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Toxicity and Sub-lethal effect of essential oils of *Eugenia aromatica* and *Piper guineense* on cowpea seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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Abstract
Under laboratory conditions (28 ± 3 ºC and 65 ± 5% relative humidity) in Akure, Nigeria, essential oils of *Eugenia aromatica* and *Piper guineense* were evaluated for their toxicity and sub-lethal effect on cowpea seed beetle *Callosobruchus maculatus* (F.). The rate of application 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5ml per 20g of seed was tested. The mating propensity of males and females of *C. maculatus* after exposure to 0.05 and 0.4ml of the essential oils of both plants were also investigated. The results showed that the lowest dose of application of 0.05ml of the essential oils of *P. guineense* and *E. aromatica* was observed to cause significant increase in oviposition with up to 370 eggs laid and eclosion of 273 adults in *C. maculatus*. The lowest dose of the essential oils of *P. guineense* and *E. aromatica* was also observed to cause significant increased propensity to mate in males and females *C. maculatus.*

Keywords: *Callosobruchus maculatus*, *sub-lethal dose* essential oil, *Eugenia aromatica*, *Piper guineense*

1. Introduction

Synthetic insecticides which are very effective in the control of *Callosobruchus maculatus* in cowpea are available, but their wide spread use has several drawbacks [1, 3, 11] and this led to the development of botanical insecticides that are easily degradable and eco-friendly. The insecticidal efficacies of essential oils extracted from plants have been widely investigated for pest control properties, some proving to be toxic [8], repellent [13], antifeedant and ovicidal in insect pest [5]. Many plant secondary metabolites, such as monoterpenoids, alkaloids or phenylpropanoids exhibit a wide spread of biological action against plant pest and were shown to be possible alternatives to synthetic insecticides against stored product pests. Pest resistance to insecticide is a worldwide phenomenon and current estimates indicate that over 500 arthropod species have developed resistant to one or more pesticides and of the resistant insect species, about 56 percent are of agricultural importance [14]. Pest population resurgence or tolerance is an ecological backlash that may accompany application of subjugation tactics commonly due to excessive application or indiscriminate use of insecticide. Insects show counter responses to the use of sub-lethal doses of insecticide [6, 2]. The use of sub-lethal doses of insecticides may induce insect resurgence, a phenomenon termed ‘Hormoligosis [14]. Population explosion of the cowpea aphids, *Aphis craccivora* in cultivated cowpeas following application of Cypermethrin (synthetic pyrethroids) was reported [9]. Also reported was resurgence of the hemlock scale, *Fiorina externa* following insecticidal application [7]. As human strive to subdue insect, these prodigious creatures are being observed to evolved fight-back mechanisms that have frustrated our effects on many fronts, in this regard it deem fit that any potential substances to be use as an insecticide should be tested not only for its efficacy but also for the ability of the pest developing resurgence, tolerance or resistance. Therefore this study investigates the toxicity and sub-lethal effects of essential oils of *Eugenia aromatica* and *Piper guineense* that have proven effectively for the control of *C. maculatus* a cosmopolitan pest of cowpea seeds in storage. In addition, effect of the sub-lethal doses of the essential oils on mating propensity of adults of *C. maculatus* also investigated.

2. Materials and Methods

2.1 Culturing of insects

The *C. maculatus* insect cultures were maintained under ambient conditions of minimum temperature of 24 ºC and maximum temperature of 32 ºC; relative humidity of 53-67% in an open laboratory. The cultures were maintained on Ife brown cowpea seed a well-known bruchids susceptible cowpea. Insect cultures were recycled once a month during which more than one hundred insects were transferred onto 200g clean Ife brown seeds in No.1 Kilner jars with meshed lids. The Ife brown cowpea cultivar used for the study was obtained from the Agricultural Research Farm of the Federal University of Technology Akure, Nigeria. The cowpea seeds were disinfested by freezing in a refrigerator for two weeks and thereafter acclimated to conditions in the laboratory.

2.2 Extraction of essential oils

The essential oils were extracted from dry powders of *E. aromatic* and *P. guineense* separately following the method of extraction described by Shaaya *et al.,* 1997. The volatile oil was extracted using steam distillation Clevenger type apparatus (Winzer®), which is made of 2000ml capacity distilling flask with a thick round neck, condenser and graduated measuring tube with a collecting tap at the end. In carrying out the steam distillation process, 120g of each of the powders of *P. guineense* and *E. aromatica* was weighed separately into a distilling flask and 300ml of water added. The apparatus was set up using a clamp on heating mantle and heated for a period of 4hrs. The volatile oil deposited on water was then collected through the attached graduated measuring tube by opening the tap. Thereafter, the distilled essential oil was dried over anhydrous sodium sulphate. There was extraction of the essential oils throughout the period of the experiment to facilitate continuous supply of essential oil for the research study. The essential oil extracted from *P. guineense* was greenish yellow in colour, while that of *E. aromatica* was colourless. The essential oils were collected separately inside a well labeled bottle with tight fitted cover and stored in a cool refrigerator at a temperature of 5 ºC prior to the period of experimental bioassay.

2.3 Experimental procedure

The effect of sub-lethal dose of the two essential oils was tested separately at different rates of application of 0.05, 0.1, 0.2, 0.3, 04, and 0.5ml/kg of cowpea seeds for their potential to induce hormoligosis in *C. maculatus*. Ten freshly emerged adults (5 males and 5 females) of adult *C. maculatus* of 0-24h old were used to infest cowpea seeds in plastic plates (8.5cm diameter) with lid. There were three replicates per treatment. Insect mortality was monitored at 42h and 48h for *P. guineense* and *E. aromatic* essential oil, thereafter; the number of eggs laid was counted and recorded. Cowpea seeds were examined for adult emergence at 21 days after oviposition and the adult that emerged were counted and number recorded.

2.4 Mating propensity of adults of *C. maculatus* exposed to seeds treated with different doses of essential plant oils.

Freshly emerged (0-24h old) adult of *C. maculatus* were placed for 3h on 20g of cowpea seeds separately treated with essential oil of *P. guineense* and *E. aromatica* essential oil at the rates of 0.05 and 0.4ml/kg, respectively. At 3 hours of exposure, beetle exposed to essential oil treated seeds was removed, marked (marked with a black point) and matched with another adult of the same sex and age on the cowpea seeds not treated with essential oil. In order to determine male mating propensity, an unexposed female was placed in a clean Petri plate and the exposed and the matching unexposed males added to the plate. Mating propensity was judge by the number of times each adult male (exposed and unexposed) gained the mounting position (mounted the female) within a period of 3 hours. After each mounting, the successful male was quickly dislodged by probing with Carmel hair brush. A successful mounting was that the aedeagus of the male has male contact with the female. The comparative male receptiveness of female exposed and unexposed to seeds treated with the essential oil was determined according to the procedure outlined for males. Mating propensity was determined for 10 individuals of both sexes.

2.5 Data analysis

Data collected on insect mortality, oviposition and adult emergence were subjected to analysis of variance (ANOVA) using SPSS 16.0. Where necessary, data were transformed before analysis (percentage data were arsine transformed while data based on counting were square root transformed). Where the ANOVA indicated significant differences between treatments, Turkey's test was used to separate means at 5% level of probability. Data collected on mating propensity of adult male and female *C maculatus* were subjected to T-test.

3. Results

Mean percentage mortality of *C. maculatus* at both 24h and 48h post treatment, with sub-lethal doses of essential oil of *P guineense* is presented in Table 1. *C. maculatus* adults were not killed in the control and treatment involving application of 0.05ml/kg of the essential oil of *P. guineense* which was significantly different from mortality observed in treatment involving 0.1 and 0.2ml/kg. Percentage mortality was significantly higher ($P<0.05$) in treatments involving 0.4 and 0.5ml/kg than in other treatments. Mean number of eggs laid by *C. maculatus* was significantly higher ($P<0.05$) in the treatment where 0.05ml/kg of *P. guineense* essential oil was used than the other treatments and control (Table 2). Eggs were not laid on cowpea seeds treated with *P. guineense* essential oil at the application rate of 0.5ml/kg but were not significantly different ($P>0.05$) from number of eggs laid on cowpea seeds treated with 0.4ml of *P. guineense* essential oil. Adult emergence was similarly significantly higher in treatment where 0.05ml of *P. guineense* essential oil was used than the other treatments and control significantly fewer adults of *C. maculatus* emerged from cowpea seeds treated with *P. guineense* essential oil at 0.4ml than those treated with 0.1- 0.3ml. There was no adult emergence from cowpea seeds treated with 0.05ml/kg of *P. guineense* oil.

Table 1: Mean percentage mortality of adult *C. maculatus* infesting seeds treated with essential oil of *P. guineense* at different application rates.

Rates of Application ml/kg of seed	Mean % of adult mortality at 24hr post treatment ± S.E.	Mean % of adult mortality at 48hr post treatment ± S.E.
0.05	0.0 ± 0.00 a	0.0 ± 0.00 a
0.1	0.0 ± 0.00 a	0.7 ± 1.55a
0.2	0.7 ± 1.55 a	0.8 ± 0.58 a

0.3	2.3 ± 1.53 b	5.0 ± 1.73 b
0.4	7.0 ± 1.73 c	8.7 ± 1.55 c
0.5	8.7 ± 1.55 c	9.3 ± 0.58 c
0.0 (control)	0.0 ± 0.00 a	0.0 ± 0.00 a

Means in each column bearing the same letter are not significantly different at 5% level of probability by Tukey's test

Table 2: Mean number of egg laid and adult emergence of *C. maculatus* infesting seeds treated with essential oil of *P. guineense* at different application rates

Rates of Application ml/kg	Mean number of eggs laid laid ± S.E.	Mean number of adult emergence ± S.E.
0.05	369.7 ± 3.56 f	263.4 ± 3.33 f
0.1	128.4 ± 2.66 d	112.4 ± 2.88 d
0.2	115.0 ± 2.01 c	33.3 ± 1.52 c
0.3	63.0 ± 1.89 b	14.0 ± 1.51 b
0.4	15.0 ± 0.31 a	3.00 ± 0.04 a
0.5	0.0 ± 0.00 a	0.0 ± 0.00 a
0.0 (control)	231.0 ± 3.15 e	202.3 ± 3.40 e

Means in each column bearing the same letter are not significantly different at 5% level of probability by Tukey's test.

At 24h post treatment, adults of *C. maculatus* were not killed in the control as well as in treatment involving cowpea seeds treated with *E. aromatica* essential oil at 0.05ml/kg, and this was not significantly different (P>0.05) from mortality produced when 0.1 and 0.2ml/kg of cowpea seed was applied (Table 3). *C. maculatus* mortality produced when *E. aromatica* essential oil was applied at 0.3, 0.4 and 0.5ml/kg of cowpea seed, was significantly higher than in other treatments. The trend observed at 48h post treatment is similar to that observed at 24h post treatment. Mean number of eggs laid by *C. maculatus* was significantly higher (P<0.05) in treatment involving 0.05ml/kg of *E. aromatica* essential oil than in other treatments including the control (Table 4). Mean number of egg laid by *C maculatus* was significantly higher in the control than treatment involving application of *E. aromatica* essential oil at 0.1-0.5ml/kg of seed. Mean number of *C. maculatus* adult emerging from seeds treated with *E. aromatica* essential oil largely followed the same pattern as the mean number of eggs laid.

Table 3: Mean percentage mortality of adult *C. maculatus* infesting seeds treated with essential oil of *E. aromatica* dry flower bud at different application rates.

Rate of Application ml/kg of seed	Mean % of adult mortality at 24hr post treatment ± S.E.	Mean % of adult mortality at 48hr post treatment ± S.E.
0.05	0.0 ± 0.00 a	0.3 ± 0.58 a
0.1	0.7 ± 1.55 a	1.3 ± 1.53 a
0.2	1.0 ±1.00 a	1.3 ± 1.53 a
0.3	7.0 ±1.73 b	9.7 ± 0.58 b
0.4	8.7 ± 0.58 b	10.0 ± 0.00 b
0.5	9.3 ± 0.58 b	10.0 ± 0.00 b
0.0 (control)	0.0 ± 0.00 a	0.0 ± 0.00 a

Means in each column bearing the same letter are not significantly different at 5% level of probability by Tukey's test.

Table 4: Mean number of egg laid and adult emergence of *C. maculatus* infesting seeds treated with essential oil of *E. aromatica* at different application rates.

Rate of Application ml/kg	Mean number of egg laid ± S.E	Mean number of adult emergence ± S.E.
0.05	315.7 ± 14.74 d	273.0 ± 10.44 c
0.1	171.7 ± 18.50 b	164.0 ± 33.15 b
0.2	175.3 ± 14.29 b	131.7 ± 71.02 b
0.3	33.7 ± 43.29 a	17. 7 ± 29.74 a
0.4	4.0 ± 3.00 a	0.0 ± 0.00 a
0.5	0.0 ± 0.00 a	0.0 ± 0.00 a
0.0 (control)	262.7 ± 23.30 c	178.3 ± 63.84 b

Means in each column bearing the same letter are not significantly different at the % level of probability by Tukey's test

Mean percentage adult mortality of *C. maculatus* at 24 and 48hr was linearly and positively correlated with increasing rates of *P. guineense* and *E. aromatica* (r = + 0.94, r = + 0.95, r = + 0.95, r = + 0.92) respectively (Table 5). Adult mortality increases with increase in application rate of the essential oils. However, both oviposition and adult emergence of *C. maculatus* were negatively correlated with increasing rate of *P. guineense* and *E. aromatica* (r = - 0.85, r = - 0.86, r = - 0.93, r = -0.1), respectively (Tables 6 and 7). Number of eggs laid and adult emergence by *C. maculatus* decreases with an increase in application of the essential oils.

Table 5: Linear Correlation and Regression between increasing doses (X) of Essential oil and *Callosobruchus maculatus* adult mortality (Y) for 24hr and 48hr.

Adult Motrality At 24hr			Adult Mortality At 48hr	
Essential oils	**Correlation. coefficient (r)**	**Regression Equation Y = a+bX**	**Correlation Coefficient (r)**	**Regression equation Y = a+bX**
P. guineense essential oil	+ 0.94	Y = -1.39+18.345X	+ 0.95	Y = -1.19+ 21.191X
E. aromatic essential oil	+ 0.95	Y = -1.04+21.922X	+ 0.92	Y= -0.73+ 24.352X

Table 6: Linear Correlation and Regression between increasing doses (X) of Essential oil and number of eggs laid by *Callosobruchus maculatus* (Y)

Essential oils	Correlation coefficient (r)	Regression equation Y = a+ bX
P. guineense essential oil	- 0.85	Y = 263. 93-57.051X
E. aromatic essential oil	- 0.93	Y = -278.68-637.635X

Table 7: Linear Correlation and Regression between increasing doses (X) of Essential oil and number of adult emergence by *Callosobruchus maculatus* (Y)

Essential oils	Correlation coefficient (r)	Regression equation Y = a+bX
P. guineense essential oil	- 0.86	Y = 198.40-40.601X
E. aromatic essential oil	- 0.91	Y = 223.11-514.26X

Males and females of *C. maculatus* exposed to 0.05ml concentration of essential oil of *P. guineense* showed significantly greater propensity to mount than unexposed individuals (Fig.1). Males and females of the beetle exposed to 0.4ml concentration of the essential oil of *P. guineense* showed significantly lower propensity to copulate than unexposed individuals. A similar trend was observed in males and females of *C. maculatus* exposed to 0.05ml and 0.4ml concentration of *E. aromatica* essential oil.

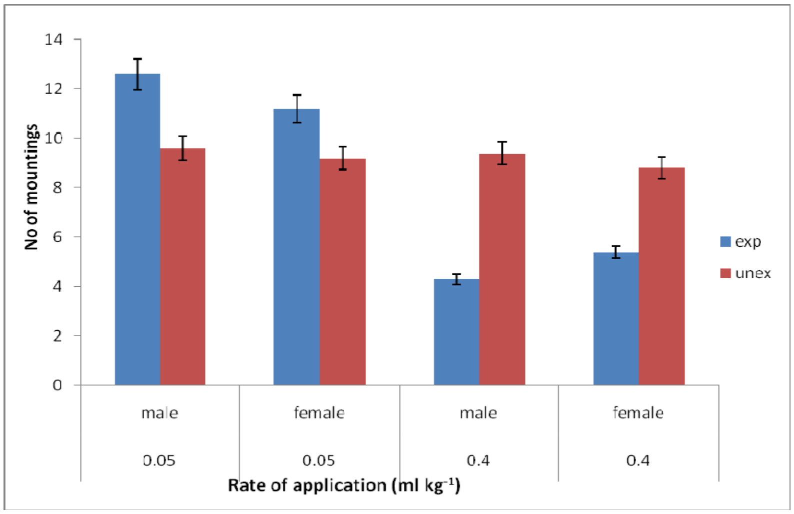

Fig 1: Number of mounting of *C. maculatus* adults exposed to two rates of essential oil of *P. guineense* for three hours exp- exposed; unex - unexposed

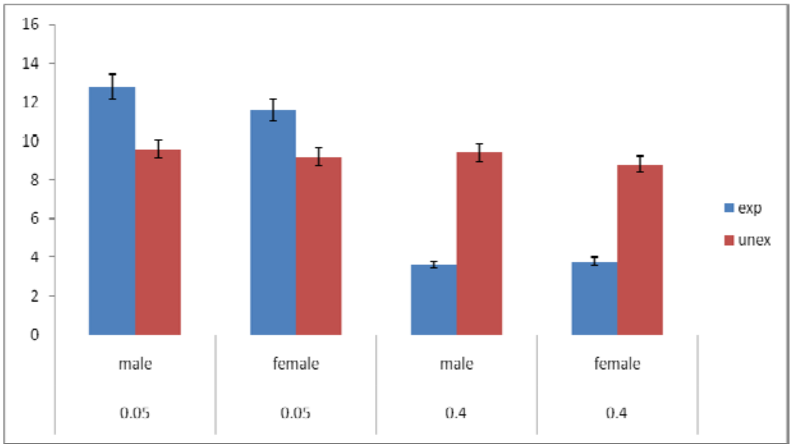

Fig 2: Number of mounting of *C. maculatus* adults exposed to two rates of essential oil of *E. aromatica* for three hours exp- exposed; unex - unexposed

4. Discussion

The result from this study shows that *C. maculatus* can develop resurgence to sub-lethal application of essential oils of *P. guineense* and *E. aromatica*. Quite interesting was the observation that at a sub-lethal application of 0.05ml of the essential oils of *P. guineense* and *E. aromatica* significantly enhanced oviposition by females of *C. maculatus* in comparison with other treatment and the control. Correspondingly, progeny production by *C. maculatus* in treatment involving application rate of 0.05ml/kg of cowpea seed was significantly higher than in other treatments and the control. The phenomenon in which a stimulatory effect is induced in insects or other arthropods by sub-lethal doses of pesticidal substances which causes females to lay more viable eggs that develop to adults is termed hormoligosis [4, 14]. Hormoligosis induces resurgence so that instead of reducing numbers of pest insects, their population actually increases. It was also reported that there was an increase in oviposition and progeny production of *C. maculatus* infesting cowpea treated with sub-lethal amounts of some insecticidal botanical oils including clove oil *Syzygium aromaticum* [2]. Hormoligosis, in the form of increased fecundity was observed in *Acanthoscelides obtectus* and *Zabrotes subfasciatus*, when milled dried leaves of *Tetradenia riparia* was applied at 1% for the control of bruchids [16].

In this study a plausible reason for the observed increase oviposition leading to a corresponding increase in progeny production in *C. maculatus* as a response to application of sub-lethal dose of essential oils of *P. guineense* and *E. aromatica* has been unravelled. The sub-lethal dose caused significant increase in propensity to mate in the exposed individuals. It has been reported that an increase in the number of copulation, increases ovarian production and fecundity of female *C. maculatus* [10]. Also reported was symptoms of lethal effects of insecticidal products from *E. aromatica* and *P. guineense* to include restlessness, hyper-excitability, rapid induction of unconsciousness, paralysis and death [12]. In this study, it is being postulated that the sub-lethal dose of the essential oils may have elicited non-lethal excitability that was responsible for the significant increase number of matings of exposed individual insects. Other possible mechanism for inducing hormoligosis in *C. maculatus* by exposure to sub-lethal doses of insecticidal essential oils may include increased longevity and oviposition period and stimulation of egg development. These can be subjected to empirical verification. Hormoligosis can be avoided by always applying a dose of the pesticide that provides economic control of the insect pest [4]. The phenomenon may however confer incidental benefit on natural enemies of pest species as it could boost their populations and thus improve their capacity to regulate the populations of pest species [4]. This may not mean much especially in stored products protection as considerable damage would already have been done to the stored products.

5. References

1 Adedire CO. Pest of Stored Cereal and Pulses in Nigeria. Edn 1, Dave Collins Publications, Akure, 2001; 1:54-94.

2 Lale NES. The biological effects of three essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Journal of African Zoology. 1991; 105:357-362.

3 Lale NES. Stored-Product Entomology and Acaralogy in Tropical Africa. Mole Publications, Maiduguri, 2002, 124-133

4 Lale NES. Dictionary of Entomology and Acaralogy. Mole Publications, Porthacourt, 2006, 294.

5 Lale NES, Abdulrahman HT. Status of varietal resistance in the management of the cowpea seed bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in the tropics. Entomological Society of Nigeria (ESN). 1998; 31:221-226.

6 Lucky TD. Insect hormoligosis. Journal of Economic Economic Entomology. 1968; 61:7.

7 Mclure MS. Resurgence of the scale insect, *Fiorinia externa* (Homoptera: Diaspididae) on hemlock following insecticide application. Environmental Entomology. 1977; 6:482-483.

8 Obeng-Ofori D. The use of botanicals by resource farmers in Africa and Asia for the protection of stored agricultural products. Stewart Postharvest Review. 2007; 6:1-8.

9 Ofuya TI. A population explosion of *Aphis craccivora* (Koch) (Homoptera: Aphididae) in cowpea protected with cypermethrin. F.A.O. Plant Protection Bulletin. 1987; 35:75-97

10 Ofuya TI. Multiple mating in males of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and its consequences. Journal of Stored Product Research. 1995; 31:71-75.

11 Ofuya TI. Beans, insect and man. Inaugural Lecture. The Federal University of Technology, Akure, Nigeria, 2003; 35:45.

12 Ofuya TI, Osadahun MJ. Effects of three plant powders on behavior, mortality and reproductive fitness of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Zoological Research. 2005; 26(6):603-608.

13 Pascua-Villalobos MJ, Ballesta-Acosta MC. Chemical variation in an *Ocimum basilicum* germplasm collection and activity of the essential oils on *Callosobruchus maculatus*. Biochemical Systematic and Ecology. 2003; 31:673-679

14 Larry PP, Marlin ER. Entomology and Pest Management. Edn. 5, Pearson Prentice Hall, New Jersey, USA, 2006, 556-586.

15 Shaaya E, Kostjukovski M, Eilberg J, Sukprakarn C. Plant oils as fumigants and contact insecticides for the control of stored product insects. Journal of Store Product Research. 1997; 33:7-15.

16 Weaver DK, Dunkel FV, Cusker JL, Puyvelde LV. Oviposition pattern in two species of bruchids (Coleoptera: Bruchidae) as influenced by the dried leaves of *Tetradenia riparia* a perennial mint (Lamiales: Lamiaceae) that suppresses population size. Environmental Entomology. 1992; 21(5):1121-1129.