transformed before analysis. Result means were separated using the LSD test ($p \leq 0.05$) [30].

3. Results

3.1 Contact toxicity of *A. cordifolia* leaf powder

Table 1 shows the percentage mortalities of *S. zeamais* in maize treated with the different doses of *A. cordifolia* leaf powder. All the doses gave significant mortalities against the insect. Toxicity increased with increasing concentrations of the *A. cordifolia* leaf powder. However, lower mortality was observed within one day after the exposure of weevils to botanical powders at all doses. A very lower amount of mortality resulted within 21 days when the weevils were exposed to the lowest dose (3.0g) of the botanical powders while a higher mortality was observed when the insects were exposed to the highest dose (5.0g) of the powder.

maize seeds

The protection provided by the *A. cordifolia* leaf powder is shown in Table 2. There were significant differences ($P < 0.05$) among the treatments in reducing the damage caused by the stored product pest. The 5.0g *A. cordifolia* leaf powder provided the highest protection (weight loss and seed damage)

and prevented the perforation of the maize seeds by the weevils and the 3.0g *A. cordifolia* leaf powder provided the

lowest protection of and provided the lowest perforation index.

Table 2: Protectability of *A. cordifolia* powder on maize seeds

Dose (g) of <i>A. cordifolia</i> leaf powder	Mean total number of seeds	Mean total number of damaged seeds	Mean % of seeds damaged	% weight loss	Weevil perforation index
Control	99.00	42.35 ± 4.11 ^b	43.45 ± 4.22 ^b	75.93 ± 3.45 ^b	50.00 ± 0.00 ^c
3.0	98.50	3.00 ± 0.07 ^a	3.19 ± 0.07 ^a	4.85 ± 1.01 ^a	3.40 ± 1.42 ^b
4.0	99.50	2.00 ± 0.03 ^a	2.20 ± 0.05 ^a	3.03 ± 0.07 ^a	5.05 ± 1.31 ^b
5.0	98.50	0.34 ± 0.01 ^a	0.58 ± 0.01 ^a	0.67 ± 0.07 ^a	0.01 ± 0.01 ^a

Each value is a mean ± standard error of four replicate means within column followed by the same letters (s) are not significantly different at ($P>0.05$) using New Duncan's Multiple Range Test

3.3 Fecundity of *S. zeamais* treated with *A. cordifolia* leaf powder on maize seeds

Table 3 presented the oviposition and % progeny development of *S. zeamais* after being exposed to various doses of plant

powders as contact insecticide. Progeny development was significantly suppressed by various plant powders with the 5.0g dose almost completely inhibiting the emergence of *S. zeamais*.

Table 3: Fecundity of *S. zeamais* treated with *A. cordifolia* powder on maize seeds

Dose (g) of <i>A. cordifolia</i> leaf powder	Oviposition	% number of progeny development
Control	50.00 ± 5.69 ^c	84.60 ± 7.53 ^c
3.0	15.00 ± 0.84 ^b	19.20 ± 2.04 ^b
4.0	8.63 ± 0.84 ^{ab}	13.80 ± 1.22 ^b
5.0	3.50 ± 1.90 ^a	0.02 ± 0.11 ^a

Each value is a mean ± standard error of four replicate means within column followed by the same letters (s) are not significantly different at ($P>0.05$) using New Duncan's Multiple Range Test

3.4 Effects of *A. cordifolia* leaf powder on viability of stored maize seeds

The percentage of maize seeds that germinated after treatment with powder of *A. cordifolia* leaf powder is presented in Table 4. At the end of seven-day germination period, all the treated

seeds recorded high germinability. The untreated maize seeds and seeds treated with *A. cordifolia* leaf powders and control had the highest percentage germination of 100%. The least percentage germination was recorded in 3g *A. cordifolia* leaf powder which had 97% viability.

Table 4: Effects of *A. cordifolia* leaf powder on viability of stored maize seeds

Dose (g) of <i>A. cordifolia</i> leaf powder	% Viability
Control	100.00 ± 0.00 ^a
3.0	97.00 ± 2.04 ^a
4.0	100.00 ± 0.00 ^a
5.0	100.00 ± 0.00 ^a

Each value is a mean ± standard error of four replicate means within column followed by the same letters (s) are not significantly different at ($P>0.05$) using New Duncan's Multiple Range Test

3.5 Repellent action of *A. cordifolia* leaf powder to *S. zeamais*.

The various doses of the *A. cordifolia* leaf powder showed different levels of repellence to *S. zeamais*. The 5g dose

recorded the highest repellence of 90% while the 3g dose recorded the lowest repellence of 60% as can be found in table 5.

Table 5: Repellency caused by *A. cordifolia* leaf powder against *S. zeamais* after 10mins in petri test of preferential zone

Dose (g) of <i>A. cordifolia</i> leaf powder	Mean (± SE) number of insects in controlled zone	Mean (± SE) number of insects in treated zone	% Repelled
Control	6.30 ± 1.50 ^c	6.30 ± 1.50 ^c	0
3.0	5.70 ± 0.58 ^b	3.30 ± 0.58 ^b	60
4.0	6.70 ± 0.58 ^{ab}	2.30 ± 0.58 ^{ab}	77
5.0	9.00 ± 1.00 ^a	0.70 ± 0.57 ^a	90

Each value is a mean ± standard error of four replicate means within column followed by the same letters (s) are not significantly different at ($P>0.05$) using New Duncan's Multiple Range Test

4. Discussion

The use of plant materials as a protectant against stored product pests is a common practice mostly in many developing countries of the world. This practice has been suggested as one of great hope for controlling stored product pests [2] due to several limitations associated with the use of synthetic insecticides and fumigants. It was observed in this research that the powders of *A. cordifolia* leaves caused adult mortality and reduced adult emergence and progeny

development of the weevils. Weight loss of maize grains treated with this plant material was also reduced [23]. The high mortality effect of powders could be due to the inability of the insects to feed on the maize grains that have been coated with powders thereby leading to their starvation [28]. This suggests that the plant has antifeedant properties [29]. It was further observed that weevils killed in the *A. cordifolia* leaf powder treated grains had unfolded metathoracic wings and outstretched elytra. This according to [20] and [14], suggest that

toxicity was not only due to only ingestion of treated grains but also to inhalation of toxicants. This implies that the plant powder may have disrupted the normal respiratory activities of these insects leading to the asphyxiation and subsequent death^[28].

The powders also significantly reduced or prevented the adult emergence of the weevils when compared to the control. This could be linked to the inability of the weevil eggs to develop adult due to the death of their larvae, which cannot cast off their old exoskeleton which typically remain linked to the posterior part of the abdomen^[24, 6]. Furthermore, the powders significantly reduced or prevented the weight loss of treated maize grains. This reduction in weight loss may be due to the inability of the larvae of the weevils to feed on the treated maize grains. Similar observation has been reported by^[20, 23, 5, 16] on maize seeds treated with plant materials.

In the present research, it is clear that using *A. cordifolia* leaf powders did not induced any change in seed viability, and this will have no impact on the local market value of treated grains. The observed repellent activity of *A. cordifolia* leaf powders to *S. zeamais* could partly be attributed to the presence of volatile constituents such as terpenoids in leaves of *A. cordifolia* leaf^[3] which are well-known repellents of phytophagous insects by acting in the vapor form on the olfactory receptors^[18].

5. Conclusion

The findings of this research revealed that, the powder of *A. cordifolia* leaf could go a long way in the quest of providing alternative wherewithal to the use of chemical insecticides for protecting maize grain in storage. Further research is required to investigate the insecticidal potential of the bark and root powders as well as the extracts of the various parts of the plant so as to integrate it into integrated pest management strategies in developing countries because they have a broad spectrum action, they are locally available, potentially less expensive to the traditional farmer and relatively less harmful to human health and the environment.

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