Efficacy of essential oil of aromatic plants against immature stages of Callosobruchus maculatus Fab. and Bruchidius atrolineatus Pic. (Coleoptera-Bruchinae)

Toufique Bello Mariama, Ali Doumma

Abstract
In this study, lethal effect of three essential oils extracted from aromatics plants, Ocimum basilicum, Ocimum gratissimum and Cymbopogon giganteus on immature stages of Callosobruchus maculatus (F.) And Bruchidius atrolineatus P. (Coleoptera-Bruchinae) are compared. For this, neonate larvae, fourth instar larvae and pupae of each species are treated with 6.25 µl; 12.5µl, 25µl and 50 µl oil of both plant. Results reveal the susceptibility of all stages of the two bruchids species to fumigation with essential oil of Ocimum basilicum, Ocimum gratissimum and Cymbopogon giganteus. Oil. Regardless to the aromatic plant, Adult emergence decreased significantly as oil concentration amount increased. For both species neonate larvae seems to be more susceptible. The study also indicates the susceptibility of C. maculatus immature stage to the three essentials oils compared to those of B. atrolineatus. Besides that, O. basilicum and Cymbopogon gayanus essentials oils were more effective than essential oil of O. gratissimum.

Keywords: Cowpea, bruchids, immature stages, essential oils, aromatic plants

1. Introduction
Cowpea (Vigna unguiculata Walp), is cultivated in a range of ecologies and cropping systems in the tropics. It is most commonly grown in dry areas along with sorghum and millet [1]. Cultivated cowpea is a herbaceous annual, belonging to the sub-tribe Phaseolinae, the tribe Phaseoleae, family Papillionaceae and order Leguminosales. The potential yield of cowpea grain in Africa is around 1.5-3.0 t. ha⁻¹, but current average yields are in the range of 0.2-0.3 t. ha⁻¹ [2]. In tropical Africa, cowpea is consumed mostly in the form of dry grain or young pods. It is a relatively cheap source of protein and provides adequate complement to a cereal-based diet [3]. In many parts of Eastern and Western Africa, the young leaves are eaten cooked or used as a condiment. The green or chopped pods are not used widely in Africa, but are popular throughout the far. Like other grain legumes, cowpeas are infested by insect pests during growth and in storage [4].

Among these species, Callosobruchus maculatus (F.) and Bruchidius atrolineatus P. play an important role as cosmopolitan pests of stored cowpeas (and to a lesser extent of other stored legumes). The adults of bruchids colonize cowpea crops in August at the end of the main rainy season, and females oviposit on the developing pods. Instars larvae of these species take place inside seeds of Vigna unguiculata (L.) Walp, and cause a huge losses of seeds during the storage in traditional granaries after the seed harvesting. In Niger, it was estimated that up to 80% of the stored cowpea can be damaged after 6 months of storage [4].

In the developing countries, the control of bruchids relies heavily on application of synthetic insecticides or store fumigation, but availability and cost of chemicals involved are major limitations to their use by small-scale African farmers. On the other hand, on-farm control of stored product pests needs to be safe for both users and consumers [5]. Plant essential oils are alternative of synthetic pesticides possess insecticidal, ovicidal, repellent and ovipositional activities against various stored product insects [6-9]. Plant essential oils are potential source of alternative compounds to be used as contact or fumigant pesticides because they include a rich source of bioactive compounds.

In the present study, we compared the lethal effect of essential oils of Ocimum basilicum, Ocimum gratissimum and Cymbopogon giganteus on larvae and pupae of C. maculatus and B. atrolineatus.
2. Material and Methods

1. Experimental conditions
All experiments in this study were conducted on the campus of Abdou Moumouni University of Niamey either in the laboratory environment or under sun exposure. The study was conducted during the period of October to December. During this period, temperatures varied from 16, 7 °C to 37.8 °C and relative humidity from 25% to 58%.

2. Bruchids rearing
Bruchids adults were collected from cowpea seeds bought on the local market of Niamey (Niger Republic). They were brought back to the laboratory and mass reared under conditions of the laboratory.

Hundred pairs of 2- or 3 d-old for both species of bruchids adults were placed during 48 h in rearing containers containing seeds of *Vigna unguiculata* of the TN 5-78 variety. The females laid eggs on these seeds and their offspring completed development within the cotyledons. The adults were isolated after emergence and used either for the production of a new generation or for experiments.

3. Extraction of essential oil
Essential oils from leaves of *Ocimum basilicum; cimum. Gratissimum* and *Cymbopogon giganteus*. Were extracted separately by the methodology used by [10]. The plant materials were dried in a well-ventilated room under shade for 1 week. 50 g dried leaves of each plant were placed in a 2 liter round-bottomed flask containing 1.2 liters of water and then hydrodistilled using a Clevenger-type apparatus for 3 hours [11]. Pure oil samples from different plant collections were collected from the plants into 2 ml vials and stored at −20 °C in a freezer until required for analysis and bioassays.

4. Biological test
For each species, the tests were carried out on neonate larvae (L1), fourth instar larva (L4) and pupae. Larvae and pupae development time for both bruchids species were reported in table 1. The different instars developing inside the seeds were determined subsequently by opening the seed and counting the number of exuvia (cephalic capsules) present inside [12]. For each larval stage, fifty seeds containing one individual were sampled from culture of each bruchids species and treated with respectively 0 µl (control) 6.25µl, 12.5µl, 25µl et 50µl concentration of each essential oil deposited on circular filter paper. The treated individuals inside the seeds were exposed during 48 h and then incubated until adult emergence.

Table 1: Development time of immature stages of *C. maculatus* and *B. atrolineatus* recorded in Niger conditions (Toufique, 2001)

<table>
<thead>
<tr>
<th></th>
<th><em>C. maculatus</em></th>
<th><em>B. atrolineatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae L1</td>
<td>4-5 days</td>
<td>4-5 days</td>
</tr>
<tr>
<td>Larvae L4</td>
<td>14-16 days</td>
<td>15-17 days</td>
</tr>
<tr>
<td>Nymphae</td>
<td>16-18 days</td>
<td>17-20days</td>
</tr>
</tbody>
</table>

3. Data analysis
All experiments were in triplicate. Emergence rate at each developmental stage was subjected to analysis of variance and differences were assessed using Newman-Keuls tests at the 5% level of significance.

4. Results and discussion
Results of the present study reveal the susceptibility of all stages of the two bruchids species to fumigation with essential oil of *O. basilicum*, *O. gratissimum* and *C. giganteus*. Regardless to the aromatic plant, Adult emergence decreased significantly as oil concentration amount increased. Statistical analysis showed that the numbers of adults that emerged from treated larvae and pupae (early and late) were significantly lower than the control. For all the aromatic plant tested, the effectiveness of the oil became more pronounced with 25 µl concentration.

The most susceptible stage of bruchids was the neonate larvae. After exposure of this stage at the low concentration reproduction of this stage was completely inhibited in the case of *C. maculatus* (table 2) whereas development of more than 90%; 96% and 88 % of *B. atrolineatus* neonate larvae was inhibited respectively by *O. basilicum*, *O. gratissimum* and *C. giganteus* oil (table 3).

Table 2: Percentage of *C. maculatus* adults emerging from the 1st stage larvae treated with different doses of three essential oils

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th><em>O. basilicum</em></th>
<th><em>O. gratissimum</em></th>
<th><em>C. giganteus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0µl</td>
<td>92 ± 4,5</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
</tr>
<tr>
<td>6,25µl</td>
<td>0 ± 0 a</td>
<td>4 ± 5,5 a</td>
<td>12 ± 13,0 a</td>
<td></td>
</tr>
<tr>
<td>12,5µl</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
<td></td>
</tr>
<tr>
<td>25µl</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
<td></td>
</tr>
<tr>
<td>50µl</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
<td>0 ± 0 a</td>
<td></td>
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</tbody>
</table>

N.B: Means followed by the same letter(s) are not significantly different at the 5% level). (Test of Newman et Keuls)

Table 3: Percentage of *B. atrolineatus* adults emerging from the 1st stage larvae treated with different doses of three essential oils

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th><em>O. basilicum</em></th>
<th><em>O. gratissimum</em></th>
<th><em>C. giganteus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0µl</td>
<td>98 ± 4,5</td>
<td>4 ± 5,5 a</td>
<td>13 ± 12,0 a</td>
<td></td>
</tr>
<tr>
<td>6,25µl</td>
<td>10 ± 7,1 a</td>
<td>4 ± 5,5 a</td>
<td>12 ± 13,0 a</td>
<td></td>
</tr>
<tr>
<td>12,5µl</td>
<td>4 ± 5,5 ab</td>
<td>0 ± 0 ab</td>
<td>0 ± 0 ab</td>
<td></td>
</tr>
<tr>
<td>25µl</td>
<td>0 ± 0 ab</td>
<td>0 ± 0 ab</td>
<td>0 ± 0 ab</td>
<td></td>
</tr>
<tr>
<td>50µl</td>
<td>0 ± 0 ab</td>
<td>0 ± 0 ab</td>
<td>0 ± 0 ab</td>
<td></td>
</tr>
</tbody>
</table>

N.B: Means followed by the same letter(s) are not significantly different at the 5% level). (Test of Newman et Keuls)

At 12.5 µl concentration, *O. gratissimum* and *C. giganteus* oils induced a mortality rate of 100%. With 6.25 and 12.5 oil concentrations, percentage of bruchids adults emerging from fourth instar larvae and pupae was relativeley important for both species. (Table 4, 5, 6 and 7). At the 25 µl *O. basilicum* oil concentration and 50µl *C. gayanus* oil concentration, the development of fourth instar larvae and pupae of *C. maculatus* was completely inhibited. Contrary results were recorded with *B. atrolineatus* for the two stages with the same oils. At 12.5 µl concentration, *O. gratissimum* and *C. giganteus* oils induced a mortality rate of 100%. With 6.25 and 12.5 oil concentrations, percentage of bruchids adults emerging from fourth instar larvae and pupae was relativeley important for both species. (Table 4, 5, 6 and 7). At the 25 µl *O. basilicum* oil concentration and 50µl *C. gayanus* oil concentration, the development of fourth instar larvae and pupae of *C. maculatus* was completely inhibited. Contrary results were recorded with *B. atrolineatus* for the two stages with the same oils.

Table 4: Percentage of *C. maculatus* adults emerging from the fourth instar larvae treated with different doses of three essential oils

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th><em>O. basilicum</em></th>
<th><em>O. gratissimum</em></th>
<th><em>C. giganteus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0µl</td>
<td>92 ± 8,4</td>
<td>54 ± 5,5 a</td>
<td>60 ± 16,4 a</td>
<td>52 ± 13,0 a</td>
</tr>
<tr>
<td>6,25µl</td>
<td>54 ± 5,5 a</td>
<td>60 ± 16,4 a</td>
<td>52 ± 13,0 a</td>
<td></td>
</tr>
<tr>
<td>12,5µl</td>
<td>26 ± 5,5 ab</td>
<td>44 ± 18,2 b</td>
<td>52 ± 4,5 a</td>
<td></td>
</tr>
<tr>
<td>25µl</td>
<td>0 ± 0 c</td>
<td>14 ± 11,4 c</td>
<td>20 ± 10,0 c</td>
<td></td>
</tr>
<tr>
<td>50µl</td>
<td>0 ± 0 c</td>
<td>2 ± 4,5 cd</td>
<td>0 ± 0 d</td>
<td></td>
</tr>
</tbody>
</table>

N.B: Means followed by the same letter(s) are not significantly different at the 5% level). (Test of Newman et Keuls)
The current results are in agreement with [13] who have reported that *Cymbopogon* oil vapour treatment for 24h could be satisfactory for controlling larval development of *C. maculatus*. This study clearly indicated that the immature stage of *C. maculatus* was more susceptible to the three essential oils than those of *B. atrolineatus*. Besides that, essential oils of *O. basilicum* and *Cymbopogon giganteus* were more effective than essential oil of *O. gratissimum*. In other hand, *C. maculatus* seemed to be more susceptible to the *O. basilicum* oil than *B. atrolineatus* which seemed to be more susceptible to *C. giganteus* oil.

Instar larvae and pupae were inside the seeds, our results suggested that oil vapours might have diffused into seeds and affected the physiological and biochemical process associated with post-embryonic development as reported by [12].

The admixture of oils extracted from common plants to cowpea seeds have been studied by many authors. [14, 15] have tested essential oils of five West African plant species and found that the plants of the genus *Ocimum* can be used as a good alternative to synthetic insecticides [16]. Reported the effect of volatile oils from *Mentha arvensis*, *Mentha spicata* and *Cymbopogon nardus* on *C. maculatus*. They observed significant reduction of the number of eggs laid, adult survival, adult emergence and seed damage compared to controls.

[17] have studied the susceptibility of *C. maculatus* and its parasitoid *Dinarnus basalis* to essential oils of 3 plants of Togo (*Ocimum basilicum*, *Cymbopogon nardus* L. and *Cymbopogon schoenanthus* L.) and found that essential oil of *Cymbopogon schoenanthus* was the most efficient in the control of bruchid populations but was also toxic to the parasitoid. Aboua et al. 2010,[18] Reported that the essential oils proved to be the most effective in reducing the population of *C. maculatus*.

Essential oils act on insects through their aroma compound which are highly volatiles, renewable and biodegradable. Current study indicates that insecticidal mode of action of the volatiles may be largely attributable to contact and fumigant action. They may be toxic by penetrating the insect body via the respiratory system or by thorax % [19].

The insecticidal activity of aromatic plant essential oil could be attributed to those known major components of oxygenated monoterpenes: 1,8-cineole (43.4%), α-terpinyl acetate (14.5%), 4-terpinen-4-ol (3.4%), α-terpinol (3.4%), and monoterpen hydrocarbons: sabinene (8.2%) and β-pinene (4.0%) [20, 21, 22]. Nevertheless, it also has been shown that minor components may contribute to the biological activity [23] such as Myrcene, α-phellandrene, and Camphene.

This study shows that volatile oils of the three aromatic plants have an insecticidal activity on immature stage of *C. maculatus* and *B. atrolineatus*. Their use will have purely to be envisaged for the safeguarding of the environment and the health of the user. Essential oils could be used as biodegradable and natural bio protector for controlling stored product pests.

Application of essential oils and their formulations to grain seeds for storage is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers. A study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors of developing countries, as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazards involved [23].

5. References

9. Makanga OBB. Composition and repellency of essential


