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Evaluation of Toxic Properties of Cotyledon Powders and Oils of Sandbox, *Hura crepitans L.* against *Sitophilus zeamais* Motschulsky (coleoptera: curculionidae)

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Abstract:

Hura crepitans cotyledons were extracted in the laboratory with n-hexane. The powder and oil extracts were assessed at the temperature of 28±2°C and relative humidity of 75±5 % for their insecticidal activity against *Sitophilus zeamais* infesting maize grains, using mortality, ovicidal, and adult emergence effect as indices. The long term protectant ability of the powder and the oil was also investigated. Results obtained showed that powder and oil of *H. crepitans* were toxic on *S. zeamais*. The powder showed 100 % mortality with dosage of 0.8 g by day 8 of exposure, while the oil showed 100 % mortality with concentration of 1.5 and 2.0 % by day 4 and 8 of exposure. The powder and oil extract drastically reduced oviposition and totally prevented adult emergence at higher powder dosages and oil concentrations. Both the powder and oil drastically reduced infestation and subsequent damage in treated grains. No damage was recorded in maize grains treated with 1.5 and 2.0 % powder and 1.0, 1.5 and 2.0 % oil of *H. crepitans*. The results obtained from this study showed that cotyledons powder and extract from *H. crepitans* are effective in controlling *S. zeamais* infesting maize grains in the store and could serve as alternative to chemical insecticides.

Keywords: *Hura crepitans*, *Sitophilus zeamais,* ovicidal, oviposition, adult emergence

INTRODUCTION

Food insecurity has been a global problem facing agricultural development. Despite the efforts been made to boost food production in many countries, over 800 million people still suffer from malnutrition (FAO, 1989). The governments of many countries focus more attention on national security instead of food security, forgetting the fact that there can be no national security without food security (Adamu, 2015). Food insecurity begets hunger and a hungry man is an angry man. An angry man as a result of hunger can perpetrate evils, such as armed robbery, kidnapping, thuggery, terrorism to mention but a few, in order to make ends meet. All these vices constitute hazards to national security. According to Adamu (2015), the current security challenges facing Nigeria is as a result of hunger and this has constituted a big risk to the development of the country. Apart from inadequate food production, the problem of hunger and malnutrition is further made more severe by inability of developing countries to store harvested crops for a long period without losing a sizeable proportion of them to pests and diseases (Adedire, 2001).

Maize, *Zea mays* L, belongs to the family Poaceae. Maize plant is regarded as versatile and with many uses since it can thrive in diverse climate; as a result, it is grown in many countries than any other crops (Maribet *et al*., 2008). It is an important cereal crop in Nigeria where it serves as a major component of their diet. Fresh ripe maize grain is boiled and eaten as food, and when dried can be used for brewing and for distillation of alcoholic drinks (Adedire *et al*., 2011). It is also processed into various foods and industrial products including starches, sweeteners, oil, beverages and fuel ethanol. Varieties of foods and other important items such as toothpaste, cosmetics, shoe polish, ceramics, explosives, adhensive, construction materials and textile containing maize components (Garcia, 1990).

Sitophilus zeamais is a serious primary and major pest of maize in Nigeria and other parts of the world, causing most of the losses incurred in maize annually (Nwana, 1993). Both the larval and adult stages of this insect attack maize grains causing severe damage to the commodity resulting in losses in weight, seed viability, and nutritive quality of the food stuff (Maribet *et al*., 2008).

Effective control of weevils is achieved mainly by the use of chemical insecticides (Jackai and Daoast, 1986). The problems of these chemical insecticides include environmental pollution and residue in food, destruction of non-target organisms, ozone layer depletion, handling, resurgent of pest, cost of the insecticides and inability of illiterate farmers to read instructions written on the labels placed on the containers of the chemical insecticide. These adverse effects have led researchers to look for substitutes to the use of these dangerous chemical insecticides (Obembe and Kayode, 2013). Currently, research efforts are being focused on the use of botanicals insecticides, such as plant powders, oils and other extracts which are relatively cheaper, biodegradable and ecologically friendly than the chemical insecticides. This present research focuses on insecticidal potential of *Hura crepitan* seed powder and seed oil extracts against *Sitophilus zeamais* infesting maize grains in the store.

MATERIALS AND METHODS

The study was conducted in Plant Science Department Laboratory, Ekiti State University, Ado Ekiti, Nigeria, under ambient temperature and relative humidity.

Insect culture

The initial culture of *Sitophilus zeamais* used for the experiment was obtained from Oba market, Ado Ekiti, Ekiti State, Nigeria, along with infested maize grains. The insects were cultured at the temperature 28±2^{o}C and 75± 5% relative humidity inside plastic container covered with muslin cloth to disallow the escape of the insect as well prevent the entry of intruding insects that may act as parasitoid. The culture was maintained by replacing the devoured seeds with new un-infested seeds.

Collection and preparation of plant materials

Dry pods of *Hura crepitans* were collected from the campus of Ekiti State University, Ado-Ekiti, Nigeria. The pods were split opened in order to collect the seeds. The seeds were de-shelled to recover the cotyledons. The cotyledons were initially exposed to mild sunlight and later air-dried in the laboratory for three weeks, so as to reduce the moisture content in order to prevent moldiness. The air-dried cotyledons were milled into fine powder using an electric Binatone blender (Model BLG 400). The powder was sieved and passed through 1 mm^2 mesh and stored in black cellophane bag until needed.

Preparation of plant oil

The cotyledon powder was measured separately into beakers and packed into thimbles and extracted with 250 ml of n-hexane in a Soxhlet apparatus at 60^{o}C. The oil was concentrated by removing the solvent using rotary evaporator. The resulting extract was further concentrated by air-drying to remove traces of the solvent. The oil was poured into a bottle and stored in a refrigerator until needed.

Effect of *Hura crepitans* cotyledon powder on mortality of *Sitophilus zeamais*

Twenty grams of clean and dis-infested maize grain was weighed into Petri dish (9 cm diameter) and *Hura crepitan* powders weighing 0.2, 0.4, 0.6 and 0.8 g were added to the maize grains in each of the Petri dishes. The powder and the maize grains were thoroughly mixed together to enhance uniform spreading of the extracts on the maize grains. Untreated maize grain was also set up which serves as the control experiment. Ten pairs of adult *S. zeamais* were introduced into each of the Petri dish and covered with the Petri plate. Each of the treated and the untreated control was replicated four times and laid in Complete Randomized Design (CRD). Beetle mortality was observed at 2 days interval for a period of 8 days. After every 2 days, the number of dead beetle were counted and recorded. The beetles were confirmed dead when there was no response to probing on the abdomen with a sharp pin

Effect of *Hura crepitans* cotyledon oil on mortality of *S. zeamais*

Twenty grams of clean and dis-infested maize grains were weighed into 9 cm diameter Petri dish and treated with 0.5, 1.0, 1.5 and 2.0 % v/w of the oil. Solvent treated and untreated maize grains serve as the control experiment. The maize grains and the oil were thoroughly mixed with glass rod to enhance uniform coating of the grains on the extract. Thereafter, the treated grains were air-dried for a period of 1 h, after which 20 adult *S. zeamais* (0-7 days old) were introduced into the Petri dish containing the treated and the control experiment. Four replicates were prepared for each treatment and the controls and laid in a Complete Randomized Design. The numbers of dead beetles were counted and recorded at 2 days interval for a period of 8 days. The beetles were confirmed dead when there was no response to probing on the abdomen with a sharp pin.

Toxicity of *Hura crepitans* cotyledon powders on oviposition and adult emergence of *S. zeamais*

Twenty grams of pristine and dis-infected maize grains were measured into each of 9 cm diameter Petri dishes and *Hura crepitan* powders weighing 0.2, 0.4, 0.6 and 0.8 g were added to the maize grains in each of the Petri dishes. The powder and the maize grains were thoroughly mixed together to enhance uniform spreading of the extracts on the maize grains. Untreated maize grain was also set up which serves as the control experiment. Two pairs of newly emerged copulating adult *S. zeamais* were introduced into each of the Petri dish and covered with the Petri plate. Each of the treated and the untreated control was replicated four times and laid in Complete Randomized Design (CRD). The set up was left in a wooden cage in the laboratory for 12 days after which the insects were removed and the numbers of egg plugs were identified and counted after staining with acid fuchsin dye solution (Frankenfeld, 1948). Thereafter, the experimental set-up was kept undisturbed in the wooden cage till the emergence of adults. The number of adults that emerged were counted and recorded

Toxicity of *Hura crepitans* cotyledon oil on oviposition and adult emergence

Twenty grams of clean and un-infested wholesome maize grains were weighed into 9 cm diameter Petri dish and treated with 0.5, 1.0, 1.5 and 2.0 % v/w of the oil. Solvent treated and untreated maize grains serve as the control experiment. The maize grains and the oil were thoroughly mixed with glass rod to facilitate uniform coating of the oil on the seeds as earlier discussed above. Thereafter, the treated grains were air-dried for a period of 1 h, after which 2 pairs of newly hatched copulating adult *S. zeamais* (0-7 days old) were introduced into the Petri dish containing the treated and the control experiment. Four replicates were prepared for each treatment and the controls and laid in a Complete Randomized Design. The experimental set-up was left in a wooden cage in the laboratory for 12 days after which the insects were removed and the numbers of egg plugs were identified and counted after staining with acid fuchsin dye solution (Frankenfeld, 1948) as discussed above. Thereafter, the experimental set-up was kept undisturbed in a wooden cage till the emergence of adults. The number of adults that emerged were counted and recorded.

Effect of *Hura crepitans* cotyledon powders on grain damage

Cotyledon powder of *Hura crepitans* at the rate of 0.2, 0.4, 0.6 and 0.8 g was added to 50 g clean and dis-infested maize grains in a transparent plastic container. The container was thoroughly shaken to enhance the spread of the powders on the maize grains. Thereafter, 10 pairs of adult beetles were introduced into the container. A control without any powder was included in the set-up. Each treatment was replicated four times. Each plastic container was covered with muslin cloth held tightly in place with rubber band to prevent the escape of insects and to facilitate adequate ventilation. The experimental set-ups were kept in a wooden cage in the laboratory for 90 days. After 90 days the seeds were assessed for seed damage and weight loss. Percentage seed damage was determined using the formula below:

$$\% \text{ damage} = \frac{\text{No. of seed damaged} \times 100}{\text{Total no. of seeds}}$$

Data analysis
All data obtained were subjected to analysis of variance and where significant differences existed, means were separated using Tukey's test.

RESULTS

Effect of *Hura crepitans* cotyledon Powder on mortality of *Sitophilus zeamais*
Adult mortality increased with increased in period of exposure. The highest mortality of 100 % was recorded in maize grain treated with 0.8 g of *Hura crepitans* powders by day 8 after treatment, and it was significantly different from 68.20 %, 78.25 % and 84.25 %, in grains treated with 0.2, 0.4, 0.6, and 0.8 g of *Hura crepitans* powders respectively. Weevil mortality in oil treated grains differ significantly ($p < 0.05$) from weevil mortality in untreated grain (Table 1)

Effect of *Hura crepitans* cotyledon oil on mortality of *Sitophilus zeamais*
Weevil mortality in oil treated maize grains differ significantly ($p < 0.05$) from weevil mortality in untreated and solvent treated grains (Table 2). All extracts concentration showed weevil mortality of 100% by days 4 and 7 of exposure. Adult mortality increased with increased in period of exposure. Weevils exposed to 1.5 and 2.0 % v/w oil concentrations showed 100 % mortality by day to day 8 period of exposure.

Toxicity of *Hura crepitans* cotyledon Powder on oviposition and adult emergence
All dosages of *Hura crepitans* powder effectively reduced oviposition (Table 3). Oviposition in powder-treated grains was significantly lower ($p < 0.05$) than that of untreated grains. The ability of the powder to reduce oviposition increased with increased in dosages of the powder. The percentage adult emergence in the untreated maize grains was significantly different from emergence in the treated seed. There was no adult emergence in grains treated with 0.4, 0.6 and 0.8 g of powders (Table 3)

Toxicity of *Hura crepitans* cotyledon oil on oviposition and adult emergence
All concentrations of *Hura crepitans* seed oils used effectively reduced oviposition (Table 4). Oviposition in oil-treated grains was significantly lower ($p < 0.05$) than that of untreated and solvent-treated grains. Oviposition was totally stopped in maize grain treated with 2.0 % v/w oil concentration. The ability of the oil to reduce oviposition increased with increased in concentration of the oil. There was emergence of adult *S. zeamais* in the untreated and solvent-treated maize grains but there was none in maize grains treated with *Hura crepitans* oil

Protectant ability of *Hura crepitans* cotyledon powder on maize grains
The cotyledon powder of *Hura crepitans* caused drastic reduction in infestation and damage of the treated maize grains compared to the untreated grains (Table 5). The untreated grains showed 85. 52 % damage as revealed by emergence holes and the egg plugs. The weight of the untreated maize grains was significantly reduced compared to the treated grains

Protectant ability of *Hura crepitans* cotyledon oil on maize grains
The cotyledon oil of *Hura crepitans* effectively prevented infestation and damage of the treated maize grains compared to the untreated grains (Table 6). There was neither seed damage nor weight loss recorded in the treated maize grains. There was 88.25 % and 85.15 % damage in the untreated and solvent-treated grains respectively as revealed by emergence holes and egg plugs.

Table 1: Percentage mortality of *S. zeamais* treated with *Hura crepitans* cotyledon powder

Dosage (g)	Percentage mortality at different days after treatment			
	2	4	6	8
0.2	23.00±2.05[d]	48.20 ±2.39[d]	62.75±1.25[c]	68.20±2.20[c]
0.4	28.20±1.20[c]	52.15±1.25[c]	70.25±3.23[bc]	78.25±1.44[b]
0.6	40.00±2.04[b]	62.75±1.25[b]	80.00±1.25[b]	84.25±0.25[b]
0.8	48.25±2.39[a]	75.33±1.20[a]	92.35±3.50[a]	100.00±0.00[a]
Untreated	0.00±0.00[e]	0.00±0.00[e]	0.00±0.00[d]	0.00±0.00[d]

Each value is a mean of ± standard error of 4 replicates. Mean within the same column followed by the same letter(s) are not significantly different at p>0.05 using Tukey's test

Table 2: Percentage morality of *S. zeamais* treated with *Hura crepitans* oil

Conc.(%v/w)	Percentage mortality at different days after treatment			
	2	4	6	8
0.5	70.24±0.22[c]	92.22±0.28[b]	62.75±1.25[b]	68.20±2.20[b]
1.0	94.24±0.28[b]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
1.5	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
2.0	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
Untreated	0.00±0.00[d]	0.00±0.00[c]	0.00±0.00d	0.00+0.00[d]
n-hexane	0.00±0.00[d]	0.00±0.00[c]	0.25±1.30[c]	3.30±0.45[c]

Each value is a mean of ± standard error of 4 replicates. Mean within the same column followed by the same letter(s) are not significantly different at p>0.05 using Tukey's test

Table 3: Effect of *Hura crepitans* cotyledon Powder on oviposition and adult emergence of *S. zeamais*

Dosage (g)	Mean number of eggs laid	Percentage adult emergence
0.2	15.22±0.75[b]	16.75±0.85[b]
0.4	9.00±1.20[c]	0.00±0.00[c]
0.8	0.00±0.00[d]	0.00±0.00[c]
Untreated	22.15±1.22[a]	53.40±1.45[a]

Each value is a mean of ± standard error of 4 replicates. Mean within the same column followed by the same letter(s) are not significantly different at p>0.05 using Tukey's test

Table 4: Effect of *Hura crepitans* cotyledon oil on oviposition and adult emergence of *S. zeamais*

Conc (% v/w)	Mean number of eggs laid	Percentage adult emergence
0.5	13.25±1.30[b]	23.40±1.45[b]
1.0	10.12±0.25[b]	0.00.±0.00[d]
1.5	4.10±0.80[c]	0.00±0.00[d]
2.0	0.00±0.00[d]	0.00±0.00[d]
Untreated	20.15±1.50[a]	58.22±3.10[a]
n-hexanne	17.15±1.55[a]	38.20±2.11[c]

Each value is a mean of ± standard error of 4 replicates. Mean within the same column followed by the same letter(s) are not significantly different at p>0.05 using Tukey's test

Table 5: Effect of *Hura crepitan* cotyledon powder on long term storage of maize grain

Conc (% v/w)	Mean number of grains	Mean percentage grains damaged	Mean percentage weight loss
0.5	191.15	22.20±1.24[b]	20.40±1.45[b]
1.0	188.20	12.25±1.00[c]	8.30.±3.10[c]
1.5	190.00	0.00±0.00[d]	0.00±0.00[d]
2.0	187.75	0.00±0.00[d]	0.00±0.00[d]
Untreated	190.25	88.25±1.33[a]	52.20±0.80[a]
n-hexanne	186.75	85.15±1.12[a]	48.25±2.11[a]

Each value is a mean of ± standard error of 4 replicates. Mean within the same column followed by the same letter(s) are not significantly different at p>0.05 using Tukey's test

Table 6: Effect of *Hura crepitan* cotyledon oil on long term storage of maize grain

Conc (% v/w)	Mean number of grains	Mean percentage grains damaged	Mean percentage weight loss
0.5	186.25	15.10±2.14[b]	20.40±1.45[b]
1.0	184.10	0.00±0.00[c]	0.00.±0.00[c]
1.5	184.00	0.00±0.00[c]	0.00±0.00[c]
2.0	187.25	0.00±0.00[c]	0.00±0.00[c]
Untreated	186.75	88.25±1.33[a]	52.20±0.80[a]
n-hexanne	188.35	85.15±1.12[a]	48.25±2.11[a]

Each value is a mean of ± standard error of 4 replicates. Mean within the same column followed by the same letter(s) are not significantly different at p>0.05 using Tukey's test

DISCUSSION

The result obtained from this finding showed that *Hura crepitans* cotyledon oil and powder were effective in controlling *Sitophilus zeamais*. The most effective is the oil because it caused 100% mortality at 0.4, 0.6 and 0.8% by 96 h post-treatment. This result is in agreement with Fekadu *et al*. (2012) who discovered that both cotton and Ethiopian mustard seed oil respectively caused 100 and 95 % mortality of *S. zeamais*.

The use of seed oils and powders has been employed in the control of *S. zeamais*. Oil of groundnut has been reported to effectively control *S. zeamais* infestation (Ivbijaro, 1984). Seed powder and oil of black pepper are known to adversely affect the biology of maize weevil and also caused high mortality (Agbaje, 1986; Lale, 1992; Adedire and Ajayi, 1996)

Oviposition by female *S. zeamais* was drastically reduced in the extract-treated maize grains as against oviposition in the untreated and solvent treated grains. It was also observed that adult emergence of *S. zeamais* was completely prevented in the oil and powder treated grains after 28 days of exposure except for grains treated with 0.2 g and 0.5 % powder and oil respectively. This implies that the oil and powder are toxic to the adult weevil, their eggs and the young larva.

Plant oils are commonly used in insect control because they are relatively efficacious against virtually all life stages of insect (Adedire, 2003). The mechanism of action may be due to repellency, contact toxicity or change in the surface tension, which could cause protoplasm coagulation (Bhaduru *et al*., 1990). It may also be partially attributed to interference with normal respiration. Most insects breathe by means of trachea which usually opens to the surface of their body through the spiracle, the blockage of the spiracle by the oil and powder may result in suffocation and death of the insect (Adedire *et al*., 2005; Rhaman and Talukder 2006; Akinkurolere *et al*., 2006). Plant oil may contain insecticidal and repellent compounds, including fatty acid and other compounds which must have affected the normal functioning of the insect (Don-Pedro, 1990).

An array of plant oil has been used for protecting maize grains from damage by coleopterous pests over a long storage period with positive results. *Hura crepitans* seed oil used in this study totally prevented damage except on grains treated with 0.5 % oil where 20.40 % damage was recorded. Adedire *et al*. (2011) reported that cashew kernel oil offered full protection to maize grains from *S. zeamais* for a period of three months.

The result obtained from this study demonstrates that *Hura crepitans* cotyledon oil and powder exhibit toxic activity to maize weevil infesting maize grains and could serve as alternative to chemical insecticides for use by resource-poor farmers and even for commercial purposes.

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