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Mothcidal activity of three botanical extracts against *Sitotroga cerealella* (Olivier, 1789) (Lepidoptera: Gelechiidae) infesting rice grains

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Abstract. The renewed awareness on the potential adverse effects associated with several synthetic insecticides has continually served as impetus to search for safer and more sustainable pesticides derived from plant origin. In this study, the mothcidal activity of *Alstonia boonei* (de wild), *Garcinia kola* (Heckel) and *Crassocephalum crepidoides* (Benth) against *Sitotroga cerealella* (Olivier, 1789) was investigated at ambient temperature (28 ± 2 °C) and relative humidity (75% ± 5%). Adult moths were exposed to 1%, 2% and 3% concentration of each oil extract and mortality was recorded at day 1, 2, 3, 4 and 5 post-treatment, respectively. The control experiments were also set-up. All the three botanicals exhibit moth mortality irrespective of the concentration and exposure time. However, only *C. crepidoides* elicited complete moth mortality (100%) irrespective of the concentration and exposure time while *A. boonei* oil extract applied at 1%, 2% and 3% showed the least moth mortality regardless of the exposure time. Similarly, 2% and 3% concentration of each botanical showed complete moth mortality at 4 and 5 days post-treatment, respectively. Least (6.60%) and highest (23.30%) adult emergence was also observed in grain exposed to 1% of *C. crepidoides* and *A. boonei*, respectively, while *G. kola* and *C. crepidoides* applied at 3% completely prevented moth emergence and loss in weight in rice grains. This study showed that oil extract of *G. kola* and *C. crepidoides* would provide a much desired wherewithal to synthetic insecticides in the management of *S. cerealella* infesting rice grains in Nigeria.

Keywords: *Alstonia boonei*; *Garcinia kola; Crassocephalum crepidoides*; *Sitotroga cerealella*; mothcidal.

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Introduction

In Nigeria, one of the most critical expectations of the populace from the government is the need to increase the nation's domestic food production; as the little food being produced are not enough, and still suffers from poor processing techniques which pre-dispose them to infestation by insect pests such as weevils, beetles and moths. The inability of the country to achieve self-sufficiency in food production is further aggravated by her over-reliance on oil proceeds which has led to the negligence of other sectors of the nation's economy for several years. The recent dwindling in the nation's income due to incessant drop in global oil price has further necessitated the need to diversify the nation's economy away from oil dependency. Agriculture has been advocated as one of the alternative wherewithal that could serve as the mainstay of the nation's economy (Adubi, 2004). This has led to the establishment of various Agricultural Research Institute all over the country saddled with the responsibility of developing production and utilization technologies for various crops in Nigeria (Olatunde, 2014). Notwithstanding, most Nigeria farmers and crop merchants still record huge post-harvest losses due to low level of literacy on modern storage techniques that could guarantee food security in Nigeria.

Rice is one of the cereal crops that could contribute to the realisation of food security in sub-Sahara Africa. Nigeria remained the largest producer, consumer and importer of rice in West-Africa with a local production of about 4.2 million tonnes (FAO, 2013). The crop plays a major nutritional role in the diet of most Nigerians and also forms an important part of their daily nourishment. Nonetheless, the country still struggles to achieve self-sufficiency in rice production due to various limitations of which insect pest infestation during storage served as one of the most important constraints. *Sitotroga cerealella* (Olivier) has been implicated as one of the destructive storage pest militating against rice production in Nigeria (Ashamo and Akinnawonu, 2012). Synthetic chemicals however remained the most effective method of managing stored product pest in the country (Chedi and Aliyu, 2010). However, recent ban by the European Union on some agricultural food export from Nigeria due to high level of pesticides has necessitated the need for a better and sustainable means of protecting farm produce during storage. Likewise, the various adverse environmental, biological and health consequences associated with most synthetic chemicals have further prompted the urgent need for better control measures.

The search for effective and eco-friendly means of pest control on rice grains has led to the touting of several botanicals as a substitute to most synthetic chemicals. This has led to the usage of several plant materials in the control of *S. cereallela* (Zaidi et al., 2004; Iqbal, 2010; Ashamo and Akinnawonu, 2012). However, high abundance of prospective insecticidal floras in Nigeria has given several researchers the impetus to constantly investigate more floras for their efficacy against this infamous pest of rice. This is needed for the actualisation of the nation's much desired self-sufficiency in rice production. Thus, the mothcidal efficacy of *Alstonia boonei* (de wild), *Garcinia kola* (Heckel) and *Crassocephalum crepidoides* (Benth) against *S. cerealella* infesting rice grains was investigated in this study.

Materials and methods

Insect culture

The infested paddy stock culture of *S. cerealella* and clean paddy rice variety (FARO-52) used in this study were obtained from Agricultural Development Project (ADP), Akure, Ondo State, Nigeria. Clean paddy rice variety (FARO-52) was disinfested in the freezer at -18 ^{o}C for two weeks and thereafter allowed to equilibrate in the laboratory for three days. 200 g of disinfested clean paddy rice variety were introduced into 1.5 L plastic containers and moths from starter culture were later reared on the paddy rice variety. The containers were covered with perforated lid and muslin cloth to prevent the escape of insects and allow air into them. The moths were reared for two generations on the paddy rice variety to eliminate the effect of parent food before being used for bioassay test. The plastic containers used for insect rearing were kept inside cages at ambient temperature of 28 ± 2 ºC and $75\% \pm 5\%$ relative humidity.

Procurement and processing of plant materials

The three plant materials used in this study were obtained from three different sources. Leaves of *G. kola* and *C. crepidoides* were sourced from Aba Oyo Village and Odopetu Market, Akure, Ondo State, respectively. Stem bark of *A. boonei* was however obtained from Fayemi Market, Ado-Ekiti, Ekiti State. Fresh leaves and stem bark of various plants were dried in the laboratory using a dry cabinet at a temperature of 40 °C and later pulverized separately using NAKAI NJ-1731 electric blender. Powders of each plant material were later sieved with a mesh size of 1 mm^2 before being separately stored in plastic containers with airtight lids for subsequent use.

To prepare the extracts, Metler beam weighing balance was used to weigh twenty grams of each pulverized plant material into a muslin cloth. The muslin cloth containing each botanical was later transferred into the thimble and extracted with methanol in a soxhlet apparatus. The extraction was carried out for 3-4 h depending on the plant material. The extraction was terminated when the solvent in the thimble became clear. The thimble was removed from the unit and the solvent recovered by distilling in the soxhlet extractor. The resulting extracts contained both the solvent and the oil. 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. This is important for the avoidance of false concentrations.

From this main stock solution, different concentrations of 1%, 2% and 3% oil concentrations were made. A concentration of 1% was made by diluting 0.1 mL of plant extract in 9.9 mL of methanol (solvent). Two percent (2%) concentration was made by diluting 0.2 mL of plant extract in 9.8 mL of methanol while 3% concentration was made by diluting 0.3 mL of each plant extract with 9.7 mL of methanol. The various concentrations were made using small glass bottles and graduated syringes. After each dilution, the syringe was rinsed with the solvent while different syringes were used for different plant material extracts.

Effect of oil extracts on mortality, adult emergence, inhibition rate (%IR) and grain weight loss

Twenty grams of paddy rice (FARO-52) seeds were weighed into 170 mL plastic containers using Metler beam PB 3002 weighing balance and 1 mL of each plant extract concentrations of 1%, 2% and 3% was separately mixed with the paddy rice. Each container was left for 1hr to allow the methanol solvent to vapourise leaving only the oil extracts of each botanical on the seeds. Two controls were set up for each plant material, one with solvent alone (Control A) and the other with neither solvent nor extract (Control B). Air space was created by cutting the centre portion of the lid of each container and replacing with muslin cloth. Five pairs of adult moths (0-24 h old) were introduced into each plastic container using aspirator. Each treatment was replicated three (3) times and adult mortality was recorded daily for 5 days. Both dead and live insects were removed on the sixth day and the experiments were left for 30 days to allow for adult emergence and the number of emerged adults was counted. Inhibition rate (%IR) in adult emergence was calculated using the method described by Tapondju et al. (2002).

$$\%IR = \frac{C_n - T_n}{C_n} x \frac{100}{1}$$

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 after 30 days was calculated using the formula below:

$$\%\text{Weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{initial weight}} \times \frac{100}{1}$$

Statistical analysis

All data on adult mortality were checked for normality based on Shapiro-Wilk Test before being subjected to one-way analysis of variance (ANOVA) ($p < 0.05$) and treatment means were separated using Tukey's Test. Analyses were carried out using SPSS 17.0 software package.

Results

Effect of oil extract on the adult mortality of *S. cereallella*

Table 1 to 3 show the effect of 1%, 2% and 3% oil extract of *A. boonei*, *G. kola* and *C. crepidoides* on adult mortality of *S. cereallella* exposed for 5 days. Of all the botanicals, *C. crepidoides* elicited complete moth mortality (100%) irrespective of the concentration and exposure time. Similarly, 2% and 3% concentration of each botanical showed complete moth mortality at 4 and 5 days post-treatment, respectively. Significantly higher (P > 0.05) moth mortality was also observed in moth treated with botanicals when compared to their counterpart in control. Solvent treated control also showed 10% moth mortality after 5 days post-treatment (Tables 1 to 3). Least moth mortality was also observed in *A. boonei* oil extract applied at 1%, 2% and 3%, respectively, regardless of the exposure time (Tables 1 to 3). Significant differences however existed in the mortality of moths exposed to 1% and 2% concentration of each botanical after day 1 (24 h post-treatment) (Table 1 and 2).

Table 1. Effect of 1% oil extracts of the three botanicals on the percentage mortality of adult *S. cerealella*.

Botanical	**Duration**				
	1	**2**	**3**	**4**	**5**
A. boonei	36.70 ± 0.33^{b}	46.70 ± 0.88^{b}	60.00 ± 0.58^{b}	83.30 ± 0.88^{b}	96.70 ± 0.33^{c}
G. kola	56.70 ± 0.96^{c}	86.70 ± 0.67^{c}	100 ± 0.00^{c}	100 ± 0.00^{c}	100 ± 0.00^{c}
C. crepidoides	100 ± 0.00^{d}	100 ± 0.00	100 ± 0.00^{c}	100 ± 0.00^{c}	100 ± 0.00^{c}
Control A	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	10.00 ± 0.58^{b}
Control B	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Means followed by the same letter along the column are not significantly different (P > 0.05) using Tukey Test.

Table 2. Effect of 2% oil extracts of the three botanicals on the percentage mortality of adult *S. cerealella*.

Botanical	**Duration**				
	1	**2**	**3**	**4**	**5**
A. boonei	46.70 ± 0.33^{b}	66.70 ± 0.88^{b}	83.30 ± 0.67^{b}	100 ± 0.00^{b}	100 ± 0.00^{c}
G. kola	83.30 ± 0.88^{c}	96.70 ± 0.33^{c}	100 ± 0.00^{c}	100 ± 0.00^{b}	100 ± 0.00^{c}
C. crepidoides	100 ± 0.00^{d}	100 ± 0.00^{c}	100 ± 0.00^{c}	100 ± 0.00^{b}	100 ± 0.00^{c}
Control A	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	10.00 ± 0.58^{b}
Control B	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Means followed by the same letter along the column are not significantly different (P > 0.05) using Tukey Test.

Table 3. Effect of 3% oil extracts of the three botanicals on the percentage mortality of adult *S. cerealella*.

Botanical	Duration				
	1	2	3	4	5
A. boonei	60.00 ± 0.00[b]	76.70 ± 0.33[b]	100 ± 0.00[b]	100 ± 0.00[b]	100 ± 0.00[c]
G. Kola	96.70 ± 0.33[c]	100 ± 0.00[c]	100 ± 0.00[b]	100 ± 0.00[b]	100 ± 0.00[c]
C. crepidoides	100 ± 0.00[c]	100 ± 0.00[c]	100 ± 0.00[b]	100 ± 0.00[b]	100 ± 0.00[c]
Control A	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	10.00 ± 0.58[b]
Control B	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Means followed by the same letter along the column are not significantly different ($P > 0.05$) using Tukey Test.

Effect of botanical extracts on the emergence and inhibition in adult emergence of *S. cerealella*

Table 4 and 5 shows the effect of oil extract of the three botanicals on the adult emergence and inhibition in adult emergence of *S. cerealella* exposed to concentrations of 1%, 2% and 3%, respectively. Significantly higher ($P < 0.05$) adult emergence was observed in grains exposed to solvent treated control (Control A) (70.00) and ordinary control (Control B) (73.30) when compared to their counterpart exposed to botanical oils (Table 4). Among the treated grains, least (6.60%) and highest (23.30%) adult emergence was observed in grain exposed to 1% of *C. crepidoides* and *A. Boonei*, respectively (Table 4). Also, *G. kola* and *C. crepidoides* applied at 2% and 3% completely prevented moth emergence with the exception of *G. kola* applied at 2% where emergence of 3.33% was observed (Table 4).

Significantly lower ($p < 0.05$) percentage inhibition rate was observed in adult emergence of grains exposed to both control when compared to their counterpart treated with botanical oil extracts (Table 5). However, complete inhibition (100%) was observed in adult emergence of grains exposed to 2% and 3% oil extracts of *C. crepidoides* and *G. kola*, respectively (Table 5). The only exception was observed in grains exposed to 2% oil extract of *G. kola* which showed 95.46% inhibition rate (Table 5). Of all the three botanicals, *A. boonei* showed the least emergence (59.29-95.46%) while the highest inhibition rate was observed in grains treated with *C. crepidoides* oil extracts (91.04%-100%) (Table 5). Median inhibition in adult emergence (72.86%-100%) was however observed in grains treated with *G. kola* oil extracts (Table 5).

Effect of oil extracts on weight loss in rice grains

The weight loss in rice grains treated with different concentrations of oil extracts from the three botanicals is prevented in table 6. Significantly higher ($P < 0.05$) weight loss was observed in grains exposed to both solvent control (15.78) and ordinary control (18.63) when compared to weight loss in grains treated with botanical extracts. At the highest experimental concentration (3%), *C. crepidoides* and *G. kola* however completely (0.00) prevented loss in weight in rice grains and this was significantly different ($P<0.05$) from weight loss observed in both control. Of all the botanicals, least reduction (0.61-8.70%) in weight loss was observed in grains exposed to *A. boonei* while the highest reduction (0.00-2.10%) in weight loss was observed in grains treated with *C. crepidoides*.

Table 4. Effect of oil extracts of three botanicals on the adult emergence of *S. cerealella*.

Botanical	Concentration (%)		
	1	2	3
A. boonei	30.00 ± 0.00[c]	23.30 ± 0.33[b]	3.33 ± 0.33[a]
G. kola	20.00 ± 0.37[b]	3.33 ± 0.33[a]	0.00 ± 0.00[a]
C. crepidoides	6.60 ± 0.33[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Control A	70.00 ± 0.57[d]	70.00 ± 0.57[c]	70.00 ± 0.57[b]
Control B	73.70 ± 0.88[d]	73.33 ± 0.88[c]	73.30 ± 0.88[b]

Means followed by the same letter along the column are not significantly different (P > 0.05) using Tukey Test.

Table 5. Effect of oil extracts of three botanicals on inhibition rate (%IR) in emergence of *S. cerealella*.

Botanical	Concentration (%)		
	1	2	3
A. boonei	59.29 ± 0.00[b]	68.25 ± 0.21[b]	95.46 ± 0.12[b]
G. kola	72.86 ± 0.13[c]	95.46 ± 0.12[c]	100 ± 0.00[b]
C. crepidoides	91.04 ± 0.21[d]	100 ± 0.00[c]	100 ± 0.00[b]
Control A	5.02 ± 0.03[a]	4.54 ± 0.00[a]	4.54 ± 0.00[a]
Control B	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Means followed by the same letter along the column are not significantly different (P > 0.05) using Tukey Test.

Table 6. Effect of oil extracts of three botanicals on weight loss in rice grains.

Botanical	Concentration (%)		
	1	2	3
A. boonei	8.70 ± 0.12[c]	4.91 ± 0.79[b]	0.61 ± 0.01[a]
G. Kola	5.86 ± 0.37[b]	0.71 ± 0.71[a]	0.00 ± 0.00[a]
C. crepidoides	2.10 ± 0.18[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Control A	15.78 ± 0.04[d]	15.78 ± 0.04[c]	15.78 ± 0.04[b]
Control B	18.63 ± 0.02[e]	18.63 ± 0.02[d]	18.63 ± 0.02[c]

Means followed by the same letter along the column are not significantly different (P > 0.05) using Tukey Test.

Discussion

The renewed awareness on the potential adverse effects associated with several synthetic insecticides coupled with high demand for safer and more sustainable agricultural practices has continuously serves as impetus for the use of pesticides derived from plant origin. Consequently, most farmers in developing countries have resorted to the use of botanicals for pest management to circumvent the high cost and potential risks associated with most synthetic chemicals. *Alstonia boonei*, *Garcinia kola* and *Crassocephalum crepidoides* are some of the botanicals in Nigeria that have been established to possess medicinal properties (Burkil, 2004; Odugbemi, 2006).

This present study therefore investigates the mothcidal activity of oil extracts of *A. boonei*, *G. kola* and *C. crepidoides* against *S. cerealella* infesting rice grains in Nigeria. Various results obtained showed that all the botanical oil extracts resulted in moth mortality, reduced adult emergence and weight loss in grains irrespective of the exposure days and concentration when compared to control. This shows that they all possessed mothcidal activity against *S. cerealella*, thus corroborating the findings of Iqbal (2010), Ashamo and Akinnawonu (2012) and Akinneye and Oyeniyi (2016) where the entomotoxic effect of several botanical pesticides against *S. cerealella* have been reported. The high mortality associated with the oils of these botanicals may be ascribed to disruption in the normal respiratory activities of adult moths leading to the asphyxiation and subsequent death (Ashamo *et al.,* 2013; Ogungbite and Oyeniyi, 2014).

Moth exposed to grains treated with oil extract of *C. crepidoides* however showed the highest mortality when compared to other botanicals. The ability of the oil extract from *C. crepidoides* to elicit highest mortality may be accredited to the pungent smell of this plant which may have increased its toxicity against adult moths. Of all the three botanicals, *C. crepidoides* showed the highest pungent smell. Moths are known to be active fliers which may have increased the rate of diffusion of *C. crepidoides* vapour through the insect spiracles. This might have led to the blockage of moth spiracles, suffocation and higher mortality observed in insects exposed to oil extract of *C. crepidoides* when compared to others.

The higher efficacy of the three botanicals, particularly *C. crepidoides* oil extract in preventing adult emergence when compared to control may also be ascribed to some toxic phytochemical compounds that have been reported in these plants. For instance, Oduse *et al*. (2012) reported the presence of tannins, flavonoids and alkaloids in *C. crepidoides*. Most of these compounds had been reported for their considerable toxicity and antifeedant effect towards insects (Yang *et al*., 2006). These compounds might have dissolved in oils and form a protective coating on the paddy rice. Tannins for example are known to induce indigestion in insects by binding with molecules such as protein and polysaccharides to form complexes which could consistently reduce the assimilation and efficiency in the exposed insect (Willmer *et al*., 2000; Lattanzio *et al*., 2005). This might have resulted in starvation of *S. cerealella* larvae leading to death and subsequent reduction in adult emergence in grains exposed to oil extract of *C. crepidoides*. Yang et al. (2006) has also reported that alkaloids and flavonoids disturb and reduced larval growth and survival which ultimately affect the life cycle of adult insects.

Oil extracts of the three botanicals also significantly reduced weight loss in treated grains when compared to control. This shows that all the three botanicals could help in preserving rice grains in Nigeria. However, grains exposed to *C. crepidoides* oil extract show the least weight loss while their counterpart exposed to *A. boonei* showed the highest weight loss. The ability of *C. crepidoides* oil extract to prevent weight loss especially at the highest experimental concentration may be attributed to the inability of the larvae of *S. cerealella* to feed on the treated rice grains. Similar observation has been reported by Akinneye and Oyeniyi (2016) on rice grains treated with *Cleistopholis patens* (Benth).

Conclusion

Various results obtained in this study showed that oil extract from *C. crepidoides* showed the highest mothcidal activity of the three botanicals due to its ability to exhibit complete moth mortality, inhibit adult emergence and prevent weight loss in treated grains at the highest experimental concentration while *A. boonei* showed the least mothcidal activity. Due to the non-toxic nature of *C. crepidoides* on humans as it is normally cooked and taken as vegetable soup in Nigeria (Oduse, 2012); further studies should be carried out on this botanical to establish the mothcidal active compounds. Crop merchants and peasant farmers should also be encourage to use oil extract from this plant in protecting their paddy rice against *S. cerealella* attacking paddy rice in Nigeria. This could help in reducing high loss usually associated with

S. cerealella infestation of stored paddy rice Nigeria.

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Conflicts of interest

Authors declare that they have no conflict of interests.

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