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*Full Length Research*

# Laboratory assessment of botanical pesticides and application strategies against kola weevil, *Balanogastris kolae* (Coleoptera : Curculoinidae)

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**Bioactivity of plant extracts viz *Lycopersicon esculentum*, *Hyptis suaveolens*, *Cymbopogon citratus*, *Loranthus braunii*, *Alstonia boonei* and *Sarcocephalus latifolius*, including their mode of action were investigated against *Balanogastris kolae* in the laboratory. Ten weevils were placed in each small muslin cloth bag and the mouth tied with an extended rope with which it was lowered half way into the 500 mls plastic container with cover containing 2mls of each botanical in fumigant test. In topical action, ten microlitres of each plant was applied directly on to the dorsal surface of the weevils in each Petri-dish with the aid of a micro-syringe. In residual method, the filter paper was drenched with 60 microlitres of the botanicals and allowed to be air-dried for 5 minutes. Ten weevils were placed in each of the Petri-dishes to be in contact with the residue of the botanicals. Each treatment was replicated four times. The Petri-dishes were perforated on the top to provide aeration and prevents suffocation of the test insects. Mortality counts were taken and recorded after every 20 minutes for 120 minutes. The results of laboratory studies showed that standard insecticide gave more quick action than the other botanical insecticides. Aqueous *C. citratus* and *H. suaveolens* produced 60% mortality within 120 minutes compared to less than 50% mortality in the cases of others, during the same period. Ethanolic extracts caused 100% mortality of exposed weevil within 20 minutes whereas the aqueous extracts achieved 10% or none for the same period. Mortality of weevils increased with high period of exposure to these potential natural insecticides under residual, topical and fumigant action in aqueous extracts. However, ethanolic extracts gave quick knock down effect and achieved high weevil mortality during the exposure periods. Hence, the results indicate that the botanicals with any of the topical or fumigant action may provide effective control of kola weevil and other field pests.**

**Key words:** Mortality, botanical, Fumigant, Residual, Topical

## INTRODUCTION

Nigeria produces about 70% of world's kola nut with an annual estimated production of 200,000 metric tonnes of fresh nuts, mostly from South west, Nigeria (CBN, 2002). The south west accounts for about 88% of the produce. On the other hand, only about 10% of the produce is exported, while the rest are consummed locally (Oluokun and Oladokun, 1999; Okunade, 2003).

Kola nut, which is primarily chewed because of its stimulants contains about 1% protein, 1.35% fats and 45% starch (Quarcoo, 1973). Its principal constituents are caffein, theobromine, tannins, phenolics, kolatin and kolanin. The presence of alkaloids and other phytochemicals makes kola nut suitable for the manufacture of pharmaceutical products and beverages. The tannin content is also a good source of dyes in textiles and thread (Esther *et al.*, 2010). It also has industrial usage for the production of drugs, soft drinks, wines, candles, beverages, animal feed formulation, liquid soap and dyes (Beattie, 1970; Daramola, 1978). Some Africans chew the nut for stamina, to ease hunger, aid digestion and as an aphrodisiac. It is also believed to dispel sleep, thirst and hunger and act as an anti-depressant. Tachie-Obeng and Browns (2006) observed that beverage could be made by boiling powdered seed in water. The nut plays important part in the cultural, social, trado-religious life of the Nigerian people. Consequently, it is valued in

Nigerian culture as a sign of friendship and peace and is consumed ("broken") at re-unions, during meetings, ceremonies and festivals. Predominantly grown in the south western states of Nigeria, the nut is celebrated with awe by the Igbo tribe of the south-east, Nigeria. They regard it in high esteem and as such often offered as a gift to traditional leaders and visitors. And in northern Nigeria, where the bulk of kola nut is chewed, it is greatly relished as a stimulant in substitute for alcoholic drinks. As a result, there is a heavy trade of kola from the humid southern regions to the northern arid parts of West Africa. The stem of the trees are used in the building industry, for making furniture, canoe and carvins. In Africa, the pods are used to ease labour pain.While the demand is rising, the production remains low due to several limiting factors amongst which are the insect pests. The insect pests of economic importance are the weevils, Balanogastris kolae and Sophrorhinus spp. They are field-to-store pests which can cause up to 100% loss if left uncontrolled in storage (Asogwa *et al*., 2008). The weevil infestation also predisposes the nuts to secondary invasion by other micro organisms, especially fungi which is further lower the market value and eventually results in total destruction of the nuts. Consequently, kola nuts storage is usually laborious because the freshness of the nuts needs to be maintained for several months. Esther *et al*., (2010) observed that the major problem encountered in kola nut trading business was basically that of storage with insect pest causing 53.33% kola nut losses which reduces the value and invariably the price of stored nuts.

The present study was conducted to test the application methods and efficacy of crude aqueous and alcoholic extracts of several local plants known to have insecticidal properties for the control of kola nut weevils using the standard insecticide as a basis of comparison. The ultimate objective is to identify botanicals that are environmentally friendly and affordable to farmers for kola nut weevil control.

## MATERIALS AND METHODS

### Insect culture and maintenance

Balanogastris kolae specimens used in this study were derived from a colony arising from naturally infested kola nuts bought from Ogunmakin market, Ogunmakin and fallen pods in CRIN farm, Ibadan. The nuts were then placed inside small polythene bags designed to keep the nuts fresh for as long as required for weevil development in place of fresh banana or Dorax sp. leaves used in the traditional storage system (Daramola, 1973). The polythene bags were perforated, and water droplets inside the bags were wiped off every other day to prevent fungal infection. The newly emerged adult B. Kolae were used to infest *Cola nitida* as appropriate in each experiment.

### Sources of plant materials

Fresh and healthy leaves *Hyptis suaveolens*, *Cympogon* spp, *Loranthus braunii*, *Alstonia boonei* and Opasin of were collected from the field at CRIN Headquarters, Ibadan. The collection was based on selection criteria such as usage of plants in folk medicine and taxonomic closeness of the plant to families known to possess biologically active compounds (Beaver, 1986; Azeez, 2012).

### Preparation of extracts

The plant extracts were prepared as described by Azeez (2006) and Oyedokun *et al.* (2011) with some modifications. The collected leaves were thoroughly rinsed in distilled water twice to remove dirts and allowed to drain on a wire mesh. 200 g each of the plant materials were weighed separately into sterile mortar and pestle. Aqueous extracts were prepared by pounding the weighed leaves of each plant materials into 200 ml of distilled water with which the stock solution of each extract was prepared. These mixtures were allowed to stay for 24 hours, heated at $60^0$C for 45 minutes in a water bath (Tecan-WB3, Techne Incorporation, New Jersey, USA), later shaken thoroughly and sifted with muslin cloth.

Similarly, ethanolic extract was prepared using 200 ml of ethanol (99.5%) (Absolute AR grade (Thornton and Ross Laboratories, England) as the solvent for 200 g fresh, healthy leaves each of plant materials following the extraction and dilution methods of the aqueous extract earlier described. The ethanolic extract was serially-diluted with distilled water at same rate as described above.

This research work was carried out in the Entomology laboratory of the Cocoa Research Institute of Nigeria, Nigeria at a temperature of 28± 30C and relative humidity 75± 5%.

### Bioassay tests

The laboratory tests were conducted using newly emerged kola weevils. The weevils were collected from the cultured lots. Treatments were replicated four times and the number of kola weevils used per replicate was ten.

**Topical application test:** Ten weevils were picked from the collection tray into a Petri-dish for each treatment. There were six treatments (pesticidal plants) and untreated or control. Ten microlitres of each plant was

**Table 1.** Mean mortality rate based on residual application.

| | Exposed period (minutes) and percentage weevil mortality | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Means | Aqueous extract | | | | | | Alcoholic extract | | | | | |
| Botanicals | No of weevil exposed | 20 | 40 | 60 | 80 | 100 | 120 | 20 | 40 | 60 | 80 | 100 | 120 |
| *L. esculentum* | 10 | 0 | 0 | 0 | 0 | 0 | 10 | 60 | 80 | 90 | 100 | 100 | 100 |
| *H. suaveolens* | 10 | 0 | 0 | 0 | 0 | 0 | 20 | 60 | 70 | 80 | 90 | 100 | 100 |
| *C. cymbopogon* | 10 | 0 | 20 | 30 | 30 | 40 | 60 | 50 | 60 | 70 | 70 | 80 | 100 |
| *L. braunii* | 10 | 0 | 0 | 0 | 0 | 20 | 30 | 50 | 50 | 60 | 80 | 90 | 100 |
| *A. boonei* | 10 | 0 | 10 | 10 | 20 | 30 | 30 | 50 | 50 | 60 | 60 | 80 | 100 |
| *S. latifolius* bark | 10 | 0 | 10 | 20 | 30 | 30 | 40 | 10 | 50 | 50 | 60 | 60 | 80 |
| *S. latifolius* leaf | 10 | 20 | 20 | 20 | 30 | 30 | 40 | 20 | 50 | 50 | 60 | 70 | 70 |
| Standard | 10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

applied directly on to the dorsal surface of the weevils in each Petri-dish with the aid of a micro-syringe (that is one microlitre/insect). Each treatment was replicated four times. The Petri-dishes were perforated on the top to provide aeration and prevents suffocation of the test insects. Mortality counts were taken and recorded after every 20 minutes for two hours (which was the maximum time period taken to achieve 100% mortality in over 90% of the Petri-dishes). A weevil was regarded as dead if it showed no signs of movement when touched lightly with a soft camel hairbrush or when it is lying flat on its black.

**Residual contact action:** This test was carried out with Petri-dishes fitted with filter papers. The filter paper was drenched with 60 microlitres of the botanicals and allowed to be air-dried for 5 minutes. Ten weevils were placed in each of the Petri-dishes to be in contact with the residue of the botanicals. Each of the 6 treatments with untreated or control was replicated four times. The Petri-dishes were perforated on the top to provide aeration and prevent suffocation of the test insects. Mortality counts were taken every 20 minutes for 2 hours using the same attributes of identifying a dead insect as earlier described.

**Fumigant action test:** The test was carried out using 500mls plastic containers (10cm x 10 cm x 15 cm) with cover and small muslin cloth bags measuring 7 cm x 10 cm. Ten weevils were placed in each bag and the mouth tied with an extended rope with which it was lowered half way into the plastic container containing 2mls of each botanical.

The numbers of weevils knocked down or killed during trials were recorded and expressed as percentage mortality. Also, the data were further subjected to the analysis of variance. Means were separated using Tukey Honest Test in order to test the levels of significance.

## RESULTS

Table 1 shows the residual action of aqueous and ethanolic extracts on percent kola weevil mortality. Aqueous *C. cymbopogon* extract recorded 60% weevil mortality at 120 minutes after exposure, while aqueous *L. esculentum* extract caused least weevil mortality (10%) at same exposure period. However, ethanolic extracts of *C. citratus*, *L. braunii* and *A. boonei* caused highest weevil mortality (100%) at 120 minutes after exposure. Whereas ethanolic extract of *S. latifolius* recorded the least mortality (10-20%). A varying high percent weevil mortality (50-100%) was recorded by ethanolic extracts of *L. esculentum*, *H. suaveolens*, *L. braunii* and *A. boonei* across durations ( 40,60,80 and 100 minutes) after exposure. The standard insecticide caused highest weevil mortality (100%) after various exposure period. Similarly, ethanolic extracts of *L. esculentum*, *H. suaveolens*, *L. braunii* and *A. boonei* recorded higher percent weevil mortality compared with aqueous extracts of the botanicals throughout exposure periods.

Table 2 shows the topical action of aqueous and ethanolic extracts on percent kola weevil mortality. Aqueous opasin extract caused highest weevil mortality (70%) at 80 minutes after exposure. Aqueous extracts caused weevil mortality at lower duration of exposure. Hence, no weevil mortality was recorded after 20 minutes of exposure. However, ethanolic extracts (*L. esculentum*, *H. suaveolens*, *C. citratus*, *L. braunii*, *A. boonei* and *S. latifolius*) including standard insecticide caused highest weevil mortality throughout exposure periods.

Table 3 shows the fumigant action of aqueous and ethanolic extracts on percent kola weevil mortality. Aqueous extracts of *H. suaveolens* and *C. citratus*, caused 60% weevil mortality at 120 minutes after exposure, while the least percent weevil mortality (10%) was recorded by aqueous *L. esculentum* extract. Thus, standard insecticide caused 100% weevil mortality the same periods. Similarly, ethanolic extracts of the botanicals(*L. esculentum*, *H. suaveolens*, *C. citratus*, *L. braunii*, *A. boonei* and *S. latifolius*) compete favourably with standard insecticide and recorded 100% weevil mortality.

Table 4 showed that standard insecticide sustained the highest weevil mortality (100%) through out exposure

**Table 2.** Mean mortality rate based on topical application.

| Botanicals | Means No of weevil exposed | Exposed period (minutes) and percentage weevil mortality | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aqueous extract | | | | | | Alcoholic extract | | | | | |
| | | 20 | 40 | 60 | 80 | 100 | 120 | 20 | 40 | 60 | 80 | 100 | 120 |
| *L. esculentum* | 10 | 0 | 0 | 0 | 0 | 0 | 50 | 100 | 100 | 100 | 100 | 100 | 100 |
| *H. suaveolens* | 10 | 0 | 20 | 20 | 40 | 60 | 60 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. cymbopogon* | 10 | 0 | 30 | 40 | 50 | 50 | 50 | 100 | 100 | 100 | 100 | 100 | 100 |
| *L. braunii* | 10 | 0 | 50 | 50 | 50 | 50 | 50 | 100 | 100 | 100 | 100 | 100 | 100 |
| *A. boonei* | 10 | 0 | 0 | 10 | 20 | 30 | 50 | 100 | 100 | 100 | 100 | 100 | 100 |
| *S. latifolius* bark | 10 | 0 | 50 | 50 | 70 | 70 | 70 | 100 | 100 | 100 | 100 | 100 | 100 |
| *S. latifolius* leaf | 10 | 0 | 0 | 0 | 20 | 30 | 50 | 100 | 100 | 100 | 100 | 100 | 100 |
| Standard | 10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 3.** Mean mortality rate based on fumigation application.

| Botanicals | Means No of weevil exposed | Exposed period (minutes) and percentage weevil mortality | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aqueous extract | | | | | | Alcoholic extract | | | | | |
| | | 20 | 40 | 60 | 80 | 100 | 120 | 20 | 40 | 60 | 80 | 100 | 120 |
| *L. esculentum* | 10 | 0 | 0 | 10 | 10 | 10 | 10 | 100 | 100 | 100 | 100 | 100 | 100 |
| *H. suaveolens* | 10 | 10 | 20 | 30 | 40 | 50 | 60 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. cymbopogon* | 10 | 10 | 10 | 30 | 40 | 40 | 60 | 100 | 100 | 100 | 100 | 100 | 100 |
| *L. braunii* | 10 | 20 | 30 | 30 | 40 | 40 | 40 | 100 | 100 | 100 | 100 | 100 | 100 |
| *A. boonei* | 10 | 20 | 20 | 30 | 30 | 30 | 30 | 100 | 100 | 100 | 100 | 100 | 100 |
| *S. latifolius* bark | 10 | 0 | 0 | 20 | 30 | 30 | 40 | 100 | 100 | 100 | 100 | 100 | 100 |
| *S. latifolius* leaf | 10 | 0 | 0 | 10 | 20 | 20 | 30 | 100 | 100 | 100 | 100 | 100 | 100 |
| Standard | 10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 4.** Bioactivity of pesticidal plants against Balanogastris kolae.

| Treatments | Duration on percent weevil mortality | | | | | |
|---|---|---|---|---|---|---|
| | 20 mins | 40 mins | 60 mins | 80 mins | 100 mins | 120 mins |
| *L. esculentum* | 44.20b | 58.00b | 60.00c | 50.00d | 42.00c | 67.000b |
| *H. suaveolens* | 44.20b | 55.80b | 65.8bc | 71.00b | 70.00b | 92.00a |
| *C. cymbopogon* | 41.7cb | 63.00b | 67.00bc | 63.8bc | 63.00bc | 80.00ba |
| *L. braunii* | 35.00cd | 50.80bc | 67.00bc | 67.00bc | 70.20b | 68.00ba |
| *A. boonei* | 41.70cb | 63.00b | 67.00bc | 63.80c | 63.00bc | 53.00c |
| *S. latifolius*/ bark | 33.3d | 48.00c | 71.00b | 68.80bc | 60.5bc | 50.00c |
| *S. latifolius*/ leaf | 40.8cbd | 45.00c | 60.90bc | 67.10bc | 73.00b | 83.00ba |
| Standard insecticide | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |

Means followed by the same letter in each column are not significantly different (P>0.05) according to Honest Tukey Test

periods. All the botanicals caused average weevil mortality within the duration. Table 5 showed the interaction among the various treatments used. They showed significant at (P<0.05) except number of replicate.

## DISCUSSION

Generally, all the botanicals including the standard insecticide tested caused varying degrees of percentage weevil mortality in different mode of actions (residual, topical and fumigant actions). The effectiveness of the botanicals were not brought to bear on the weevil mortality by the residual method of application at the lower period of exposure in aqueous extracts. This showed that the residual method of application did not enhance the toxicity of botanicals because it took a long period of time before the effect of toxicity was established

**Table 5.** 120 minutes exposure of *Balanogastris kolae.*

| ANALYSIS OF VARIANCE | | | | |
|---|---|---|---|---|
| **SOURCE** | **SS** | **Df** | **MS** | **MSR** |
| Botanicals | 18.48 | 7 | 2.64 | 7.66* |
| Methods | 14.04 | 2 | 7.02 | 20.38* |
| Solvent | 46. 02 | 1 | 46.02 | 133.62* |
| Replicate | 0.44 | 3 | 0.15 | $0.42^{N.S}$ |
| Botanical x Method | 21.96 | 14 | 1.57 | 4.55* |
| Methods x Solvent | 14.04 | 2 | 7.02 | 20.38* |
| Botanical x Method x Solvent | 40.44 | 21 | 1.93 | 5.59* |

*Significant at (P> 0.05; NS - Non significant

on the weevils. Mortality of weevils increased with high period of exposure to these potential natural insecticides under residual, topical and fumigant action. Previous works have demonstrated the potency of some botanicals to cause insect mortality (Ivbijaro and Agbaje, 1986). The residual mode of action of aqueous extract was very slow and may be regarded as slow poison. Consequently, a very low percent mortality was recorded at earlier period of exposure. However, no mortalities were recorded in the earlier period of exposure with residual and topical application. Therefore, the ethanolic extracts applied in topical and fumigant acton were more effective in the control of kola weevil. The ethanolic extracts of *L. esculentum*, *H. suaveolens*, *C. citratus* and *A. boonei* including standard insecticide achieved highest mortality throughout exposure periods. The plants do not have specific insecticidal effect but toxicity of these materials may be attributed to fumigation or repellency effect. Adu-Acheampong *et al*., (2000) reported that leaf extract was used in the control of caspid. Also, the effectiveness of crude neem extract and the commercial neem products against cocoa capsids demonstrated in the earlier studies (Adu-Acheamong *et al*., 2000) is an indication of the potential that exists for the use of crude neem extracts and neem-based commercial pesticides for cocoa capsid control.

The direct contact of extracts (aqueous and ethanolic) with the weevil enhanced quick mortality. Hence, all the botanicals; *L. esculentum*, *H. suaveolens*, *C. citratus*, *L. braunii*, *A. boonei* and *S. latifolius* achieved high weevil mortality throughout the exposure periods. However, ethanolic extract of all the botanicals, *H. suaveolens*, *C. citratus* and *A. boonei* were lethal to the kola weevil. Tobih, (2011) had previously rated *C. citratus* as a superior repellent or antifeedant botanicals to the yam beetle. The botanicals showed quick knockdown effect on the weevil which is similar to standard insecticide used. The topical application bring quick contact of botanicals with the body of weevils. This affords the spread of botanicals (both aqueous and ethanolic extracts) that touch every parts of treated weevils. In a similar findings, Barbara *et al*., (2010) reported that topical applications of *H. suaveolens* and *H. spicigera* on insects showed that both essential oils had an effective insecticidal activity.

In similar experiment, *C. citratus, A. boonei, H. suaveolens, L. braunii, A. indica* and *L. esculentum* had been found to also cause mortality in bruchids (Azeez, 2012). High fumigant action of the botanicals could be measured by the immediate weevil mortality caused by ethanolic extracts. Though, there was low fumigant action in aqueous extracts. Also, ethanolic extract showed superior action over aqueous extract because it releases fumes which killed the weevil immediately. This is attributed to toxic effect exerted by distrupting normally respiration activity of the weevil, hence results in asphyxiation and subsequent death (Grainge *et al*., 1983). Ethanolic extracts (*H. suaveolens*, *C. citratus* and *A. boonei*) as well as standard insecticide achieved high weevil mortality at the early exposure period. It thus compared favourably with the standard insecticide in achieving quick weevil mortality. This is corroborated by the Oyedokun *et al*. (2011) who reported that ethanolic extracts of *Phyllanthus amarus, Acassia albida* and *Tithonia diversifolia* resulted in a significantly higher percentage mean mortality (64-91%) of termite and that the bio-insecticidal activities of the ethanolic extract was potientiated by the extraction. Oparaeke *et al*. (1998) reported that leaf extracts of *O. gratissium*, *C. citratus*, *Vernonia* spp, *Eupatorium* spp, *H. suaveolens* and *L. esculentum* exhibited varying degrees of suppressing of pests population, reduced pods damage significantly and ensured higher grain yield than the untreated control. This is similar to the findings of Shazia *et al*. (2006) who reported that black pepper powder gave significantly better results than the control in suppressing bruchid survival. The results indicate that the botanicals with any of the topical or fumigant action may provide effective control of kola weevil and other field pests. However, field trials of the tested botanical insecticides and optimum concentrations should be carried out to further confirm insecticidal activity.

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