Biotoxic efficacy of two horticultural plants against infestation of *Sitophilus oryzae* on stored maize

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Abstract

Since botanicals still remained the most promising tool that could obviate the use of chemical insecticides, this study investigated the biotoxic effect of *Acacia auriculiformis* and *Acalypha goddsefiana* powder and oil against *Sitophilus oryzae.* The powder of the plants were tested at 0.0g (control), 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0g dosages while their extracts were prepared at 1, 2, 3, 4 and 5% concentrations per 20g of maize seeds. Solvent control was also prepared for the oil extracts bioassay. Mortality, adult emergence, % inhibition rate and % weight loss were observed. LD_{50} and LC_{50} of the powders and oils of the two botanicals were calculated at 48 hours of application. The powders of both plants achieved complete weevil mortality within 72h of application at 2.0g and their effect was significantly ($p<0.05$) different from other dosages except 1.0g dosage. Also, the extracts of both plant achieved 100% insect mortality within 48h hours of application at 5%. However the powder and oil of *A. auriculiformis* appeared more effective than that of *A. goddsefiana* in term of mortality as reflected by their lethal dosage and concentration. However, *A. goddsefiana* powder and extract appeared more effective than that of *A. auriculiformis* in term of protectability as they greatly reduced the emergence of the adult weevil and their ability to cause

weight loss of the maize grains. Only the higher dosages and concentrations of the plants were able to achieve 100% inhibition rate.

Keywords

Sitophilus oryzae; *Acacia auriculiformis*; *Acalypha goddsefiana*; Lethal concentration; Protectability; Biototoxic

Introduction

Food security is one of the current challenges which have attracted much attention of the government of all the countries of the world. In Nigeria, all those connected with agricultural production have been showing serious anxiety because losses of agricultural produce are one of the major problems thwarting the economic growth of the country. Ajibola [1] in his report opined that the amount of food waste in Nigeria in 1990s during postharvest is enough to feed more than 200 million people. Insect attack both on the field and in storage together with other factors such as poor handling, adverse climate condition among other have been pointed out to be the key factors exacerbating the postharvest loss in Nigeria as more than 40% grains production are loss through these ways [2,3]. In attempt to trim down food wastage in the country and to ensure food security, the Federal Government of Nigeria, took some measures such as building of silos across all the six geopolitical zones of the country to at least ensure more than 1.3 million tones of the nation agricultural produce are stored in good condition [2,3]. Despite the effort of the government, high level of postharvest losses is still of great deal as many of the foods in the markets are imported from overseas in other to ease the shortfall in food availability.

Maize (*Zea mays L.*) is an important staple cereal crop for most people in sub-Saharan Africa. It provides families with much needed nutrients, such as carbohydrates, proteins, fats, vitamin B and minerals [4,5]. Besides being a major source of food for both human and animals worldwide, it is also processed into various industrial products such as fuel ethanol and starches [6]. In spite of the advantages derive from this ponderous crop, insects including weevils, beetles and moths incessantly attack it both on the field and in storage where their malevolence deed is more pronounced [3,7]. Sitophilus oryzae is very popular among other insects that attack maize grains because of its nefarious deed to other important grains like rice. In most developing countries including Nigeria where the level of literacy among the

farmers is still low and government intervention in term of adequate storage facilities provision is minimal, the production and market price of this weighty cereal has dropped steadily over the years [5].

Different methods are however being employed by the entomologists of the world to restraint the activities of *S. oryzae* and other stored product insect pests. Some of the control methods include physical control, mechanical control, chemical control and host-plant resistance. Among the different control methods used, chemical control method is the most popular and successful means of controlling insect pest up till now [8]. In recent years, chemical control method is losing its popularity because they were found associated with many quandaries frustrating their widespread use nowadays. Such perils include pest resurgence and resistance, lethal effects on non-target organisms, the risk of user's contamination, food residues and environmental pollution [3,9-11].

Plant species and their derivatives have been suggested by researchers to be a new thoroughfare of insect control as they are believed to be eco-friendly and less toxic to mammals. More so, even before the discovery of the most popular synthetic insecticides in the 1930s, botanicals have been the key missile in the farmer's magazine. Up till now, in spite of the effectiveness of many botanical insecticides, their insecticidal activity is yet to be comparable to many synthetic chemical insecticides; and the once that are believed to be comparable with chemical insecticides have not commanded more than 1% of the global insecticide market [8,10] because they are believed to live for a short period of time and to lose their potency over time [12]. Therefore, need for search of other botanicals that could be insecticidal in nature become necessary. More so that the tropical parts of the world including Nigeria is well endowed with medicinal plants that could be insecticidal in nature.

Acacia auriculiformis and *Acalypha godseffiana* are horticultural plant with medicinal values. These plants have been used in the treatment of rheumatism, malaria, cough and tooth ache [13,14].

However, the insecticidal activity of these botanicals has not been fully investigated like many other common botanicals on which many researches have been done. Therefore, this present study investigated the phytotoxic efficacy of *Acalypha godseffiana* and *Acacia auriculiformis* against infestation of *Sitophilus oryzae* on stored maize and against *Sitophilus oryzae*.

Materials and methods

Insect culture

The culture of *S. oryzae* was obtained from an infested maize grain at Crop, Soil and Pest management Laboratory, Federal University of Technology, Akure, Nigeria. This was reared on non-infested clean maize grains obtained from Oba market, Akure, Ondo State, Nigeria. The experiment was setup in the laboratory at temperature of 28±2°C and relative humidity of 75±5%.

Collection of plant materials and maize seeds

The leaves of *Acacia auriculuformis* and *Acalypha godseffiana* were sourced fresh from Teaching and Research farm, Horticultural garden, Federal University of Technology, Akure, Ondo State. Collected plants were taken to botany section of FUTA Horticultural garden for identification. The yellow variety of maize grains, used for the experiment, was obtained from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The seeds were cleaned of foreign matter and disinfested by being kept in a freezer at -5°C for 30days. They were then air-dried to avoid mouldiness of the grains.

Preparation of plant powders and extracts

The collected plant materials were air-dried in an open space in the laboratory. The leaves were separately pulverized into fine powder using electric Binatone blender and the powders were further sieved to pass through 1mm2 perforations. The powders were stored in different air-tight plastic storage containers arranged in the laboratory shelve prior to use. 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0g of the powders were weighed using Metler beam weighing balance. The oil extracts of the two botanicals were prepared using Soxhlet extractor and n-hexane was used as solvent. The extraction was carried out for 3-4 hours depending on the plant material. The extraction was terminated when the solvent in the thimble became clear. The solvent was separated from the oil using rotary evaporator, after which the oil was exposed to air so that traces of the volatile solvents evaporate, leaving the oil extract. From this main stock, different concentrations of 1, 2, 3, 4 and 5% oil concentrations were made.

Effect of Acacia auriculiformis and Acalypha godseffiana Powders on Mortality, Adult Emergence of S. oryzae and Weight Loss of treated seeds

Twenty grams of the maize grains were weighed into Petri-dishes. Plant powders weighing 0.0g (control), 0.1g, 0.2g, 0.4g, 0.6g, 0.8g, 1.0g and 2.0g was weighed and thoroughly mixed with the maize seeds inside the Petri-dishes using glass rod. The experiment was set up in a complete randomized design and each treatment was replicated three times. Five pairs of newly emerged (0-24h) *S. oryzae* were separately introduced into those treated maize grains immediately and weevil mortality was assessed at 48 and 72hours post treatment. Both dead and live insects were removed on the fourth day and experiments were left for 42days to allow for emergence of F1 generation and the number of adult emerged was counted. Inhibition rate (%IR) in adult emergence was calculated using Eq(1).

$$\%IR = \frac{Cn - Tn}{Cn} \times 100 \quad (1)$$

where C_n is the number of insects that emerged in the control treatment and T_n is the number of adult insects that emerged in the treated grains.

The weight loss of the stored grains was calculated using Eq(2):

$$\%weight\ loss = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100 \quad (2)$$

Effect of Acacia auriculiformis and Acalypha godseffiana oil on mortality, adult emergence of S. oryzae and weight loss of treated seeds

20g of maize gains was weighed into Petri-dishes and plant extracts concentration of 1, 2, 3, 4 and 5% was separately mixed with the maize and was left for 1hr to ensure the escape of the N-hexane solvent. Two control experiments were set up, one with solvent alone and one with neither solvent nor extract (untreated control). Five pairs of newly emerged (0-24h) adult insects were introduced into containers with treated grains and were arranged in a complete randomized design and each treatment was replicated three times. Adult mortality was observed after 48 and 72 hours of application. Both dead and live insects were removed on the fourth day and experiments were left for 42 days to allow for emergence of F1 generation and the number of adult emerged was counted. %IR and the weight loss of the stored grains were calculated using the formulas described above.

Statistical analysis

All the data obtained from the research were subjected to one-way analysis of variance at 5% significant level and means were separated with New Duncan's Multiple Range Tests using SPSS version 17. Also data, obtained from weevil's mortality, were subjected to regression analysis to calculate the LD_{50} of the powders and LC_{50} of the extracts using probit analysis [15].

Results

Effect of A. auriculiformis and A. godseffiana powder at different dosages on the mortality of adult S. oryzae

Table 1 presents the effect of *A. auriculiformis* and *A. godseffiana* powder at different dosages against adult *S. oryzae.* The effectiveness of the powder increased with increase in dosage and period of application. At all levels of dosage used, the powder achieved above 13% insect mortality within 48hours of application. However, 2.0g of *A. auriculiformis* powder achieved the highest insect mortality of 96.67% within 48 hours of exposure and it effect was significantly ($p<0.05$) different from other dosages except its 1.0g dosage which recorded 86.67% weevil mortality. 2.0g of *A. auriculiformis* powder showed consistency in its potency as it was the only dosage that achieved complete weevil mortality within 72 hours of application. At all levels of dosage, powder of *A. auriculiformis* recorded high mortality of *S. oryzae* than powder of *A. godseffiana*.

Toxic effect of A. auriculiformis and A. godseffiana oil extract at different concentrations against adult S. oryzae

The mortality of *S. oryzae* exposed to different concentrations of *A. auriculiformis* and *A. godseffiana* oil extract was presented in Table 2. The effect of the oil on the adult weevil varied with increase in concentration as well as period of exposure. The oils achieved above 35% insect mortality within at all periods of application but none of the concentration was able to achieve complete weevil mortality at 48hrs of exposure except 5% concentration of *A. auriculiformis*. Nevertheless, 5% concentration of *A. godseffiana* oil achieved 100% mortality of the insect after 72hrs of exposure. At levels of application, the effect of the oils are significantly ($p< 0.05$) different from the controls.

Table 1. Percentage mortality of adult S. oryzae exposed to different dosages of *A. auriculiformis* and *A. goddsefiana*

	Dosage (g)	% Mortality in hours	
		48	72
A. auriculiformis	0.1	33.33±0.66^{c}	33.33±0.67^{b}
	0.2	50.00±0.58^{d}	53.33±0.33^{d}
	0.4	56.67±0.33^{d}	58.67±0.33^{d}
	0.6	58.67±0.88^{de}	63.33±0.33^{de}
	0.8	76.67±0.33^{f}	78.33±0.67^{e}
	1.0	86.67±0.88^{fg}	96.67±0.33^{f}
	2.0	96.67±0.33^{g}	100.00±0.00^{f}
A. goddsefiana	0.1	13.33±0.67^{b}	20.00±1.15^{b}
	0.2	30.00±1.53^{c}	38.00±1.53^{bc}
	0.4	23.33±0.33^{c}	40.00±0.58^{c}
	0.6	26.67±0.33^{c}	43.33±0.33^{c}
	0.8	46.67±0.67^{d}	46.67±0.33^{cd}
	1.0	50.00±0.58^{d}	60.00±0.58^{d}
	-2.0	70.00±0.58^{f}	93.33±0.67^{f}
	Control	0.00±0.00^{a}	0.00±0.00^{a}

Each value is Mean ± S.E of three replicates.
Values followed by the same letter on the same column are not significantly (p>0.05) different from each other using New Duncan's Multiple Range Test.

Table 2. Percentage mortality of *Sitophilus oryzae* exposed to *Acacia auriculiformis* oil at different concentrations

	Dosage (g)	% Mortality in hours	
		48	72
A. auriculiformis	1	36.67±0.88^{b}	48.00±0.58^{b}
	2	50.00±0.58^{c}	63.33±0.33^{cd}
	3	70.00±0.58^{d}	80.00±0.00^{e}
	4	90.00±1.00^{e}	95.00±1.15^{f}
	5	100.00±0.00^{e}	100.00±0.00^{f}
	Treated control	0.00±0.00^{a}	2.00±0.24^{a}
	Untreated control	0.00±0.00^{a}	0.00±0.00^{a}
A. goddsefiana	1	53.33±0.67^{c}	55.00±0.28^{bc}
	2	55.63±0.27^{c}	59.00±0.67^{c}
	3	56.67±0.88^{c}	70.00±0.58^{d}
	4	70.00±1.15^{d}	90.00±0.58^{f}
	5	90.00±0.57^{e}	100.00±0.00^{f}
	Treated control	0.00±0.00^{a}	2.00±0.24^{a}
	Untreated control	0.00±0.00^{a}	0.00±0.00^{a}

Each value is Mean ± S.E of three replicates. Values followed by the same letter on the same column are not significantly (p>0.05) different from each other using New Duncan's Multiple Range Test.

Lethal dose (LD50) and lethal concentrations (LC50) of A. auriculiformis and A. godseffiana powder and oil in S. oryzae after 48h

Table 3 shows that lower amounts of *A. auriculiformis* powder and extract was required to achieve 50% mortality of the weevil when compared to the amount required using *A. godseffiana* powder and extract. This shows that the *A. auriculiformis* was more toxic than the *A. godseffiana*. However, fiducial limits revealed that a lower amount of extract of both *A. auriculiformis* (1.66-2.00) and *A. godseffiana* (2.04-2.51) were required to cause 50% mortality in *S. oryzae* when compared to the amount needed by their powders. This further revealed that *S. oryzae* were more susceptible to *A. auriculiformis* powder and extract than *A. godseffiana* powder and extract.

Table 3. Lethal dose (LD_{50}) and lethal concentration (LC_{50}) of *A. auriculiformis* and *A. goddsefiana* powders and extracts required to achieve 50% mortality in *S. oryzae* after 48 hours post treatment

LD_{50} (gm)		LC_{50} (%)	
A. auriculiformis	*A. goddsefiana*	*A. auriculiformis*	*A. goddsefiana*
2.47 (2.19-2.71)	3.73 (3.45-4.01)	1.84 (1.66-2.00)	2.34 (2.04-2.51)

Values in brackets represent 95% fiducial limits

Adult emergence, Inhibition rate (%IR) in adult emergence of S. oryzae and weight loss in maize grains treated with A. auriculiformis powder

The effect of *A. auriculiformis* and *A. godseffiana* powder on the emergence of adult *S. oryzae* and their ability to cause seed weight loss was presented in Table 4.

Table 4. Number of adult emergence, inhibition rate (%IR) in adult emergence of S. oryzae and weight loss in maize grains treated with A. auriculiformis powde

Dosages (g)	Mean number of adult emergence	%IR	%weight loss
0.1	7.00±0.58^{c}	49.72±8.61^{b}	12.26±0.86^{c}
0.2	5.67±0.33^{bc}	60.87±4.32^{c}	10.93±0.25^{c}
0.4	4.67±0.33^{b}	68.22±0.79^{c}	6.37±3.19^{b}
0.6	0.00±0.00^{a}	100.00±0.00e	0.00±0.00a
0.8	0.00±0.00^{a}	100.00±0.00e	0.00±0.00a
1.0	0.00±0.00^{a}	100.00±0.00e	0.00±0.00a
2.0	0.00±0.00^{a}	100.00±0.00e	0.00±0.00a
0.1	6.33±0.88^{c}	56.42±6.50^{bc}	11.79±1.36^{c}
0.2	5.33±0.88^{bc}	63.77±5.22^{c}	10.53±0.42^{c}
0.4	4.33±0.33^{b}	70.30±2.46^{cd}	10.07±0.14^{c}
0.6	0.00±0.00a	100.00±0.00e	0.00±0.00a
0.8	0.00±0.00a	100.00±0.00e	0.00±0.00a
1.0	0.00±0.00a	100.00±0.00e	0.00±0.00a
2.0	0.00±0.00a	100.00±0.00e	0.00±0.00a

Each value is Mean ± S.E of three replicates. Values followed by the same letter on the same column are not significantly ($p>0.05$) different from each other using New Duncan's Multiple Range Test

The powder at different dosage significantly reduced or prevented the emergence of the adult weevil and its effect is directly proportional to the increase in dosage. At lower dosage (0.1, 0.2 and 0.4g), the powder was unable to prevent the emergence of the weevil but greatly reduced the number of insect that emerged and their effect was significantly ($p<0.05$) different from the control. The powder at 0.6, 0.8, 1.0 and 2.0g dosages prevented the emergence of the weevil and therefore recorded 100% inhibition rate. This powder prevented the weight loss of the protected maize grain. However, at 0.1, 0.2 and 0.4g the powder of *A. godseffiana* showed more protectability effect than the powder of *A. auriculiformis* but its effect was not significantly ($p>0.05$) different from the powder of *A. auriculiformis*.

Adult emergence, Inhibition rate (%IR) in adult emergence of S. oryzae and weight loss in maize grains treated with A. auriculiformis and A. godseffiana oil

Table 5 presented the effect of *A. auriculiformis* and *A. godseffiana* oil extract on the emergence of adult *S. oryzae* as well as its ability to cause seed weight loss. The effect of the oils is relatively proportional to the increase in their concentrations. The oils greatly reduced the emergence of the adult weevil and recorded high percentage inhibition rate. Only the 1% concentration of the oils was unable to prevent the emergence of the insect as they only recorded 91.05 and 90.12% inhibition rate respectively. However, the effect of the oils at all levels of concentration were significantly ($p<0.05$) different from the controls.

Table 5. Number of adult emergence, inhibition rate (%IR) in adult emergence of *S. oryzae* and weight loss in maize grains treated with *A. auriculiformis* oil

Concentrations (%)	Mean number of adult emergence	%IR	%weight loss
1	2.00 ± 0.58^{b}	91.05 ± 1.80^{b}	8.53 ± 0.55^{b}
2	0.00 ± 0.00^{a}	100.00 ± 0.00^{c}	0.00 ± 0.00^{a}
3	0.00 ± 0.00^{a}	100.00 ± 0.00^{c}	0.00 ± 0.00^{a}
4	0.00 ± 0.00^{a}	100.0 ± 0.00^{c}	0.00 ± 0.00^{a}
5	0.00 ± 0.00^{a}	100.00 ± 0.00^{c}	0.00 ± 0.00^{a}
1	1.33 ± 0.88^{b}	90.12 ± 1.82^{b}	5.42 ± 1.13^{b}
2	0.00 ± 0.00^{a}	100.00 ± 0.00^{c}	0.00 ± 0.00^{a}
3	0.00 ± 0.00^{a}	100.0 ± 0.00^{c}	0.00 ± 0.00^{a}
4	0.00 ± 0.00^{a}	100.00 ± 0.00^{c}	0.00 ± 0.00^{a}
5	0.00 ± 0.00^{a}	100.00 ± 0.00^{c}	0.00 ± 0.00^{a}
Treated control	14.67 ± 0.88^{c}	2.02 ± 0.84^{a}	80.42 ± 2.33^{c}
Untreated control		0.00 ± 0.00^{a}	82.09 ± 0.13^{c}

Each value is Mean ± S.E of three replicates.
Values followed by the same letter on the same column are not significantly ($p>0.05$) different from each other using New Duncan's Multiple Range Test

Discussion

Plants and their derivatives have played an integral role in the well-being of human race as they not only serve as food for man but also serve as source of medicine to man and animals. Insects, being major antagonists to the man combat against hunger have posted alarming threats to human security in term of food provision. However, because of the urgent need to control the evil works of these insects, man has overwhelmingly relied on the use of synthetic chemical insecticides which have been noted to link with numerous perils which lead to their ban in many developed countries [3,5,16,17]. In order to replace these problems associated chemical insecticides, man have returned to natural products from plants since they are believed to be safer to both human and environmental health. However, many botanicals in the man's habitats are yet to be exploited for their insecticidal potential. Therefore, increasing the data bank of plants with insecticidal efficacy is of essential need.

The result obtained from this research showed that both powder and extract of *A. auriculiformis* and *A. godseffiana* are effective against adult *S. oryzae* and its ability to cause weight loss of the stored maize grains. The effectiveness of the two plants varied with types of materials used (powder or oil extract), dosage or concentration used and period of exposure. The powder and extract of these botanical achieved high weevil mortality within 48hours of application but the extracts are more effective than the powders as reflected by their lethal dosage and concentration. Also the powder and extract of *A. auriculiformis* effect high mortality than the powder and extract of *A. godseffiana* has shown by the amount of dosage and concentration required by them to achieve 50% mortality of the *S. oryzae*. The aptitude of these powders and extracts to effect high mortality rate of the insect could be due to their ability to disrupt the normal respiratory activity of the insect. Also the powders and extracts may have blocked the spiracle which is the main breathing organelle of insects thereby causing suffocation. In addition, the ability of the oils of these botanical to effect high mortality of the insect than their powders could be due to high secondary metabolites contained in them as suggested by Isman [17]. Also, the plant powders and extracts may have inhibitory effect on the detoxifying enzyme of *S. oryzae* as many botanical extracts such as neem and *Nicotiana tabacum* had been acclaimed to have significant effect on wide range of insect's detoxifying enzymes [10,18].The result obtained in the research acquiesced with the work of Ileke and Oni [19] as well as Ogungbite and Oyeniyi [5] in which botanical oils and powders were used to protect maize grain against *S. zeamais* and *S. oryzae.*

Furthermore, it was noted in this work that the powders and extracts of these botanical significantly reduced the amount adult that emerge on the treated grains when compared to the controls. The oils however reduced the number of adult emerged on the protected grains than the powders as reflected by their percentage inhibition rate (%IR). It was also noted that the powder and oil of *A. godseffiana* showed more effectiveness on the adult emergence of the weevil than the powder and extract of *A. auriculiformis*. Moreover, the lower adult emergence recorded on the treated maize grains could be due to high mortality recorded by the oils and the powders because high mortality could reduce mating ability of the insect and in turn reduce number of egg laid. The low number of adult emerged on the treated seed may also be due to the death of the insect larvae which was unable to entirely shed off their old exoskeleton which typically remained linked to the posterior part of the abdomen [20]. This result was in accordance with the work of Ogungbite and Oyeniyi [5] as well as Ileke and Ogungbite [21] in which the extract of different botanicals reduced the number of adult insects that emerged from treated maize grains. The percentage weight loss caused by the insect was greatly reduced even at lower concentration and dosage of the oils and powders respectively. The ability of the powder and extract of the two botanicals to reduce or prevent the weight loss of the treated grains could be due to the reduction in the number of adult weevil that emerged. The inability of the larvae of the insect to feed on the treated maize grains could also contribute to the reduction or prevention of seed weight loss. Terpinoids, Tannins, Flavonoids, Alkaloids, Saponins, Glycosides and Steroids have been reported present in *A. auriculiformis* and *A. godseffiana* [22]. Many of these phytochemical compounds had been reported to have antifeedant effect, growth effects and also possessed considerable toxicity toward insect [23,24]. For example, alkaloid had been reported to have inhibitory effect on oviposition of insects [24]. This result was in support of Oigiangbe *et al.*, [20] which stated that alkaloid found in *A. boonei* disrupted growth and reduced larva survival as well as disruption of life cycle of insects [24].

Conclusions

The result obtained from this study showed that both the powder and extract of *A. auriculiformis* and *A. godseffiana* are effective against *S. oryzae* and they can therefore be

incorporated into integrated pest management system since they are medicinal plants. However, for the control of already infested maize grains the powder and extract of *A. auriculiformis* could be employed since it effects high mortality on the insect than the powder and extract of *A godseffiana* but for the protection of stored maize, powder and extract of *A. godseffiana* could be used as it showed the high protectability effect against the insect. However, more research is still required as regard the mode of action of the plants, their toxic effect on mammals, their effect on detoxifying enzyme of insects as well as characterization and purification of their active compounds.

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