



Entomocidal properties of Christmas bush, *Alchornea cordifolia* (Schum. & Thonn.) bark powder against the maize weevil, *Sitophilus zeamais* motschulsky (Coleoptera: Curculionidae) in storage

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Abstract

The maize weevil (*Sitophilus zeamais*) is a very destructive pest of maize. Synthetic chemicals have mainly been used extensively to control this pest and this has led to serious health and environmental problems. The present study investigated the entomocidal properties of bark powders of *Alchornea cordifolia* against *Sitophilus zeamais* on stored maize in the Integrated Science Education Department laboratory of the University of Education, Winneba, Central Region, Ghana, at a temperature of $30\pm 2^{\circ}\text{C}$ and $75\pm 5\%$ relative humidity. Bark powders were added as admixtures to 20.0 g of grains at the following rates of 1g, 2g, and 3g to assess contact toxicity, damage assessment, progeny production, repellency and seed germination ability. Results indicated that the plant material was toxic to the insect ($P < 0.05$). The bark powder of *A. cordifolia* applied at 3g greatly induced the highest mortality of 95% after 21 days, repelled almost 92% of the weevils, significantly inhibited adult emergence and seed damaged by the weevils up to about 97% and 98.6% respectively compared to other concentrations ($P < 0.05$). The bark powder also had no effect on germination. The present study revealed that *A. cordifolia* bark powder can be used as good alternatives to chemical insecticides against *S. zeamais* and its incorporation into traditional storage pest management and integrated pest management is strongly recommended in developing countries.

Keywords: protectability, *Sitophilus zeamais*, entomocidal, *Alchornea cordifolia*

1. Introduction

In many developing countries, security of food has been threatened due to infestation of their farm produce by many stored product insect pests starting from the field to storage where it is more pronounced [1]. Loss of stored grains which amounts to 5-10% in the temperate countries and 20-30% in the tropical zones has been associated with the infestation by many stored product insect pests [2, 3]. Maize (*Zea mays*) one of the major food staples of the world, belongs to the family Gramineae and is one of the most important cereal crops grown widely throughout the world in diverse agro ecological environments [4]. Maize is however, exposed to numerous insect pests and the most predominant ones are *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) and *Sitophilus zeamais* Mosh (Coleoptera: Curculionidae) [4]. Both the larvae and adults of the two insects feed on the grain causing loss in viability, quality and value, weight and nutrition [5]. This situation results in an increase in food insecurity and poverty level of farmers especially in developing countries like Ghana. *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is the most widespread and destructive major insect pest of stored maize throughout the world [5]. It is an internal feeder causing considerable loss to cereals by affecting the quantity as well as quality of the grains [6]. 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 [7].

Currently, synthetic insecticides are widely used indiscriminately in most developing countries like Ghana to control insect pests of stored products leading to food poisoning, destruction of natural enemies and non-target species, pest resistance, turning innocuous species into pests and contamination of food, environmental pollution through air and as a residue in food which leads to health risk of man and animal [8]. Thus, most researchers now focus on the use of non-chemical technologies to store grains in order to reduce the use of synthetic insecticides. *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 [9, 10], found out the leaves of the plant was effective in controlling the stored products insect pests through suppressing oviposition and progeny development, contact toxicity and repellency activities. There is therefore the pressing need to find out whether other parts of the plant such as the bark also has any insecticidal properties in addition to its numerous medicinal properties. This paper

reports the results of laboratory investigations on the entomocidal properties of *Alchornea cordifolia* bark powder 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 $30\pm 2^\circ\text{C}$ and relative humidity was $70\pm 5\%$. The study was carried out from October to November 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 $30\pm 2^\circ\text{C}$, and relative humidity of $70\pm 5\%$) The moisture content of maize grain was adjusted to 12 to 13% [11]. After three weeks of oviposition, the parent weevils were sieved out after oviposition. Later the grain were kept in the laboratory for adult emergence while the emerging generation of same age insects re-cultured at temperature of $30\pm 2^\circ\text{C}$, and relative humidity of $70\pm 5\%$. The F1 generation was used for the experiment.

2.2 Collection and preparation of plant materials

Barks of *Alchornea cordifolia* plant were collected from the Gomoa Otapirow area of the Central Region of Ghana early in the morning. They were rinsed in clean water to remove sand and other impurities, air dried at room temperature in the laboratory for 15 days, after which, ground into very fine powder using an electric blender. The powders were further sieved to pass through 1mm^2 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

S. zeamais were collected from infested stock of maize grains from the Mandela market at Agona Swedru in the Central Region of Ghana and reared on whole maize grains in the laboratory at $30\pm 2^\circ\text{C}$, 70% RH. Insects were sieved using a 2 mm impact test sieve into a bowl covered with a nylon mesh and left under the sun for 3 hours. By this process, insects infested with mites died while the surviving ones were collected and washed in 1% sodium hypochloride solution [12]. Insects were dried by placing them on filter paper before transferring them into a glass jar containing 500 g of grains, which had previously been sterilized in an oven at 40°C for 6 hours. 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* bark powders on adult mortality, oviposition and progeny development of *Sitophilus zeamais*

a) Contact toxicity of *Alchornea cordifolia* bark powder

Twenty pairs of *S. zeamais* were introduced into the a clean sterilized 250ml plastic containers containing 20g of uninfested sterilized maize seeds at 0, 1, 2, and 3g% (w/w) of *Alchornea cordifolia* bark powder, while in the control treatment there was no plant material added. The *Alchornea cordifolia* bark 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 experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. 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 *Alchornea cordifolia* bark powder. Adults were considered dead when probed with sharp objects and there were no responses [13]. Percentage adult mortality was corrected using [14] 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

Grains treated with the bark powders were kept inside the laboratory for further 30 days to assess for the emergence of the first filial generation (F1). The containers were sieved out and newly emerged adult *S. zeamais* were counted and recorded for one week. The percentage adult emergence was calculated using the method of [15].

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

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 [16] as follows:

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

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 [27] as follows:

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

Weevil Perforation Index (WPI) used by [17], quoted by [18] 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 enhancement of infestation by the weevil or negative protectability of the plant material tested.

d) Repellency test

The repellency effect of the plant bark powder against maize weevil was assayed using the method of preferential zone on a filter paper described by [19] 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. 10 unsexed adult insects were starved for 24hrs in a clean glass jar. 30g of sterilized maize seeds were placed at the center of the two extreme zones of the petri dish. Plant powders (0, 1, 2, and 3g) 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. The experiment was conducted in triplicate for each dose of the plant powders in CRD. The process was repeated for maize weevil using maize. Percent repellency was calculated using the formula proposed by [20];

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

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 [19] as shown below:

Table 1: Percent Repellency (PR) classes ranked by [19].

Class	PR proportion (%)	Description
0	PR < 0.01	Not repellent
1	0.1 < PR ≤ 20	Fair repellent
2	20.1 ≤ PR ≤ 40	Moderate repellent
3	40.1 ≤ PR ≤ 60	Good repellent
4	60.1 ≤ PR ≤ 80	Very repellent
5	80.1 ≤ PR ≤ 100.0	Perfect repellent

Source: [19]

Table 2: Percentage mortality of adult *S. zeamais* treated with *A. cordifolia* bark powders

Dose (g) of <i>A cordifolia</i> bark powder	Mean % Mortality + SE on Days after treatment			
	1	7	14	21
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
1.0	12.00 ± 1.01 ^b	32.10 ± 3.80 ^b	58.00 ± 4.63 ^b	72.30 ± 3.41 ^b
2.0	18.30 ± 3.49 ^{bc}	43.40 ± 1.89 ^{bc}	69.80 ± 4.08 ^{bc}	87.00 ± 2.68 ^{cd}
3.0	32.20 ± 2.79	60.00 ± 5.45 ^d	89.00 ± 3.81 ^d	95.30 ± 3.14 ^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

Percent repellency less than one was considered zero [21]. Data from repellency test was analyzed using chi square test to assess the repellency activity of the various powder doses of *Alchornea cordifolia* bark and the susceptibility of the weevils. PR₅₀ was calculated using [22] 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 maize treated with the bark powders and their interactions on seed germination and viability was examined after 21 days of 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 [23]. The 50 grain sub-samples were germinated on moistened 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 [24] 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

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 (SPSS, 2007). 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 ≤ 0.05) [24].

3. Results

3.1 Contact toxicity of *A. cordifolia* bark powder

A. cordifolia bark powders tested at 5% level showed various bioactivities against *S. zeamais* (Table 2). There was a significant difference (p < 0.05) in mortality of the insect amongst the treatments; with the 3g *A. cordifolia* bark powder giving the highest mortality after 21 days. Toxicity increased with increasing concentrations of the *A. cordifolia* bark powder. However, lower mortality was observed within one day after the exposure of weevils to botanical powders at all doses.

3.2 Protection ability of the *A. cordifolia* bark powder on maize seeds

Grains treated with *A. cordifolia* bark powders showed significant difference ($p < 0.05$) in the reduction of damage caused by *S. zeamais* and (Table 3). The 3g *A. cordifolia* bark

powder provided the highest protection (weight loss and seed damage) and prevented the perforation of the maize seeds by the weevils and the 1g *A. cordifolia* bark powder provided the lowest protection of and provided the lowest perforation index.

Table 3: Protectability of *A. cordifolia* bark powder on maize seeds

Dose (g) of <i>A. cordifolia</i> bark powder	Mean total number of seeds	Mean total number of damaged seeds	Mean % of seeds damaged	% weight loss	Weevil perforation index
Control	98.50	43.30 ± 4.13 ^b	48.31 ± 3.42 ^b	78.43 ± 3.36 ^b	54.00 ± 0.20 ^c
1.0	99.50	1.81 ± 0.06 ^a	2.03 ± 0.07 ^a	3.74 ± 1.21 ^a	2.10 ± 1.35 ^b
2.0	99.00	1.05 ± 0.04 ^a	1.16 ± 0.06 ^a	2.02 ± 0.06 ^a	3.01 ± 1.20 ^b
3.0	98.50	0.22 ± 0.06 ^a	0.49 ± 0.01 ^a	0.46 ± 0.06 ^a	0.12 ± 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

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

Table 4 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 3g dose almost completely inhibiting the emergence of *S. zeamais*.

Table 4: Fecundity of *S. zeamais* treated with *A. cordifolia* bark on maize seeds

Dose (g) of <i>A. cordifolia</i> bark powder	Oviposition	% number of progeny development
Control	49.23 ± 4.58 ^c	81.10 ± 6.23 ^c
1.0	14.12 ± 0.34 ^b	18.12 ± 2.10 ^b
2.0	7.52 ± 0.67 ^{ab}	11.28 ± 1.09 ^b
3.0	2.12 ± 1.80 ^a	0.04 ± 0.21 ^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* bark powder on viability of stored maize seeds

The percentage of maize seeds that germinated after treatment with powder of *A. cordifolia* bark powder is presented in Table 5. 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* bark powders at 2.0g and 3.0g concentrations had the highest percentage germination of 100%. The least percentage germination was recorded in 1g *A. cordifolia* bark powder which had 99% viability.

Table 5: Effects of *A. cordifolia* bark powder on viability of stored maize seeds

Dose (g) of <i>A. cordifolia</i> bark powder	% Viability
Control	100.00 ± 0.00 ^a
1.0	99.00 ± 1.02 ^a
2.0	100.00 ± 0.00 ^a
3.0	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* bark powder to *S. zeamais*.

Table 6 show the mean (±SD) of maize weevil repelled by different doses of *A. cordifolia* bark powder after 10 minutes of exposure. The chi-square test was conducted on counts. The result reveals that the highest concentration (3g) of *A. cordifolia* bark powder had a strong repellent effect of 92% for the maize weevil described according to [19] as perfectly repellent while the 1g dose recorded the lowest repellence of 67%.

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

Dose (g) of <i>A. cordifolia</i> bark powder	Mean (± SE) number of insects in controlled zone	Mean (± SE) number of insects in treated zone	% Repelled
Control	7.20 ± 1.50 ^c	7.30 ± 1.50 ^c	0
1.0	6.56 ± 0.69 ^b	4.26 ± 0.42 ^b	57
2.0	6.60 ± 1.48 ^{ab}	2.21 ± 0.41 ^{ab}	82
3.0	9.12 ± 1.03 ^a	0.81 ± 0.51 ^a	92

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

Safe storage of grains and food products against insect damage is a serious concern [25]. Around the world, residual chemical insecticides are currently the method of choice for the control of stored-product insects [26], but extensive use has resulted in the conversion of innocuous species to pests and the evolution of resistant forms [27] in addition to environmental contamination and health hazards. In order to protect human health and the environment, scientists have resorted to the use of plants to protect grains under storage. Several powders and extracts of different plants have been shown to possess insecticidal activity against stored product pests [28, 29, 30, 31, 10].

In the current study, mortalities were recorded when the maize was treated with the plant material. This suggests the promising potential of the plant material for controlling *S. zeamais*. From the mortalities observed, acute toxicity was not prominent as compared to chronic toxicities because mortalities progressively increased with days after treatment.

The increase in mortality post-treatment period was also due to the fact that the insects had no escape routes and were confined in the treatment to pick up the lethal dosage as the time of exposure increased ^[32].

The powders of *A. cordifolia* bark also significantly suppressed F1 progeny development. This could be due to reduced egg-laying and increased mortalities of egg and perhaps the females dying before they could lay eggs ^[33]. This confirms earlier works done by ^[10], who reported that *A. cordifolia* bark powder decreased fecundity of *S. zeamais*. The decrease in fecundity may also be due to the inhibition of free insect locomotion by the plant powder thereby inhibiting mating. The effect of the plant bark powder on oviposition could also be due to respiratory impairment which probably affects the process of metabolism of the insects ^[34].

The results further revealed a very efficacious repellency potential of *A. cordifolia* bark powder against the maize weevil with all doses repelling at least 60% of the insects within 10mins of exposure. This support strongly that *A. cordifolia* bark powder have a repellency effect on this insect pest. Though the mode of action and the constituent active ingredient elucidating this repellency behaviour was not revealed in this study, the repellency behavior of the insect pest may be due to suffocation and inhibition of different biosynthetic process of the insect's metabolism ^[35].

All the treated seeds and the control recorded high germinability. The ability of the plant bark powder to increase seed germination could be attributed to the suppression of seed borne fungi that could have killed the embryo of the seeds leading to germination failure ^[36]. According to ^[36], parts of some higher plants exert antifungal activity against fungi. The antifungal principle of *A. cordifolia* bark powder tested in this study is unknown, but could be associated to the phenolic compounds known for their antifungal activities ^[37]. Powders of *A. cordifolia* bark significantly reduced or prevented the weight loss of treated maize grains. At the highest experimental dosage and concentration, the plant material completely prevented weight loss in infested 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, suggesting that the plant has antifeedant properties ^[29]. Similar observation was reported by ^[10] on maize seeds treated with *A. cordifolia* leaves powder.

5. Conclusion

The present study has shown that *Alchornea cordifolia* bark powder has entomocidal properties against *S. zeamais* and thus could go a long way in the quest of providing alternatives to the use of chemical insecticides for protecting maize grain in storage. Further research is required to investigate the insecticidal potential of the root powder 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 are locally available, potentially less expensive to the traditional farmer and relatively less harmful to human health and the environment.

6. References

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