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The insecticidal activities of two indigenous plants against *Sitophilus zeamais*, Motschulsky [Coleoptera: Curculionidae] in maize grains

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Abstract
The insecticidal activities of *Cleistopholis patens* and *Eugenia aromatica* (powder and ethanolic extracts) on maize weevil was carried out to determine its efficacy on mortality of *Sitophilus zeamais*, percentage reduction of the maize weevil, percentage seed damage and Weevil Perforation Index (WPI) as an index of insecticidal activity. Five different levels of concentration were used (0.5g, 1.0g. 1.5g. 2.0g and 2.5g w/w of powder), (0.5ml, 1.0ml,1.5ml, 2.0ml and 2.5ml w/v of ethanolic extracts) and (0.1g, 0.2g, 0.3g, 0.4g and 0.5g w/w of powder) for the percentage reduction, seed damage and WPI. The plant powders and extracts showed significant ($p<0.05$) level of toxicity to the maize weevil at different exposure periods and concentration. At all levels of concentration using the plant powder no mortality was obtained at 24h exposure period with *C. patens*. The highest mortality of 46.67% was obtained at 96h with 2.5g concentration of *C. patens* powder. *Eugenia aromatica* powder was able to evoke 66.67% adult mortality at 96h post-treatment periods. Ethanolic extracts of *C. patens* caused lowest mortality (6.67%) at concentration of 1.0ml at 24h exposure period. The highest mortality (86.66%) caused by *C. patens* ethanolic extract was observed at concentration of 2.5ml at 96h exposure period. Ethanolic extracts of *E. aromatica* caused lowest mortality (13.33%) at concentration of 0.5ml at 48h exposure periods and achieved total mortality (100%) at concentration of 2.5ml at 96h exposure period. Seed damage brought about by feeding activities of the weevil reduced as the concentrations of powders increased. Percentage damage of maize grains reduced as concentration increased using both powders. It is observed that the ethanolic extracts of both plants are much more effective in insect pest control than powders.

Keywords: *Sitophilus zeamais*, *Cleistopholis patens*, *Eugenia aromatica*, percentage reduction, percentage damage, weevil perforation index

Introduction
The maize weevil, *Sitophilus zeamais*, is from a family Curculionidae. Female insect deposits white egg in a hole made through the grain. It cover hole with a plug, a waxy secretion. *S. zeamais* lay one egg per grain and it can lay 300-400 eggs during life time. The complete development can last 36 days and adult lives up to 8 months. This species can attacks both standing plants, stored cereal products and process cereals. *Sitophilus zeamais* Motschulsky, is one of the major pests of maize in storage. Maize production in Nigeria has increased over the years, it serves as food for human consumption, feeds for animal and livestock, raw material for industries and for foreign exchange. According to Throne [1], maize weevil cause a great economic loss to maize in storage, especially in tropical and subtropical regions where storage is been done without chemical protectants. Infestation starts occurring from the field and if proper storage management is not done prior to maize grain storage, insect population builds up rapidly [2].
Synthetic insecticides have remained most effective method in protecting stored grains against pests' infestation [3]. It has an effect on environment and human health, especially when not use according to the specification and this has been a major concern to its usage. The present focus in research is to find alternative methods that are harmless to the environment and also effective in controlling insect infestation, and one of such methods involve the use of insecticides derived from plant origin. It is of no doubt that man is enriching with plants which possesses bio pesticide and medicinal properties, especially in Africa. Many researchers have reported botanicals with insecticidal properties [3, 4] and there is need to constantly search for more and better botanicals with better potential in the control of *S. zeamais* in maize. This will help to reduce the economics loss on stored grains due to insect infestation and minimize

Havoc cause by synthetic insecticide. This project is sought to evaluate the insecticidal activities of *Eugenia aromatica* and *Cleistopholis patens* as bio pesticides in the management of *S. zeamais* in maize grain.

Materials and Methods

Insect Culture

Sitophilus zeamais was obtained from stocks maintained at the Biology Postgraduate Research Laboratory, Federal University of Technology, Akure, Nigeria. Adult *S. zeamais* was bred in the laboratory at 28 ±2 °C, 70% relative humidity (RH). The feed medium used was whole maize grains, purchased from the Oja-Oba market, Akure, Ondo State, Nigeria. Maize grains used for this study were refrigerated for 72 hrs at -4 °C in a deep freezer to kill all live forms of the insect if present. After 72 hrs, the seeds were air dried in the ventilated laboratory and later kept in a polythene bag. Fifty pairs of *S. zeamais* were introduced into one litre glass jars containing 400 g of weevil-susceptible maize grains. The jars were covered with nylon mesh held in place with rubber bands and kept in a wire mesh cage of dimension (60 x 50 x 60) cm. Freshly emerged adults of *S. zeamais* were raised from the culture.

Collection and Preparation of Plant Powders

The stem and bark of *C. patens* and cloves of *E. aromatica* respectively were collected in large quantities from Ota-Sun market, Ondo East Local Government and Oba market, Ondo town, washed and air dried thoroughly. The plant parts were ground to fine powder and pulverized powder were then sieved through 0.2mm² wire mesh and the fine powder was stored in plastic containers with screw cap lids.

Preparation of Extract

The plant powders each weighing 20g were soaked in 100% ethanol for 72 hrs. The mixture was thoroughly stirred for 30 minutes at 12 hrs interval and was filtered with muslin cloth. Solvent was allowed to evaporate naturally by exposing the filtrate in the laboratory. The solid extract obtained was kept in a dark bottle in a freezer prior to use.

Contact Toxicity of Plant Powders to Adult of *S. zeamais*

Twenty grams of maize grain was weighed in plastic containers of equal volume and then treatment was administered at different levels (0.5g, 1.0g, 1.5g, 2.0g and 2.5g). The powder and grains were thoroughly mixed together using a glass rod. The cover was perforated and lined with muslin cloth on the inside to allow aeration and avoid escape. The untreated were set up to be the control of the experiment. Each dosage and control was replicated three times. Twenty *S. zeamais* (≤ 24 hr old) adults were introduced into the plastic containers containing the treated and untreated maize grains. Plastics were covered with the lid, the experiment was arranged in completely randomized design in the laboratory. Mortality was recorded at 24hrs, 48 hrs, 72 hrs and 96 hrs post treatment. Insects were considered dead on failure to respond to probing of the abdomen with pin head.

Effect of Ethanol Extract of Powders on Adult Mortality of *S. zeamais*

Twenty grams of maize grain was weighed into plastic containers of equal volume and then treatment was added to it at different concentrations of 0.5%, 1.0%, 1.5%, 2.0% and 2.5%. The ethanol and untreated were set up to be the control of the experiment. Each dosage and control was replicated three times. The treated seeds inside the plastic container were mixed with the aid of a glass rod to ensure uniform coating of the seeds with the extract. Treated grains were kept open for 1 hour to allow ethanol to completely evaporate before bioassay was conducted. Twenty *S. zeamais* (≤ 24 hr old) adults were introduced into the plastic containers containing the treated and untreated maize grains. Plastics were covered with the lid, the experiment was arranged in completely randomized deign in the laboratory. Mortality was recorded at 24hrs, 48 hrs, 72 hrs and 96 hrs post treatment. Insects were considered dead on failure to respond to probing of the abdomen with pin head.

Effect of Plant Powders on Oviposition and Adult Emergence of *S. zeamais*

Twenty (10 males and 10 females) *S. zeamais* of (≤ 24 hr old) adults were introduced into the plastic containers containing the treated (0.5g, 1.0g, 1.5g, 2.0g and 2.5g.) and untreated maize grains. The adult were removed after ten days and the experiment was kept in the insect breeding wire mesh cage pending adult emergence. At the end of seven weeks, the adults that emerged were counted as the first filial generation till when there was no further emergence observed for 20 days. The percentage reduction of the insects was calculated using the formula below:

$$\% \text{ reduction} = \frac{100 - (\text{No of F1 adults from treated sample})}{\text{No of F1 adults from control}} \; x\ 100$$

Damage Effect and weevil perforation index

The percentage damage (PD) and weevil perforation index (WPI) for each plant treatment on the grain were calculated using the formula below:

$$\text{PD} = \frac{\text{Total number of treated grains perforated}}{\text{Total number of grains}} \; x\ 100$$

$$\text{WPI} = \frac{\text{percentage of treatteded grains perforated}}{\text{Percentage of control grain perforated} + \text{percentage of treated grain perforated}} \; x\ 100$$

Data Analysis

The data obtained was subjected to Analysis Of Variance (ANOVA) and mean were separated using Duncan's Multiple Range test at α = 0.05. Percentage reduction effects of the plants were also subjected to Paired sample test using SPSS version 21.0.

Results

Contact Toxicity of *C. patens* to Adult *S. zeamais*

Table 1 shows the percentage mortality of *S. zeamais* in grains treated with *C. patens* at different concentrations and exposure periods. No mortality was observed at 24 hrs post-treatment periods at all levels of concentrations. At 48 hrs post-treatment period, mortality (13.33%) was observed at concentration of 2.5g of *C. patens*. This was significantly different ($p<0.05$) from mortality observed at other concentrations. At 72 hrs post-treatment period, 0.5g concentration had no mortality (0.00%) while highest mortality (40.00%) was observed at concentration of 2.5g. Mortality recorded for other concentration (1.0g -2.0g) at 96 hrs post-treatment period were the same (13.33%) and were significantly different ($p<0.05$) from control (6.67%) and 2.5g (46.67%).

Table 1: Contact Toxicity of *C. patens* to Adult *S. zeamais* (Mean % ± S. E)

	Exposure Time (hours)			
Conc. (g w/w)	**24**	**48**	**72**	**96**
0.5	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	6.67 ± 6.67ᵃ
1.0	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	6.67 ± 6.67ᵃ	13.33 ±13.33ᵇ
1.5	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	6.67 ± 6.67ᵃ	13.33 ±13.33ᵇ
2.0	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	6.67 ± 6.67ᵃ	13.33 ±13.33ᵇ
2.5	0.00 ± 0.00ᵃ	13.33 ± 6.67ᵇ	40.00 ± 11.55ᵇ	46.67 ± 6.67ᶜ
Untreated	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ

Means followed by the same letter are not significantly different (*p*>0.05) down the column by Duncan's New Multiple Range Test.

Contact Toxicity of *E. aromatica* to Adult *S. zeamais*

Table 2 shows the percentage mortality of *S. zeamais* in grains treated with *E. aromatica* at different concentrations and exposure periods. At 24 hrs post-treatment period, lowest mortality (6.67%) was observed at concentration of 1.5 and 2.0g while highest mortality (13.33%) was observed at concentration of 2.5g of *E. aromatica*. At 48 hrs post-treatment period, concentration 0.5g (6.67%) and 1.0g (6.667%) were not significantly different (*p*>0.05) and likewise 1.5g (20.0%) and 2.0g (20.0%). Highest mortality (33.33%) was observed at concentration of 2.5g. At 72 hrs post-treatment period, mortality ranged from 26.67% to 46.67%. At 96 hrs, 2.0g (60.0%) was not significantly different (*p*>0.05) from 1.5g (53.33%) and 2.5g (66.67%). Untreated (control) had no mortality (0.00%).

Table 2: Contact Toxicity of *E. aromatica* to Adult *S. zeamais* (Mean % ± S. E)

	Exposure Time (hours)			
Conc. (g w/w)	**24**	**48**	**72**	**96**
0.5	0 ± 0.00ᵃ	6.67 ± 6.67ᵇ	26.67 ± 6.67ᵇ	40.00 ± 2.89ᵇ
1.0	0 ± 0.00ᵃ	6.67 ± 6.67ᵇ	26.67 ± 6.67ᵇ	40.00 ± 0.00ᵇ
1.5	6.67 ±6.67ᵇ	20.00 ±1.55ᶜ	33.33 ±6.67ᶜ	53.33 ±6.67ᶜ
2.0	6.67 ±6.67ᵇ	20.00 ±0.00ᶜ	33.33 ±6.67ᶜ	60.00 ±0.00ᶜᵈ
2.5	13.33 ± 6.67ᶜ	33.33 ±6.67ᵈ	46.67 ± 2.01ᵈ	66.67 ± 1.73ᵈ
Untreated	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ

Means followed by the same letter are not significantly different (*p*>0.05) down the column by Duncan's New Multiple Range Test.

Effect of Ethanol Extract of *C. patens* on Adult Mortality *S. zeamais*

Table 3 shows the percentage mortality of *S. zeamais* in grains treated with *C. patens* ethanolic extract at different concentrations and exposure periods. At 24 hrs post-treatment period, lowest concentration (6.67%) was observed at concentration of 1.0ml and 1.5ml while highest mortality (20.0%) was observed at concentration of 2.5ml of *C. patens* extract. At 48 hrs post-treatment period, there was significant difference (*p*<0.05) at all level of treatment except between 0.5ml (6.67%) and Ethanol control (6.67%). Highest mortality (46.67%) was observed at concentration of 2.5ml. At 72 hrs post-treatment period, lowest mortality (13.33%) was in 0.5ml concentration and highest mortality (66.67%) was in 2.5ml concentration. The mortality was significantly different (*p*<0.05) in all treatments at 96 hrs of exposure. The highest mortality recorded was 86.67% (2.5ml).

Table 3: Effect of Ethanol Extract of *C. patens* on Adult Mortality *S. zeamais*

	Exposure Time (hours)			
Conc. (g w/v)	**24**	**48**	**72**	**96**
0.5	0 ± 0.00ᵃ	6.67 ± 6.67ᵇ	13.33 ±6.67ᵇ	33.33 ±6.67ᶜ
1.0	6.67 ±6.67ᵇ	20.00 ±1.55ᶜ	33.33 ±6.67ᶜ	46.67 ±13.33ᵈ
1.5	6.67 ±6.67ᵇ	26.67 ±6.67ᵈ	40.00 ±0.00ᵈ	66.67 ± 1.73ᵉ
2.0	13.33 ± 6.67ᶜ	33.33 ±6.67ᵉ	53.33 ±6.67ᵉ	73.33 ± 6.67ᶠ
2.5	20.00 ± 0.00ᵈ	46.67 ± 6.67ᶠ	66.67 ± 1.73ᶠ	86.66 ± 6.67ᵍ
Untreated	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ
Ethanol	0.00 ± 0.00ᵃ	6.67 ± 6.67ᵇ	13.33 ±6.67ᵇ	20.00 ± 0.00ᵇ

Means followed by the same letter are not significantly different (*p*>0.05) down the column by Duncan's New Multiple Range Test.

Effect of Ethanol Extract of *E. aromatica* on Adult Mortality *S. zeamais*

Table 4 shows the percentage mortality of *S. zeamais* in grains treated with *E. aromatic* ethanolic extract at different concentrations and exposure periods. At 24 hrs post-treatment period, 0.5ml had no mortality (0.0%) while highest mortality (40.0%) was observed at concentration of 2.5ml of *E. aromatic* extract. There was significant difference (*p*<0.05) in all level of treatment at 48 to 96 hrs post-treatment period. Mortality ranged from 13.33% to 53.33% at 48hrs, while it ranged from 33.33% to 73.333% at 72 hrs, At 96 hrs post-treatment period, total mortality (100%) was achieved in 2.5ml concentration.

Table 4: Effect of Ethanol Extract of *E. aromatica* on Adult Mortality *S. zeamais*

	Exposure Time (hours)			
Conc. (g w/v)	24	48	72	96
0.5	0.00 ± 0.00[a]	13.33 ±6.67[c]	33.33 ±6.67[c]	40.00 ±11.55[c]
1.0	20.00 ±0.00[b]	33.33±6.67[d]	46.67±13.33[d]	66.67 ± 6.67[d]
1.5	26.67 ±6.67[c]	46.00 ±6.67[e]	66.67 ± 1.73[e]	80.00 ± 0.00[e]
2.0	33.33 ± 6.67[d]	46.00 ±6.67[e]	66.67 ± 1.73[e]	86.67 ± 6.67[f]
2.5	40.00 ± 0.00[e]	53.33 ±6.67[f]	73.33 ± 6.67[f]	100.00 ± 0.00[g]
Untreated	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Ethanol	0.00 ± 0.00[a]	6.67 ± 6.67[b]	13.33 ± 6.67[b]	13.33 ± 6.67[b]

Percentage Reduction of Adult *S. zeamais* on maize grains

Table 5 shows the effect of the plant powders on adult emergence and development at different concentrations. At all levels of powder concentrations, the powders significantly reduced the number of adults that emerged. The reduction in the number of adult weevil that emerged increased with increase in the concentration of the powders. The highest percentage reduction (68.40%) and (95.83%) were observed at concentration of 0.5g in *C. patens* and *E. aromatica* respectively. There was significant differences ($p<0.05$) between the two plants percentage reduction except in 0.2g concentration.

Table 5: Percentage Reduction effect on Adult *S. zeamais*.

Concentration (g w/w)	Plant materials	Mean ±SE	df	t - value	p-value
0.1	*C. patens*	37.07 ± 2.42	1	-5.493	0.032
	E. aromatica	57.37 ± 4.88			
0.2	*C. patens*	54.50 ± 10.63	1	-2.464	0.133
	E. aromatica	77.26 ± 6.04			
0.3	*C. patens*	56.37 ± 3.95	1	-5.971	0.027
	E. aromatica	80.50 ± 10.03			
0.4	*C. patens*	61.23 ± 8.59	1	-4.869	0.040
	E. aromatica	86.53 ± 1.65			
0.5	*C. patens*	68.40 ± 3.41	1	-47.510	0.001
	E. aromatica	95.83 ± 4.16			

SE - Standard Error, df – Degree of Freedom

4.6 Damage assessment of maize grains treated with plant powders

The effect of plant powders on the ability of *S. zeamais* to cause seed damage and Weevil Perforation Index (WPI) is presented in Table 6. The highest WPI (49.57%) was observed at concentration of 0.1g and the lowest WPI (23.94%) was observed at 0.5g in *C. patens*. The lowest WPI (3.63%) was observed at concentration of 0.5g while the highest WPI (29.24%) was observed at concentration of 0.1g in *E. aromatica*. Meanwhile WPI obtained at concentration 0.2g and 0.3g of *E. aromatica* powders not significantly different ($p>0.05$) from each other.

Table 6: Damage assessment of maize grains treated with plant powders

Plant powder	Conc.	Total no. of grains	No. of grains damaged	Grains Undamaged	PD	WPI
C. patens	0.1	161	20	141	12.44 ±0.78[a]	49.57 ± 4.17[a]
	0.2	161	14	147	8.71 ±1.31[b]	31.92 ± 5.45[b]
	0.3	173	14	159	7.72 ±1.14[c]	29.03 ± 2.06[c]
	0.4	173	12	161	6.95 ±1.10[d]	27.23 ± 4.89[d]
	0.5	171	10	161	5.83 ± 0.39[e]	23.94 ± 2.31[e]
E. aromatica	0.1	167	10	157	6.00 ±1.33[d]	29.24 ±2.96[d]
	0.2	163	5	158	3.06 ±0.61[c]	17.95 ±3.61[c]
	0.3	170	4	166	2.39 ±1.20[bc]	14.75 ±7.39[c]
	0.4	171	3	168	1.75 ±0.64[b]	11.24 ±0.96[b]
	0.5	171	1	170	0.58 ±0.58[a]	3.63 ±3.63[a]
	Control	167	27.5	139.5		

Discussion

In this study, plant materials used shows pesticide potential on *S. zeamais*. Traditionally, plant materials were been used as insecticidal, as many of the plants were discovered to have bio pesticide potential [3, 5, 4]. Generally, toxicity of the plant materials, both the powder and the extract increased as the concentration and exposure time increased.

The results obtained from this research work showed that between the two plant powders used, the powder as well as ethanolic extract of *E. aromatica* was able to cause high mortality of the maize weevil. However, *C. patens* also showed little mortality effect on the insect pest, but its potency was low even at higher concentration when compare with *E. aromatic. C. patens* powder was found not to have any effect at 24 and 48 hrs of exposure. The high mortality effect of *E. aromatica* on the weevil may be attributed to contact toxicity as suggested by Idoko and Adesina [6]. Eugenol is a major component of *E. aromatica* and has been said to give it, its characteristic aroma. It is possible that eugenol is responsible for the mortality effects present in *E. aromatica*. This work agrees with findings of Akinneye and Ashamo [7] that *E. aromatica*, *Uvaria afzelli*, *Aframonium melequeta* and *Zingiber officinales* were able to evoke 100% mortality on *Plodia interpunctella*.

Also, the mortality effect of these two plants may be due to inability of the weevils to feed on treated maize seeds which have been coated with the powders and extracts thereby leading to starvation and eventually death. Also the powders may have blocked the spiracles of the weevil or disrupted the normal respiratory activity of the weevil leading to asphyxiation and subsequent death.

Ethanolic extracts of *C. patens* and *E. aromatica* were observed to be more effective in achieving mortality than powders. *E. aromatica* extract evoked 100% mortality on *S. zeamais*. This was also revealed by the Weevil Perforation Index (WPI), *E. aromatica* had the least WPI when compare with *C. patens.* This may be attributed to the fact that the active components of the plant powders were properly dissolved in the solvent hence increasing the rate of penetration of the active components into the cuticle to cause insect mortality rather than coarse powder. Some oils and ethanolic extracts also have pungent smell which fills and disturbs the respiratory tract of insects causing death. Adedire and Akinneye [8] also reported the effectiveness of leaf extract (*Tithonia diversifolia*) on the development of *S. zeamais*. The high potency of plant extracts in this study also agrees with the research that showed extract of coconut, sunflower, sesame and mustard, alone and in combination with eucalyptol, eugenol or camphor have been found to be toxic to *S. zeamais* in wheat and maize-treated grains [9].

The high percentage reduction of the plant powders on *S. zeamais* inability to emerge may be due to the death of the insect larva which may be as a result of the inability of the larva to fully cast off their exoskeleton which remains linked to the posterior part of their abdomen [10].

Conclusion

This research work showed that ethanolic extracts of plants examined are much more effective than powders which may be as a result of easy penetration of the active components of the plant materials because of the dissolution in liquid medium. In the treatment of maize grains with bio pesticides, ethanolic extracts will give quicker and better results.

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