Effectiveness of the Powders of *Securidaca longipedunculata* (Fres.) as Bioinsecticides against Cowpea Beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae)

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Abstract Insect pests cause a great deal of post-harvest losses of stored food products worldwide and especially in the tropics where food products are usually susceptible to attack by insects between the periods of harvest, storage and consumption. In the present study, powders obtained from different parts of *Securidaca longipedunculata* (Fres.) (leaf, stem bark and root bark) were tested against the cowpea beetle, *Callosobruchus maculatus* in the laboratory at ambient tropical conditions of temperature and relative humidity. The powders were applied at 0.0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0 g. Insect mortality was observed for four (days). The result obtained showed that mortality of the insect increased with increased in concentration and exposure time. The powder obtained from the root bark recorded the highest insect mortality of 71.25 % within 96 h of exposure at the highest dosage of 1.0 g this was followed by the stem bark causing 65 % at the same level of concentration. The LD50 revealed the root bark powder to be the most effective as 3.17 % was required to obtain 50% insect mortality within 96 h of application as reflected by the regression probit analysis. The least toxic powder on *C. maculatus* was leaf powder. It was also observed that the tested powders significantly (P<0.05) reduced oviposition and percentage adult emergence when compared with the control. The results obtained from this research work showed that root bark of *S. longipedunculata* contains components of higher toxicity potential.

Keywords Mortality; Lethal dosage; Oviposition; Adult emergence; Cowpea; Plant powder; *Vigna unguiculata*; Synthetic insecticides

Background Farmers store their products for several reasons which include (i) making food available at all times to man (ii) making food / diet available to livestock (iii) making raw materials available to industries. Storage of Agricultural products has been hampered drastically as a result of infestation by insect pests (Udo, 2011). As in field crops, a wide range of insect pests attacks stored products and the commonest being beetles and moths (Udo, 2011). The primary insect attacking cowpea seed during storage is *Callosobruchus maculatus*. The larvae bore into the seed which becomes unsuitable for human consumption and loose viability (Taylor, 1981). *C. maculatus* is known to cause up to 100 % loss of stored cowpea and estimates have shown that over 30 million U.S. dollar is lost as a result of cowpea damage in Nigeria (Udo and Epidi, 2009). Infestation usually starts in the field just before harvest and the insects’ developmental stages are carried into the stored where the population builds up rapidly (Ilelke et al., 2012).

Farmers still depend on the use of synthetic insecticides in the management of stored product especially under large scale production (Gbaye and Holloway, 2011). The continued and intensive usage of these insecticides has produced some undesirable toxic effects on non-target biotic components of the ecosystem (Ojo and Ogunleye, 2013). Other potential difficulties associated with the continuous use of these chemical insecticides include; high cost of procurement, pest resurgence and resistance, poisonous residue accumulation in foods and poor knowledge of application (Ileke and Oni, 2011). To solve these problems, entomologists all over the world are in search for botanical insecticides which are safe and environmentally friendly for the control of these insects’ pests
(Isman, 2006). Currently, research efforts are focused on the use of botanical products as alternatives to synthetic insecticides. These plant products which include ashes, plant powders, extracts and oils have been confirmed to be cheaper, safe, and eco-friendly (Adedire et al., 2011; Ojo and Ogunleye, 2013). There is no doubt that botanical insecticides are an interesting alternative to insect pest control, and on the other hand only a few of the more than 250,000 plant species on our planet have been properly evaluated for this purpose (Khalequzzaman and Osman Goni, 2009). Use of plant products for infestation control in stored grains therefore seems to offer desirable solutions, especially in developing tropical countries where plants are found in abundance everywhere throughout the year (Khalequzzaman and Osman Goni, 2009).

Securidaca longepedunculata (Fres.) is a semi deciduous shrub used as a traditional medicine in many parts of Africa against a number of invertebrate pests, including insects infesting stored grains (Burkill, 1997). In Africa (Ghana; Nigeria) the plant decoction is prescribed by Ghananian healers to treat asthma and other diseases associated with smooth muscle contraction (Rakuumbo et al., 2006). In Nigeria, extracts from various parts of the plants has been reported to possess both gastrointestinal and trypanocidal effects (Olajide et al., 1999).

1 Materials and Methods
1.1 Preparations of Callosobruchus maculatus cultures
The culture of Callosobruchus maculatus was obtained from naturally infested cowpea seeds, Vigna unquiculata Walp bought from Oja-Oba Market in Ikole-Ekiti, Ekiti- State, Nigeria. The adult C. maculatus were cultured in the laboratory on clean uninfested Ife brown variety collected from Agricultural Development Project (ADP), Ikole Ekiti, Ekiti State, Nigeria. 750 g of the cowpea seeds was measured into a Kilner jar, thereafter, twenty (20) pairs of adult C. maculatus (10 male :10 female) were introduced into the Kilner jar covered with muslin cloth to let in air but prevent the escape of beetles (Jambere et al., 1995). The insects were allowed to lay eggs seven (7) days. After that all the insects both alive and dead were then removed to allow new generations to emerge and after 28 days of adult removal, the progeny that emerged were used for re-culturing. Subsequently, insects that emerged were used for the different experiments. Insect rearing and the experiment were carried out at the laboratory, Department of Crop Science and Horticure, Federal University Oye Ekiti (FUOYE), Ekiti State, Nigeria at ambient temperature of 28±2°C and 75±5 % relative humidity.

1.2 Collection of plant materials and cowpea seeds (Vigna unquiculata)
The plants evaluated for insecticidal activity were Securidaca longepedunculata (leaf, stem-bark and root-bark).These plant parts were collected from a farmland along Itapaji Ekiti, Ekiti State and taken into the laboratory. The root bark and stem bark were washed thoroughly with water and cut into small pieces. The plant parts are then air-dried in the laboratory for 30 days. Each plant part was pulverized separately into fine powders using Professional Blender (JTC OmniBlendV, Model TM-800). The powders were further sieved to pass through a 40 mesh sieve and kept in the refrigerator to retain its quality before use. The Ife brown variety of cowpea seeds used for the experiment was bought from the Agricultural Development Project (ADP), Ikole Ekiti, Ekiti State, Nigeria. The seeds were cleaned of foreign materials and disinfested by keeping in deep freezer at -5°C for 168 h to kill all hidden infestations. This is done because all the life stages, especially the eggs are very susceptible to cold (Koechner, 2003). The disinfested cowpea seeds were then air dried in the laboratory to prevent mouldiness (Adedire et al., 2011).

1.3 Contact toxicity assay
To assess the toxic effects of the plant powders on C. maculatus, twenty grammes (20 g) of clean and disinfested cowpea seeds of susceptible Ife brown were weighed using Electronic balance (Model XY3000C) into 160 ml plastic cups. After this, 0.2 g, 0.4 g, 0.6 g, 0.8 g and 1.0 g of the pulverized plant powders was weighed and mixed with the cowpea seeds. Plastic cups were shaken thoroughly to ensure adequate mixing of cowpea seeds and plant powders. 20 newly emerged adults of C. maculatus (0-2 day old) were introduced into each of the treatments. Each treatment was replicated four times. The control experiment had only the cowpea seeds and beetles i.e no plant powder was involved in the control experiment. The mortality of the beetles was monitored.
daily for 4 days. Dead beetles were those who neither moved nor respond to pin probing. Percentage adult mortality was corrected using Abott’s formula (Lale, 2006) thus:

\[ P_T = \frac{P_o - P_c}{100 - P_c} \times 100 \]

Where \( P_T \) = corrected mortality (%); \( P_o \) = observed mortality (%); \( P_c \) = control mortality (%)

1.4 Effect of plant powders on oviposition and adult emergence of *C. maculatus*

Twenty grammes (20 g) of clean and uninfested cowpea seeds of susceptible Ife brown variety was weighed into 160 ml plastic cups. Then 0.2 g, 0.4 g, 0.6 g, 0.8 g and 1.0 g of various plant powders were weighed and thoroughly mixed with the 20 g of disinfested seeds. The seeds in the control contained no plant powders. Five (5) pairs (5 male: 5 female) of adult *C. maculatus* (0-2 day old) sexed according to the methods described by Odeyemi and Daramola (2000) were introduced into the treated and untreated (control) cowpea seeds. Male *C. maculatus* have comparative shorter abdomen and the dorsal side of the terminal segment is sharply curved downward and inward. In contrast, the females have comparative longer abdomen and the dorsal side of the terminal segment is only slightly bent downward. The females also have two dark visible spots on their elytra (Odeyemi and Daramola, 2000). Four replicates of the treated and untreated control were laid out in Complete Randomized Block Design. The plastic cups were covered with muslin cloth to let in but prevent the escape of beetles (Jambere *et al.*, 1995). The experiments were left for 7 days for the insects to oviposit. The total number of eggs laid by *C. maculatus* on cowpea seeds were counted and recorded after allowing the insects to lay eggs for 7 days. The Percentage adult emergence was calculated as described below:

\[ \% \text{ Adult emergence} = \frac{\text{Total number of adults emerged}}{\text{Total number of eggs laid}} \times 100 \]

1.5 Statistical analysis

Data obtained from all the parameters 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. In addition, data obtained from beetles’ mortality were subjected to regression analysis to calculate the LD<sub>50</sub> and LD<sub>95</sub> of the powders after 96 h of application using probit analysis.

2 Results

2.1 Effect of *S. longepedunculata* powders on mortality of *C. maculatus*

Beetle mortality of *C. maculatus* on cowpea seeds treated with the plant powders *S. longepedunculata* is presented in Table 1. Adult mortality in the treated cowpea was significantly (P<0.05) different from beetle mortality in the control. The percentage adult mortality remained 0% in the control experiment throughout the period of exposure. However, low insect mortality was observed in all the treatments after 72 h of application. The highest beetle mortality of 71.25% was recorded in cowpea seeds treated with the root bark at the highest dosage of 1.0 g within 96 h of application and it was significantly different from beetle mortality of 65.0% and 46.25% in cowpea seeds treated with the powders of *S. longepedunculata* stem bark and leaf at the same concentration and period of exposure.

2.2 Lethal dose (LD<sub>50</sub> and LD<sub>95</sub>) of plant powders required to achieve 50% and 95% mortality in *C. maculatus* after 96 h post treatment

The lethal dose (LD<sub>50</sub>) and (LD<sub>95</sub>) of the plant powders is presented in Table 2. The result obtained indicated that to achieve 50% and 95% mortality in the beetle, the plant powders must be applied at higher dosage except the powder obtained from the root bark in which low dosage is required for 50% (2.56-4.01) and 95% (5.10-6.45). The powder obtained from leaf appeared to be the least effective as indicated by their amount of dosages required to cause 50% (3.53-7.01) and 95% (8.34-11.14) mortality.
Table 1 Cumulative percentage mortality of *Callosobruchus maculatus* on cowpea seeds treated with different concentrations of plant powders of *S. longepedunculata*

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Dosage (g)</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>0.2</td>
<td>5.00±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.00±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.75±2.39&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>26.25±1.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>6.25±1.25&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>8.75±2.39&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>26.25±3.14&lt;sup&gt;def&lt;/sup&gt;</td>
<td>36.25±4.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>6.25±1.25&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>8.75±2.39&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>28.75±2.39&lt;sup&gt;de&lt;/sup&gt;</td>
<td>36.25±3.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>6.25±1.25&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>11.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>28.75±2.39&lt;sup&gt;de&lt;/sup&gt;</td>
<td>37.50±1.44&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>10.00±2.04&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>13.75±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>28.75±2.39&lt;sup&gt;de&lt;/sup&gt;</td>
<td>46.25±3.75&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stem bark</td>
<td>0.2</td>
<td>8.75±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>12.50±1.44&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>20.00±2.88&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>51.25±2.39&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>0.4</td>
<td>11.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>12.50±1.44&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>21.25±3.75&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>61.25±3.75&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>11.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>12.50±1.44&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>25.00±2.04&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>61.25±2.39&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>0.8</td>
<td>11.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>16.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>26.25±3.14&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>62.50±4.33&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>11.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>16.25±3.14&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>26.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>65.00±3.53&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Root bark</td>
<td>0.2</td>
<td>5.00±2.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>16.25±3.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.50±4.33&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>8.75±2.39&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>18.75±2.39&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>26.25±1.25&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>66.25±4.26&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>11.25±2.39&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>18.75±1.25&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>30.00±3.53&lt;sup&gt;e&lt;/sup&gt;</td>
<td>67.50±3.22&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>12.50±1.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.75±2.39&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>30.00±4.56&lt;sup&gt;e&lt;/sup&gt;</td>
<td>67.50±3.22&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>13.75±1.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.00±2.04&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>40.00±3.53&lt;sup&gt;e&lt;/sup&gt;</td>
<td>71.25±3.22&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Each value is a mean ± standard error of three replicates. Values followed by the same alphabet are not significantly (p>0.05) different from each other using Duncan’s Multiple Range Test

Table 2 Lethal dosage of *S. longepedunculata* powders require to achieve 50 and 95% mortality of *Callosobruchus maculatus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slope± S.E</th>
<th>Intercept± S.E</th>
<th>X&lt;sup&gt;2&lt;/sup&gt;</th>
<th>LD&lt;sub&gt;50(FL)&lt;/sub&gt;</th>
<th>LD&lt;sub&gt;95(FL)&lt;/sub&gt;</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>0.64±0.048</td>
<td>-0.521±0.044</td>
<td>824.829</td>
<td>6.52</td>
<td>9.45</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(3.53-7.01)</td>
<td>(8.34-11.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem bark</td>
<td>0.67±0.046</td>
<td>-0.542±0.044</td>
<td>97.60</td>
<td>5.42</td>
<td>7.98</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(3.18-6.36)</td>
<td>(6.87-9.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root bark</td>
<td>0.64±0.048</td>
<td>-0.521±0.043</td>
<td>1320.456</td>
<td>3.17</td>
<td>5.68</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.56-4.01)</td>
<td>(5.10-6.45)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: S.E.: Standard Error, X<sup>2</sup>: Chi Square, LD: Lethal dosage, FL: Fiducial limit

2.3 Effect of *S. longepedunculata* powders on oviposition and adult emergence by *C. maculatus*

All the plant powders of *S. longepedunculata* tested in this study reduced the number of eggs laid by *C. maculatus* (Figure 1). Oviposition by *C. maculatus* was significantly lower (P<0.05) in powder-treated seeds than in untreated (control) seeds. The highest number of eggs (162.25) was laid on control (untreated) seeds. The least eggs (54.50, 58.75 and 70.75) were laid on cowpea seeds treated with root bark, stem bark and leaf at the highest dosage of 5.0% w/w and it was significantly (P<0.05) different from other dosages of plant powders.

The highest percentage adult emergence (88.59%) was obtained on the control (untreated) seeds (Figure 2). The least percentage adult emergence (27.52% and 29.78%) were obtained on cowpea seeds treated with the powders of root bark and stem bark of *S. longepedunculata* at the treatment level of 5.0% w/w and it was significantly different from other dosages of the plant powders.
Figure 1 Oviposition of *C. maculatus* on cowpea seeds exposed to different dosages of different parts of *S. longepedunculata*

Figure 2 Percentage adult emergence of *C. maculatus* on cowpea seeds exposed to different dosages of different parts of *S. longepedunculata*

3 Discussion

The use of plant powders in the control of stored products insects is an ancient practice (Ileke and Olotuah, 2012; Ojo and Ogunleye, 2013). In this study, the insecticidal activities of the leaf, stem bark and root bark of *S. longepedunculata* as contact insecticides against *C. maculatus* infesting cowpea seeds were evaluated. The results obtained from the experiment showed that the powder of *S. longepedunculata* particularly the root bark was very potent against *C. maculatus* causing 71.25% mortality at the rate of 1.0 g / 20 g within 96 h of exposure. Plant powders have been used to suppress the population of storage pests (Ogunleye *et al*., 2004; Ojo and Ogunleye, 2013). In this study, the observed high mortality recorded on cowpea seeds treated with the root bark may be due to the strong choky odours it produced which could asphyxiate insects by blocking the spiracles (Amusan and Okorie, 2002). Most insects breathe by means of trachea which usually open at the surface of the body through spiracles, the plant powders that were mixed with the seeds might have blocked these spiracles thereby leading to suffocation and death of the insect (Obembe and Kayode, 2013). Insecticidal property of any plant material would depend on the active constituents of the plant material (Asawalam *et al*., 2007). Efual *et al*. (2016) reported that the root powder of *S. logepedunculata* contains 2-hydroxy-benzoic acid methyl ester (methyl salicylate, 1) which is responsible for its biocide effect against the stored grain insects. The result from this investigation is similar to the observation of Efual *et al*. (2016) who reported the insecticidal, anti-ovipositant, ovicidal and repellent properties of *S. logepedunculata* against *S. zeamais* and *C. maculatus*. 
The results obtained from this study showed that the three plant powders significantly reduced oviposition and adult emergence of *C. maculatus* on cowpea treated seeds. The effect of the powders on oviposition could be due to respiratory impairment which probably affects the process of metabolism and consequently other systems of the body of the insects (Adedire *et al.*, 2011; Ileke *et al.*, 2014). The beetles were unable to move freely within the treated cowpea seeds due to the presence of the powders and this might have affected mating activities and sexual communication (Ojo and Ogunleye, 2013). The resultant reduced adult emergence could be to the fact the *C. maculatus* lay eggs on the seed coat thus bringing the eggs and larvae in close contact with the plant powders as reported by Adedire and Lajide (2001). It is evident in this research work that root bark powder of *S. longepedunculata* is effective in controlling the population of *C. maculatus*. The powder could be mixed with stored cowpea seeds in order to protect them against *C. maculatus*. The root bark powder could therefore serve as alternatives to synthetic insecticides for use by resource-poor farmers who store small quantities of the seeds for their consumption, sales and planting since the powder is readily available.

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