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Deterrent effects of citrus peel oils on oviposition and adult emergence of the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

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ABSTRACT

Oil extracts of the fruit peels of five citrus species namely Citrus tangerina, C. limonium, C. paradisi, C. aurantifolia and C. sinensis were investigated for their effect on oviposition and adult emergence of the cowpea weevil, Callosobruchus maculatus on cowpea seeds, Vigna unguiculata. The oils were extracted by hydrodistilation and applied on the cowpea seeds in concentration series of 5.5ml and 2.75ml. The results indicated that a significant ($P < 0.05$) reduction in oviposition and adult emergence of C. maculatus were recorded in all the treatments with citrus oil compared to the control. Oviposition deterrence in the weevils was highest in treatments with C. sinensis (72 - 79%) and lowest with C. tangerina (62 - 68%). Reduction in emergence in the treatments with the essential oils ranged between 89 and 98%, oils of C. paradisi and C. sinensis recorded the highest effect (98%) while C. tangerina recorded the lowest effect (89%). The effectiveness of the citrus peel oils in deterring oviposition and reducing emergence of the cowpea bruchid could be explored and used as biocide in the management of this cowpea pest.

Key words: Citrus, cowpea weevil, emergence, oil extract, oviposition.

INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* (F.) is a major pest of cowpea (*Vigna unguiculata* (L.) Walp.). It is a cosmopolitan pest with initial infestation starting in the field and continuing in storage where the weevil population expands rapidly causing substantial damage [10, 25, 17, 22].

The life cycle of *C. maculates* takes between 3 - 5 weeks, eggs are laid stuck on the outside of the pods by the female or directly on the seeds if the pods have dehised [6, 15]. The oviposition period takes six days; the female lay about ninety eggs each, about 60-70 eggs are deposited during oviposition in the first three days [13]. The eggs hatch after three days to scarabeiform larvae which undergo four larval instars. These larval stages are the most notorious and destructive causing great damage to seeds [14].

The subsequent pupal period last for seven days. Pupation takes place in a chamber just under the seed testa and is characteristically noticeable as a round “window” through which the progeny emerges on the twentieth day and an emergence hole is conspicuously left behind on the seed surface. [21] reported material loss of about 25% of the seed from which it emerged.

Efforts at reducing the effects of bruchid attack on seeds involve the use of chemical pesticides such as methyl bromide and phosphine amongst others. Despite successes recorded in the use of chemical pesticides, the egg stage of insects is the most difficult stage to control with fumigants [11]. Moreover, the use of chemical methods for reducing or eliminating bruchids in stored cowpea seeds have many drawbacks such as its effects on human health, pest resurgence, residual toxicity and environmental hazards [9, 16, 23].

An alternative for chemical control is the use of natural products from plants [3, 12]. Botanical pesticides are non-toxic to humans, readily available, cheaper and biodegradable than conventional pesticides [4]. Numerous plants have been shown to produce compounds with pesticidal activity [3, 8, 24]. The products from plant parts possess phytochemicals which have been shown to have potentials for oviposition deterrency and reproductive inhibitors [2].

Oil extracts from plants such as groundnut and palm have been shown to have ovicidal effect on bruchid egg and effective in reducing emergence of cowpea weevil and causing early mortality of emerged adults [5, 7]. [19] also reported that oils extracted from kernels of the Mee plant (*Malhuca longifolia*) significantly reduced the oviposition and egg hatchability of *C. maculatus* in Sri Lanka.

Essential oils from plants are therefore likely to provide effective, biodegradable and environmentally safe pesticides. The fruit peels of citrus species have been reported for their insecticidal properties and promising control measure of stored cowpea bruchids especially the adult stage of *C. maculatus* [11]. The use of citrus fruit peel oil in combating the early life stages of the cowpea seed pest could be an important approach which has not been given necessary attention in the management of pulse pest. Citrus plants are grown widely in Nigeria and could offer an opportunity for developing them as alternatives to chemical pesticides in protecting stored cowpea seeds from weevil attack. The present study is aimed at evaluating the effect of oil extracts of citrus fruit peels on oviposition and adult emergence of the cowpea seed bruchid, *Callosobruchus maculatus* (L.) Walpers.

MATERIALS AND METHODS

Treatment of Seeds

The seeds of cowpea, *Vigna unguiculata* (L.) Walpers, used for this experiment were purchased from the central Oba Market, Benin, Southern Nigeria. The seeds were disinfected of any living pest by placing in a beaker in an oven regulated at 60^0C for 24 hours period.

Preparation of Stock Culture

A stock culture of the cowpea seed weevils, *Collosobruchus maculatus* (Fabricius) was obtained by infesting cowpea seeds in four Bama bottles, covered with nylon netting held in place by means of a rubber band until adult insects emerged. Laboratory conditions were 30 $\pm$ 1^0C and 65 $\pm$ 5% r.h. The stock culture was sieved of *C. maculatus* with a sieve of 2mm net mesh into a tray rubbed at the edges with vaseline to prevent any of the non-flight form of the bruchid from coming out. An aspirator or pooter was used in conveying the newly emerged normal males and females.

Extraction of Citrus Oils

The barks of five species of citrus fruits (*Citrus tangerina, C. limonium, C. paradisi, C. aurantifolia* and *C. sinensis)* were peeled and sun dried for 7 days with 8h sunlight. Ground materials were obtained by grinding the dry peels into fine powder by means of mortal and pestle. 180g of each powdered peel was placed in a conical flask, the essential oil extract was obtained by hydrodistilation using a modified Clevenger-type apparatus. Extracted oil was stored in a refrigerator at 5^0C.

Experimental Design

Seeds of disinfected cowpea were counted out into two sets of 200 seeds each for the five citrus applications. The first set was placed in a bowl and 5.5 millilitre of each citrus oil was applied, shaken and allowed to dry. The other set was similarly treated with 2.75ml citrus oils. Another set represented the control and no oil was applied to this. Each of 200 seeds for both the citrus oils as treatments and that of the control was sorted out to 50 seeds each into small plastic containers perforated at the top lid in such a way that it can hold a nylon netting of about 1 x 1mm mesh size. The experiment was in four replicates. Each specimen container was infested with five normal males and females of newly emerged. *C. maculatus* and then allowed to oviposit for 6days till no live bruchid was left at the

end of each oviposition trial, eggs laid by the insects on cowpea seed were counted using a magnifying glass, the results obtained with the treated seeds were compared to control.

STATISTICAL ANALYSIS

The various values obtained were expressed as means ± standard error and percentages. Analysis of variance (one-way ANOVA of the SPSS version 6) were employed to test for significant difference among the data. Statistical significance was set at $P < 0.05$.

RESULTS

Table 1 shows oviposition activity of *Callosobruchus maculatus* after the cowpea seeds were treated with five citrus fruit peel oils.

Table 1. Oviposition deterrent activity of citrus oils on *C. maculatus.*

Treatment	Dose concentration of oil / 50 seeds	Mean Number of eggs laid ± S.E.	Percent Detergency
C. tangerina	5.5ml	22.7 ± 3.3[a]	68%
	2.75ml	24.7 ± 2.4[ab]	62%
C. limonium	5.5ml	20.7 ± 3.6[a]	71%
	2.75ml	21.7 ± 1.5[ab]	66%
C. paradisi	5.5ml	18.5 ± 2.2[a]	74%
	2.75ml	19.7 ± 3.3[a]	70%
C. aurantifolia	5.5ml	16.7 + 2.5[a]	76%
	2.75ml	19.0 + 1.4[a]	71%
C. sinensis	5.5ml	15.0 + 1.6[a]	79%
	2.75ml	18.0 + 2.7[a]	72%

** Means in a column having the same letter in their superscript do not differ significantly from each other at 5% significant level.*

The mean values of eggs laid by *C. maculatus* on cowpea seeds treated with citrus oils varied between 15 to 22.7 for the 5.5ml concentration of citrus oil. At 2.27ml oil concentration, the average number of eggs laid ranged from 18 to 24.7. The results showed that treatment means were not significantly different from each other. There was however a significant difference ($P < 0.05$) in the number of eggs oviposited when compared with the control setting. The number of eggs laid in the control was significantly higher (65.5 - 72eggs) than the oil treated samples. Mean oviposition was highest in the treatment with *C. tangerina* (22.7 - 24.7) and lowest in *C. sinensis* (15-18). The mean number of eggs laid by *C. maculatus* due to effect of 5.5ml concentration of citrus oils in descending order on cowpea seeds is as follows: *C. tangerina* (22.7), *C.limonium* (20.7), *C.paradisi* (18.5), *C.aurantifolia* (16.7) and *C. sinensis* (15.0). At this concentration a respective percent reduction of 68, 71, 74, 76 and 79% were obtained compared to the control. While with 2.75ml concentration of citrus oil mean oviposition in the descending order of the various treatment are: *C. tangerina* (24.7), *C. limonium* (21.0), *C.paradisi* (19.7), *C.aurantifolia* (19.0), and *C. sinensis* (18.0). A ratio reduction of 62, 66, 70, 71 and 72% respectively were obtained compared to the control. The trend of mean oviposition is presented graphically in Fig 1.

Table 2. Adult Emergence of *C. maculatus* on Cowpea seeds treated with essential oils of citrus species.

Treatment	Dose concentration of oil extract / 50seeds	Mean number of Adult Emergence	% reduction in adult emergence
C. tangerina	5.5ml	1.0 ± 0.4[a]	89%
	2.75ml	1.0 ± 0.04[a]	91%
C. limonium	5.5ml	1.0 ± 0.4[a]	89%
	2.75ml	1.2 ± 1.2[a]	89%
C. aurantifolia	5.5ml	0.7 ± 0.7[a]	92%
	2.75ml	1.0 ± 0.4[a]	91%
C. paradisi	5.5ml	0.2 ± 0.2[a]	98%
	2.75ml	0.2 ± 0.2[a]	98%
C. Sinensis	5.5ml	0.2 ± 0.2[a]	98%
	2.75ml	0.2 ± 0.2[a]	98%

** Means in a column having the same letter in their superscript do not differ significantly from each other at 5% significant level.*

Table 2, shows mean values of adult emergence of *C. maculatus* in the experimental and control set up. The graphical trend is presented in Fig. 2. Emergence was very low in experimental culture with citrus oils as treatment and high in control. Emergence in experiments treated with 5.5ml concentration of citrus oil recorded higher numbers (4) with *C. limonium* and *C. tangerina*, there was lower emergence (0.12) in treatments with *C. paradisi*

and *C. sinensis.* In the case of treatments with 2.27ml concentration of the citrus oils, the mean emergence ranged from 1 to 5 progeny.

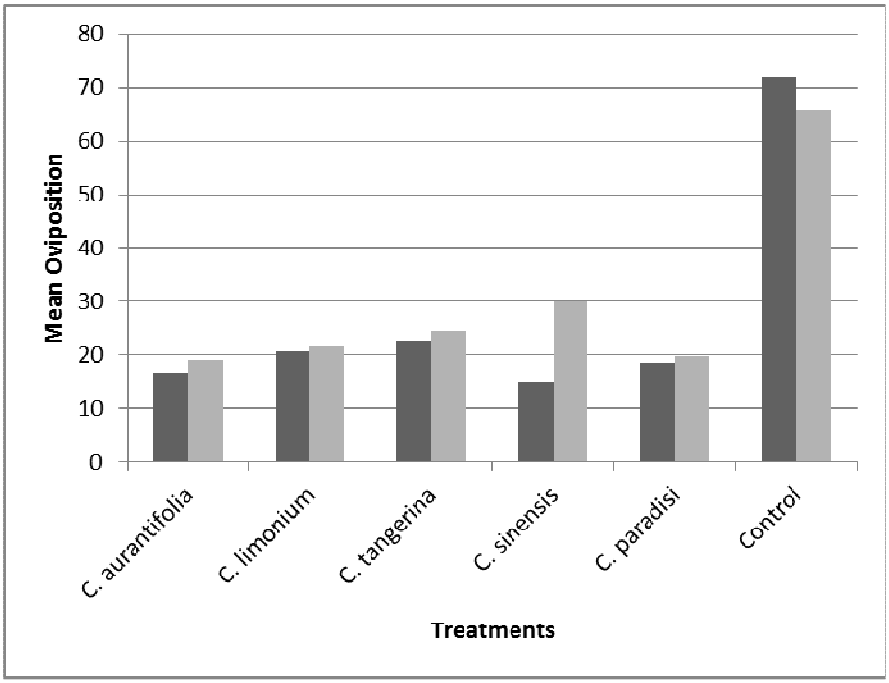

Fig. 1: Graph showing mean oviposition of *Callosobruchus maculatus* on cowpea seeds after treatment with five citrus peel oils.

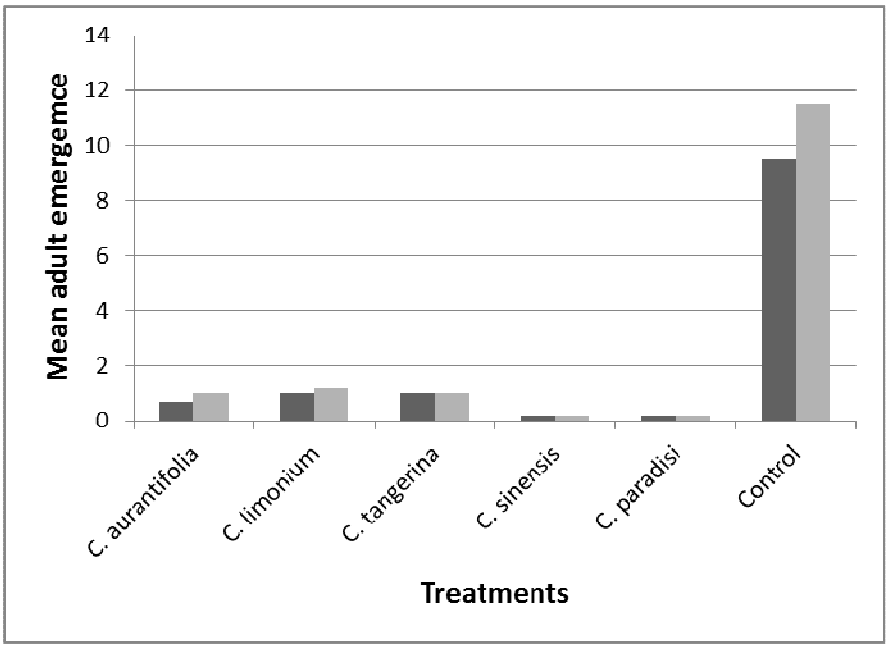

Fig. 2: Graph showing mean adult emergence of *Callosobruchus maculatus* after treatment of seeds with citrus peel oil.

The mean adult emergence in the descending order of the treatments with 5.5ml essential oil used on cowpea seeds are: *C. tangerina* (1.0 ± 0.4), *C. limonium* (1.0 ± 0.4), *C autantifolia* (0.7 ± 0.2) *C. paradisi* (0.2 ± 0.02), C. sinenesis (0.2 ± 0.2), the control recorded 9.5 ± 0.6. Treatment with 2.75ml essential oils recorded mean adults emergence in

the following descending order: *C. limonium* (1.2 ± 1.2), *C. tangerina* (1.0 ± 0.4), *C. aurantifolia* (1.0 ± 0.4), *C. paradisi* (0.2 ± 0.02) and *C. sinensis* (0.2 ± 0.02); the control recorded 11.5 ± 0.6. The computed deterrency figures averaged between 89 and 100% reduction in emergence in the treatments with 5.5ml essential oil concentration when compared with the control and between 91 to 98% reduction in treatments with 2.75 ml essential oil compared to the control.

DISCUSSION

The results of this study revealed that the essential oils extracted from the fruit peels of the five citrus species had a profound effect on oviposition and adult emergence of *C. maculatus.* The oil extracts caused a significant ($P < 0.05$) oviposition deterrence and reduction of progeny production of cowpea bruchid in all the oil treatments compared to the control where no oil was applied.

Oviposiiton in *C. macnlatus* on seeds of cowpea was significantly affected due to treatment of citrus oils. Application of the five citrus essential oils to cowpea seeds at rate of 2.75 or 5.5 ml per 50 seeds gave promising levels of control in terms of reduction of mean number of eggs laid by the weevils. It appears that the plant oil extracts might posses oviposition deterrent principles since all of them significantly reduced oviposition of *C. maculatus;* the reduction averaged between 68 and 79% compared to the control. In an earlier study, [9] revealed that *C. aurantifolia* peel extract shows more than 50% oviposition deterrent activity and attributed this deterrency to changes induced in physiology and behaviour in the adult of *C. maculatus* as reflected by their egg laying capacity. [24] has also reported that reduced number of eggs laid on cowpea seeds treated with citrus materials could be a result of disrupting the mating and sexual communications as well as deterring females from laying eggs. [1] also showed that reduction in egg laying of *C. maculatus* could be due to application of essential oils of citrus sp could be the involvement of the oil vapours in ovarian changes similar to those caused by chemosterilisants by blocking female egg laying.

The citrus oils also exhibited significant insecticidal activity against the weevils at the emergence stage. The efficacy of the oil extracts was much stronger against the emergence stage than the egg laying. This study revealed a percentage reduction in emergence of between 89 and 98% as against 62 - 79% oviposition deterrrency. A similar trend was also noted [9]. [18] had earlier pointed out that when eggs are laid on citrus oil treated seeds the toxic substance present in the oil extract may enter into the egg through chorion so that further embryonic development is suppressed and this could lead to a higher percentage reduction in adult emergence. The oils of *C. paradisi* and *C. sinensis* were more potent in inhibiting adult emergence followed of *C. aurantifolia* and *C. limonium;* the oil extract of *C. tangerina* was the least potent. This finding corroborates with the findings of [11] who worked on fumigant activity of citrus oils against cowpea seed beetle, *C.maculatus* and reported that oils of *C. paradisi* and *C. sinensis* proved most effective.

The early life stages of the insect in this study is of considerable importance because it is these stages that determine the number of adult beetles that will eventually reproduce in the F_2 generation. If fewer adults emerge due to application of suitable control measures against the early formative stages, it may result in drastic reduction in the population of the beetles which hitherto would have become potential pests of cowpeas. Generation time can have a significant effect on population growth. [20] have shown that if there is no check in the life history of an organism, the population may grow without limits. This innate capacity for growth is expressed as exponential. The life history adaptations of the cowpea bruchid favour very rapid exponential growth, the increase in the numbers of emergent individuals rapidly accelerates the size at which the population of bruchid grows in storage cowpeas. The application of appropriate control measures such as citrus oils as in this case can reduce this innate capacity for growth. The results of this study have revealed that oil extracts derived from fruit peels of citrus species applied on cowpea seeds were able to cause substantial reduction in oviposition and emergence of the cowpea seed pest, *Callosobruchus maculatus.* The oils could therefore be explored as potential biopesticide against the early life stages of this pest and as a suitable control measure in the management of weevils infesting stored cowpeas.

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