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POWDERS AND EXTRACTS OF *Syzygium aromaticum* AND *Anacardium occidentale* AS ENTOMOCIDES AGAINST THE INFESTATION OF *Sitophilus oryzae* (L.) [Coleoptera: Curculionidae] ON STORED SORGHUM GRAINS

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ABSTRACT

Sitophilus oryzae is a major pest of sorghum (*Sorghum bicor* L.) grains in storage in Nigeria. Thus a study was conducted to evaluate the entomocidal bustle of *Syzygium aromaticum* and *Anacardium occidentale* at ambient temperature of 28±2 ºC and 75±5% relative humidity. The powders were applied at rates of 0.5, 1.0, 1.5, 2.0 and 2.5 g per 20 g of sorghum grains, while oils were applied at rates 1, 2, 3 and 4% per 20 g of sorghum grains. Both the powders and oils of *S. aromaticum* and *A. occidentale* showed insecticidal effects on survival of the *S. oryzae*. However, the powder from *S. aromaticum* was more effective than that of *A. occidentale*; as it achieved 100% weevil mortality at all the tested concentrations within 96 hours of application. The oils caused more mortality on adult *S. oryzae* than their powder counterparts. Similarly, the oils of *S. aromaticum* are more effective than those of *A. occidentale.* Both the oils prevented adult emergence of the weevil and weight loss of the sorghum grains at all concentrations. The only exception was the oil of *A. occidentale* which recorded 1.2 and 0.89% adult emergence and seed weight loss, respectively.

Key Words: Entomocide, oils, *Sorghum bicolor*, weevil mortality

RÉSUMÉ

Sitophilus oryzae est une peste majeure des grains de sorgho (*Sorghum bicor* L.) en stockage au Nigeria. Ainsi une étude a été réalisée pour évaluer les effets entomocides de *Syzygium aromaticum* et *Anacardium occidentale* à une temperature ambiante de 28±2 ºC et une humidité relative de 75±5%. Les poudres étaient appliquées aux taux de 0,5 ; 1 ; 1,5 ; 2,0 et 2,5 g par 20 g de sorgho grains, tandis que les essences étaient appliqués aux taux de 1, 2, 3 et 4% par 20 g de sorgho grains. Aussi bien la poudre que les essences de *S. aromaticum* et *A. occidentale* présentent des effets insecticides sur *S. oryzae*. Cependant, la poudre de *S. aromaticum* était plus efficace que celle de *A. occidentale*; car celle-ci assure une mortalité totale (100%) des charançons du riz pour toutes les concentrations testées et ceci en 96 heures d'application. Les essences étaient plus efficaces que la poudre sur les adultes du charançon du riz. De même, les essences de *S. aromaticum* sont plus efficaces que celles de *A. occidentale.* Toutes les deux essences empêchent l'émergence des adultes et la perte pondérale des grains de sorgho pour toutes les concentrations appliquées. La seule exception était les essences de *A. occidentale* qui ont permis une émergence d'adultes de 1.2% et une perte pondérale en grains de sorgho de 0.89%.

Mots Clés: Entomocide, essences, *Sorghum bicolor*, mortalité de charançon du riz

INTRODUCTION

Losses caused by weevils are of great importance in grain storage systems. Their activities on stored produces lead to loss of both quality and quantity. *Sitophilus oryzae* is one of insect pests of sorghum in store whose control has relied profoundly on synthetic chemical insecticides (Akinkurolere, 2007; Ileke and Olotuah, 2012; Akinneye and Ogungbite, 2013). Chemical insecticides have been the cornerstone upon which pest management practices are based, despite having some downsides which has thwarted their widespread application. Such drawbacks include resistance by major insect pests, of development high procurement cost and hazards to both human health and environment (Garriga and Caballero, 2011; Ashamo *et al*., 2013).

Public awareness concerning the adverse effects of chemical insecticides has led researchers to find new boulevard of insect control in order to replace these unfriendly synthetic insecticides. Currently, there is a steady increase in the use of plant products as cheaper and ecologically safer means of controlling insect pests of stored grains, especially in the tropics (Ileke and Oni, 2011; Akinneye and Ogungbite, 2013).

In spite of the effectiveness of many botanical insecticides, the risk caused by their use is often comparable to many synthetic chemical insecticides (Begum *et al*., 2013). Therefore, there is still need to identify other pesticidal plants that can comparably contend with the effectiveness of chemical insecticides.

Clove (*Syzygium aromaticum*) and cashew (*Anacardium occidentale*) are plant species with medicinal importance (Obaineh and Agunbiade, 2013; Diego *et al.,* 2014). These plants have also been noted to be insecticidal in nature (Ileke and Oni, 2011; Khan *et al.,* 2014). The insecticidal potential of these two plants have not been fully investigated as it was done for other plants like neem, nicotiana spp and many other popular plants.The objective of this study was to investigate the entomocidal bustle of *S. aromaticum* and *A. occidentales* against *S. oryzae*, one of the major insect pests of sorghum grains in storage.

MATERIALS AND METHODS

Insect culture. The culture of *S. oryzae* was obtained from infested sorghum grains at the Environmental Biology and Fisheries Research Laboratory, Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. This was reared on clean sorghum grains obtained from the Agricultural Development Project (ADP) Akure, Ondo State. Because all the life stages of *S. oryzae*, in particular, the eggs are very sensitive to cold, the grains were first cleaned and disinfested by keeping them at -5 °C for 7 days. The grains were then air dried to prevent mouldiness before introduction of insects. The experiment was setup in the laboratory at ambient temperature (28±2 °C) and with relative humidity (5±5%).

Sorghum grains and plant materials. Sorghum grains used in this study were collected from the Agricultural Development Project (ADP), Akure, Nigeria and were disinfested as described above. *Syzygium aromaticum* and *A. occidentale* were sourced fresh from Akola Farm, Igbara-odo Ekiti, Nigeria. The seeds and nuts were dried in an open laboratory and grounded into very fine powder using an elastic blender (Supermaster, model SMB 2007, Japan). The powders were further sieved through 1 mm² perforations and stored at 4 ºC in separate plastic containers with tight lids, prior to use.

Oil extracts preparation. The oil extracts were made from the powders of *S. aromaticum* and *A. occidentale* and methanol was used as solvent. About 150 g of *S. aromaticum* and *A. occidentale* powders were soaked separately in extraction bottles containing 450 ml of absolute methanol each for 3 days. The mixtures were stirred occasionally with a glass rod. The resulting mixture was filtered using a double layer of Whatman No. 1 filter paper. The solvent for each sample was evaporated by using a rotary evaporator at 30 to 40 °C, with rotary speed of 3 to 6 rpm, for 8 hours (Udo, 2011). The resulting oils were air-dried in order to remove traces of solvents. From this stock solution, oil

concentrations of 1, 2, 3 and 4% were prepared by diluting 0.1, 0.2, 0.3 and 0.4 ml of extract in 9.9, 9.8, 9.7 and 9.6 ml of solvent, respectively (Ashamo and Akinnawonu, 2012; Ileke *et al*., 2013).

Plant powders toxicity. The powders of *S. aromaticum* and *A. occidentale* were thoroughly mixed with 20 g of sorghum grains in 250ml plastic containers at rates 0.5, 1.0, 1.5, 2.0 and 2.5 g. Untreated sorghum grains were set as controls in the experiment. Ten pairs of newly emerged adults of *S. oryzae* were introduced into each container and covered with lids. The experiment was replicated four times in a completely randomised design.

Weevil mortality was observed daily for 4 days. The weevils were confirmed dead when there was no response to probing with sharp pin on the abdomen. After the fourth day, both dead and live insects were removed from each container.

The experiment was kept inside an insect cage for another 30 days, to allow for the emergence of the first filial (F_1) generation. The number of adults that emerged from each replicate was recorded. The grains were re-weighed using a Metler weighing balance and weight loss percent was determined as follow:

Plant oils toxicity on mortality. A series of 1, 2, 3 and 4% oils of *S. aromaticum* and *A. occidentale* were separately mixed with 20 g of disinfested sorghum grains in 250 ml plastic containers. The oils were thoroughly mixed with the grains. Ten pairs of newly emerged adults *S. oryzae* were introduced into each of the containers, including two controls, one treated with methanol and another without the solvent.

The set ups were replicated four times in a completely randomised design. Weevil mortality was observed daily for 4 days. The weevils were confirmed dead as described above. Both dead and live insects were removed on the fifth day and the experiment was kept for 30 days to allow emergence of F_1 generation. The number of adults that emerged from each replicate was recorded and weight loss was determined as described above.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Scientists (SPSS) 17.0 Software (SPSS, Inc., 2007). Means were separated using Turkey's Test.

RESULTS

Effect of powders The effect of *S. aromaticum* and *A. occidentale* powders is presented in Table 1. Weevil mortality increased with the concentration and time of exposure to the powders. *Syzygium aromaticum* at 2.5 g concentration achieved 100% mortality within 48 hours. This was significantly ($P<0.05$) different from other concentrations, except powder of *A. occidentale* at 2.5 g which recorded 97.5% weevil mortality. Both powders achieved complete insect mortality at all tested concentrations within 96 hours of application, except powder of *A. occidentale* at 0.5 g and 1.0 g concentrations, which could not achieve complete insect mortality within the same time of exposure.

Adult emergence of *S. oryzae* and weight loss of sorghum grains. Table 2 presents the effect of *S. aromaticum* and *A. occidentale* powders on adult emergence of *S. oryzae* and sorghum grain weight loss. Powders at all concentrations had significant effects on the emergence of the adult weevil. *Syzygium aromaticum* at rates of 1.5, 2.0 and 2.5 g completely prevented emergence of the adult weevil. *Anacardium occidentale* powder was able to prevent adult emergence of weevils at rates 2.0 and 2.5 g per 20 g of sorghum grains. *Syzygium aromaticum* at rates of 1.5, 2.0 and 2.5 g was able to prevent weight loss, but its effect was not significantly ($P>0.05$) different from grains treated with *A. occidentale* powder at rates 2.0 and 2.5 g.

***Syzygium aromaticum* and *A. occidentale* oils.** The effect of *S. aromaticum* and *A. occidentale* oils on mortality of *S. oryzae* is presented in Table 3. There were significant ($P<0.05$) differences in the effectiveness of the oil extracts within 24 and 48 hours of application. Three and 4% concentrations of the extract of *S. aromaticum*

TABLE 1. Mortality of adult *S. oryzae* on sorghum grains treated with *S. aromaticum* and *A. occidentale* powders

Plant material	Treatments (g 20 g^{-1}) of sorghum	Mean (%) mortality ± S.E in hours			
		24	48	72	96
Syzygium aromaticum	0.5	10.00±2.04^{b}	32.50 ±7.50^{b}	55.00±2.89^{b}	100.00±0.00^{c}
	1	27.50±2.50^{cd}	40.00±4.08^{bc}	95.00±2.89^{c}	100.00±0.00^{c}
	1.5	45.00±2.89^{e}	82.50±7.50^{d}	100.00±0.00^{d}	100.00±0.00^{c}
	2	60.00±4.08^{f}	87.50±2.50^{d}	100.00±0.00^{d}	100.00±0.00^{c}
	2.5	77.00±2.50^{g}	100.00±0.00^{e}	100.00±0.00^{d}	100.00±0.00^{c}
Anacardium occidentale	0.5	20.00 ±4.08^{c}	37.50±2.50^{bc}	57.50 ± 2.50^{b}	80.00 ± 4.08^{b}
	1	32.50 ±7.50^{d}	42.50 ±7.50^{c}	77.50 ± 2.50^{c}	89.00 ± 2.89^{b}
	1.5	62.50± 7.50^{f}	82.50± 7.50^{d}	100.00±0.00^{d}	100.00±0.00^{c}
	2	66.00±4.08^{f}	88.00±2.89^{d}	100.00±0.00^{d}	100.00±0.00^{c}
	2.5	70.00± 4.08^{g}	97.50± 2.50^{e}	100.00±0.00^{d}	100.00±0.00^{c}
Control	0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0. 00±0.00^{a}

Each value is a mean ± standard error of four replicate. Means followed by the same letter along the column are not significantly different (P>0.05) using Turkey's Test

TABLE 2. Effect of *S. aromaticum* and *Anacardium occidentale* powders on adult emergence of *S. oryzae* and weight loss of sorghum grains

Plant material	Concentration (g 20 g^{-1}) of sorghum grain	Adult emergence	Grain weight loss (%)
Syzygium aromaticum	0.5	4.75±0.85^{d}	3.50±0.89^{c}
	1	2.50±0.27^{c}	1.75±0.04^{b}
	1.5	0.00±0.00^{a}	0.00±0.00^{a}
	2	0.00±0.00^{a}	0.00±0.00^{a}
	2.5	0.00±0.00^{a}	0.00±0.00^{a}
Anacardium occidentale	0.5	4.50 ± 0.27^{d}	3.20 ± 0.71^{c}
	1	2.00 ± 0.17^{bc}	1.50 ± 0.27^{b}
	1.5	1.88 ± 0.24^{b}	1.10 ± 0.17^{b}
	2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2.5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control	0	52.50±7.50^{e}	70.50±4.24^{d}

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (P>0.05) using Turkey's Test

achieved 100% weevil mortality within 24 hours of application; while only 4% of *A. occidentale* oil achieved complete insect mortality.

Effect of oils on adult emergence and weight loss. The effect *S. aromaticum* and *A. occidentale* oils on adult emergence of *S. oryzae* and weight loss of sorghum grains is presented in Table 4. At all the tested concentrations, plant oils prevented the emergence of the adult weevil and weight loss of the grains; except *A. occidentale* oil at 1% concentration, which recorded 1.24% adult emergence. However, the effect of the 1% concentration was not significantly (P>0.05) different from other concentrations.

TABLE 3. Mortality of adult *S. oryzae* in sorghum seeds treated with oil extract of *S. aromaticum*

Plant materials	Concentration (%)	Mean (%) mortality ±S.E at 24 to 96 hr post treatment			
		24	48	72	92
Syzygium aromaticum	1	50.00±4.08^{d}	87.50±2.50^{c}	100.00±0.00^{c}	100.00±0.00^{c}
	2	75.00±2.89^{f}	100.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{c}
	3	100.00±0.00^{g}	100.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{c}
	4	100.00±0.00^{g}	100.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{c}
Anacardium occidentale	1	45.00 ± 2.89^{c}	60.00 ± 4.08^{b}	100.00±0.00^{c}	100.00±0.00^{c}
	2	60.00 ± 4.08^{e}	85.00 ± 2.89^{c}	100.00±0.00^{c}	100.00±0.00^{c}
	3	80.00 ± 4.08^{f}	100.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{c}
	4	100.00±0.00^{g}	100.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{c}
	Solvent control	6.00±2.04^{b}	17.50±2.50^{b}	30.00±4.08^{b}	37.50±2.50^{b}
	Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (P>0.05) using Turkey's Test

TABLE 4. Effect of oil extract *S. aromaticum* on adult emergence of *S. oryzae* and weight loss of sorghum grains

Plant materials	Conc. (%)	Number of adult emergence	Weight loss (%)
Syzygium aromaticum	1	0.00±0.00^{a}	0.00±0.00^{a}
	2	0.00±0.00^{a}	0.00±0.00^{a}
	3	0.00±0.00^{a}	0.00±0.00^{a}
	4	0.00±0.00^{a}	0.00±0.00^{a}
Anacardium occidentale	1	1.20±0.24^{a}	0.89±0.04^{a}
	2	0.00±0.00^{a}	0.00±0.00^{a}
	3	0.00±0.00^{a}	0.00±0.00^{a}
	4	0.00±0.00^{a}	0.00±0.00^{a}
	Solvent control	45.00±2.89^{b}	72.50±7.50^{b}
	Control	52.50±7.50^{b}	77.50±2.50^{b}

Each value is a mean ± standard error of four replicate. Means followed by the same letter along the column are not significantly different (P>0.05) using Turkey's Test

DISCUSSION

From the result obtained in this research, it was observed that the powders and oils of *S. aromaticum* and *A. occidentale* have considerable mortality effects on adult *S. oryzae* (Tables 1 and 2). Mortality of adult insects increased as the exposure period to the powders and oils increased, showing that the toxic volatile components of the powders and oils of these plants have some levels of doggedness. The high mortality recorded by the oils of these plants could be associated with the fact that oil extracts usually embody all the secondary metabolites, which have high toxic effects on insects (Forim *et al*., 2012). Powders and oils have been reported to inhibit locomotion of adult insects (Adedire *et al*., 2011; Ileke *et al*., 2013).

The weevils were unable to move freely because of powders and oils presence and this might have affected mating activities and sexual communication (Ileke and Olotuah, 2012). One of the main mechanisms of action of plant extracts is their ability to penetrate the chorion of insects'

eggs *via* the micropyle, thereby leading to the death of developing embryos through asphyxiation (Don-Pedro, 1989a; b; Ileke and Olotuah, 2012; Ashamo *et al*., 2013). The result of this study indicated that the powders and oil extracts of clove seeds and cashew nuts had a direct effect on post embryonic survival of this insect which resulted in prevention of adult emergence.

Reduction in progeny growth may be due to early mortality and partial or complete retardation of embryonic development (Dike and Mbah, 1992). The effect of the powders and the oils on the adult weevil emergence might be because of the poor laying capacity of the weevil due to physiological changes induced by them. The effect of clove seeds and cashew nuts may also be due to the death of the insect larvae which was unable to fully cast off their exoskeleton which typically remained linked to the posterior part of their abdomen (Oigiangbe *et al*., 2010).

With an increasing awareness of environmental pollution, the natural-products concept has returned to the global arena as a proposed alternative for pest control. Natural products reportedly have reduced environmental consequences which have led to the emergence of several studies (Forim *et al*., 2012).

Prior to the development and commercial success of synthetic insecticides beginning in the 1940s, botanical insecticides were major weapons in the farmer's arsenal against crop pests (Isman, 2008). In many villages of Africa, farmers often mix plant materials (e.g. *Piper guineenses* and neem with stored grains against pest infestation (Akinkurolere, 2007).

The ability of clove seed and cashew nut powders and oils to prevent the weight loss of the treated grains may be due to the high mortality rate of the adult weevils and inability of the eggs to emerge, thereby reducing metabolic activities of insects. The result of this work is in agreement with previous reports (Oparaeke and Bunmi, 2006; Adedire *et al*., 2011); whereby *A. occidentale* oil prevented weight loss of bambara groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata* L.) seeds infested by *C. maculatus* and *C. subinnotatus* respectively.

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