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

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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 0C 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)

	C. maculatus	*B. atrolineatus*
Larvae L1	4-5 days	4-5 days
Larvae L4	14-16 days	15-17 days
Nymphae	16-18 days	17-20days

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

	Emergence rate (%)			
	Control	***O. basilicum***	***O. gratissimum***	***C. giganteus***
0µl	92 ± 4,5			
6,25µl		0 ± 0 a	0 ± 0 a	0 ± 0 a
12,5µl		0 ± 0 a	0 ± 0 a	0 ± 0 a
25µl		0 ± 0 a	0 ± 0 a	0 ± 0 a
50µl		0 ± 0 a	0 ± 0 a	0 ± 0 a

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 1ststage larvae treated with different doses of three essential oils

	Emergence rate (%)			
	Control	***O. basilicum***	***O. gratissimum***	***C. giganteus***
0µl	98 ± 4,5			
6,25µl		10 ± 7,1 a	4 ± 5,5 a	12 ± 13,0 a
12,5µl		4 ± 5,5 ab	0 ± 0 ab	0 ± 0 ab
25µl		0 ± 0 ab	0 ± 0 ab	0 ± 0 ab
50µl		0 ± 0 ab	0 ± 0 ab	0 ± 0 ab

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. Contrairy 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

	Emergence rate (%)			
	Control	***O. basilicum***	***O. gratissimum***	***C. giganteus***
0µl	92 ± 8,4			
6,25µl		54 ± 5,5 a	60 ± 16,4 a	52 ± 13,0 a
12,5µl		26 ± 5,5 ab	44 ± 18,2 b	52 ± 4,5 a
25µl		0 ± 0 c	14 ± 11,4 c	20 ± 10,0 c
50µl		0 ± 0 c	2 ± 4,5 cd	0 ± 0 d

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

Table 5: Percentage of *B. atrolineatus* adults emerging from the fourth instar larvae treated with different doses of three essential oils

	Emergence rate (%)			
	Control	*O. basilicum*	*O. gratissimum*	*C. giganteus*
0µl	80 ± 21,2			
6,25µl		56 ± 8,9 a	50 ± 10,0 a	26 ± 27,9 b
12,5µl		30 ± 21,2 ab	24 ± 30,5 ab	12 ± 16,4 c
25µl		6 ± 8,9 d	6 ± 5,5 d	0 ± 0 cd
50µl		0 ± 0 cd	2 ± 4,5 cd	0 ± 0 cd

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

Table 6: Percentage of *C. maculatus* adults emerging from pupae treated with different doses of three essential oils

	Emergence rate (%)			
	Control	*O. basilicum*	*O. gratissimum*	*C. giganteus*
0µl	78 ± 8,4			
6,25µl		60 ± 12,2 a	34 ± 20,7 b	34 ± 11,4 b
12,5µl		36 ± 20,7 b	26 ± 11,4 bc	34 ± 15,2 b
25µl		0 ± 0 c	14 ± 11,4 cd	4 ± 5,5 c
50µl		0 ± 0 c	0 ± 0 cd	0 ± 0 cd

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

Table 7: Percentage of *B. atrolineatus* adults emerging from pupae treated with different doses of three essential oils

	Emergence rate (%)			
	Control	*O. basilicum*	*O. gratissimum*	*C. giganteus*
0µl	76 ± 11,4			
6,25µl		50 ± 14,1	66 ± 20,7	22 ± 8,4
12,5µl		22 ± 11,0	26 ± 11,4	4 ± 5,5
25µl		2 ± 4,5	12 ± 4,5	0 ± 0
50µl		0 ± 0	4 ± 5,5	0 ± 0

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 larvae development of *C. maculatus*. This study clearly indicated that the immature stage of *C. maculatus* was more susceptible to the three essentials oils than those of *B. atrolineatus*. Besides that, essentials 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 stocks 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 *Dinarmus basalis* to essential oils of 3 plants of Togo (*Ocimum basilicum* L, *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 monoterpene 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 [22] 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* andt *B. atrolineatus*. Their use will have purely to be advised 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

1. Singh BB, Ajeigbe HA, Tarawali SA, Fernandez RS, Abubaker M. Improving the production and utilization of cowpea as food and fodder, Field Crops Res 2003; 84:169-177.
2. Romain HR. Crop Production in Tropical Africa. Directorate General for International Cooperation (DGIC), Brussels, Belgium, 2001, 165-187.
3. Adenekan MO, Okpeze VE, Ogundipe WF, Oguntade MI. Evaluation of Moringa oleifera powders for the control of bruchid beetles during storage nternational Journal of Agricultural Policy and Research. 2012; 1(10):305-310.
4. Opolot HN, Agona A, Kyamanyawa S, Mbata GN, Adipala E. Integrated field management of cowpea pests using selected synthetic and botanical pesticides. Crop Protection 1986; 25:1145-1152.
5. Chiasson H, Vincent C, Bostanian NJ. Insecticidal properties of a Chenopodium-based botanical. Journal of Economic Entomology. 2004; 97:1378-83
6. Tripathi NN, Kumar N. Putranjiva roxburghii oil. A potential herbal preservative for peanuts during storage. Journal of Stored Product Research. 2007; 43:435-442.
7. Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR. A review on prospects of essential oils as biopesticide in insect-pest management. Journal of Pharmacognosy and Phytotherapy. 2009; (5):52-63.
8. Aboua LRN, Seri-Kouassi BP, Koua HK. Insecticidal Activity of Essential Oils from Three Aromatic Plants on Callosobruchus maculatus F in Côte D'ivoire, European Journal of Scientific Research. 2010; 39(2):243-250.
9. Makanga OBB. Composition and repellency of essential

oil of Tagetes minuta from different zones of Kenya against brown tick, Rhipicephalus appendiculatus. Master of Sciences. University of Kenya, 2012, 120.

10. Clevenger JF. Apparatus for the determination of volatile oil. Pharm Assoc 17: 346, 1928.
11. Ketoh GK, Koumaglo HK, Glitho IA, Auger J, Huignard J. Essential oils residual effects on Callosobruchus maculatus (Coleoptera: Bruchidae) survival and female reproduction and cowpea seed germination. Int J Trop Insect Sci. 2005; 25:129-133.
12. Ketoh GK, Koumaglo HK, Glitho IA and Huignard J. Comparative effects of Cymbopogon schoenanthus essential oil and piperotone on Callosobruchus maculatus development, Fitoterapia 2006; 77:506-510.
13. Keita SM, Vincent C, Schmidt JP, Belanger A. Essential oil composition of Ocimum basilicum LO. gratissimum L. and O. suave L. in the Republic of Guinea, Flavour Fragr J. 2000; 15:339-341.
14. Ketia SM, Vincent C, Schmit JP, Annason JT, Belonger. A Efficacy of essential oil from Ocimum basilicum l and O. gratissimum L. applied as an insecticidal fumigants and powder to control Callosobruchus maculatus J Stored Prod. 2001; 37:339-349.
15. Raja N, Albert S, Ignacimuthu S, Dorn S. Effect of plant volatile oils in protecting stored cowpea Vigna unguiculata (L) walpers against b Callosobruchus maculatus (F) (Coleoptera: Bruchidae) infestation. Journal of Stored Products Research. 2001; 37(2):127-132.
16. Ketoh GH, Glitho AI, Huignard J. Susceptibility of bruchid Callosobruchus maculatus (Coleptera: Bruchidae) and it`s parasitoid Dinarmus basalis (Hymenoptrea: Pteromalidae) to three essential oils, J Econ Entomol. 2002; 95:174-182.
17. Eman EA, Abass MH. Chemical composition and efficiency of five essential oils against the pulse. Beetle Callosobruchus maculatus (F) on Vigna radiata seeds, The American-Eurasian Journal of Agriculture and Environmental Science. 2010; 8:411-419.
18. Aboua LRN, Seri-Kouassi BP, Koua HK. Insecticidal Activity of Essential Oils from Three Aromatic Plants on Callosobruchus maculatus F in Côte D'ivoire. European Journal of Scientific Research. 2010; 39:243-250.
19. Islam R, Khan RI, Al-Reza SM, Jeong YT, Song CH, Khalequzzaman M. Chemical composition and insecticidal properties of Cinnamomum aromaticum (Nees) essential oil against the stored product beetle Callosobruchus maculatus (F) Journal of the Science of Food and Agriculture. 2009; 89(7):1241-1246.
20. Nyamador WS, Ketoh GK, Amévoin K, Nuto Y, Koumaglo HK, Glitho IA. Variation in the susceptibility of two Callosobruchus species to essential oils. Journal of Stored Products Research. 2010; 46(1):48-51.
21. Waliwitiya R, Isman MB, Vernon RS, Riseman A. Insecticidal activity of selected monoterpenoids and rosemary oil to Agriotes obscurus (Coleoptera: Elateridae) Journal of Economic Entomology. 2005; 98(5):1560-1565.
22. Iacobellis NS, Lo Cantore P, Capasso F, Senatore F. Antibacterial activity of Cuminum cyminum L and Carum carvi L. essential oils. Journal of Agricultural and Food Chemistry. 2005; 53(1):57-61.
23. Pandey Abhay K, Singh P, Palni Uma T, Tripathi NN. Use of essential oils of aromatic plants for the management of pigeon pea infestation by pulse bruchids during storage, Journal of Agricultural Technology. 2011; 7(6):1615-1624.