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Effectiveness of citrus oils as cowpea seed protectant against damage by the Cowpea Bruchid *Callosobruchus maculatus* (F) (Coleopteran: Bruchidae)

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ABSTRACT

The effectiveness of essential oils Citrus aurantofolia, Citrus limonium, Citrus sinensis and Citrus paradisi were evaluated as cowpea seed protectant against damage by the cowpea bruchid, Callosobruchus maculates in a laboratory set up at 2.75 and 5.5 ml oil using hydrodistillation technique. The results showed that cowpea seed damage in all the citrus oil treated experiments was significantly low and ranged between 0.50 and 2.50% compared tot the control (23.00%). Weevil perforation index (WIP) ranged from 2.12 to 9.81 and indicate a highly positive protectant ability of the essential oils. The computed percentage protectant ability of the citrus oils showed that the essential oils resulted in 90.19 - 92.0% protectant ability in C. limonium, 92.00 - 93.87% in C. aurantifolia and 97.80% in C. sinensis and C. paradisi. The results indicate that citrus peel essential oils are highly effective as cowpea seed protectant against damage by C. maculates and may be used as safe pesticide for the management of stored cowpeas.

Key words: Citrus peel oil, cowpea bruchid, protectant, seed damage.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walpers, is a major food crop in tropical countries and popularly used as protein suppliment for meat and fish; moreover, it contains digestible carbohydrates and lysine [4]. The seeds of this crop is however vulnerable to insect pests of which the cowpea beetle, *Callosobruhus maculatus* (Coleoptera: Bruchidae) is the most important [15].

Callosobruchus maculatus is a major field to storage pest of cowpea with initial infestation starting in the field and expanding rapidly during seed storage. Losses due to infestation of between 87 to 100% within 3-5 months of storage have been reported [30, 28, 27]. The occurrence of this cowpea pest therefore constitutes a major problem contributing to huge food shortage in tropical and subtropical countries of the world.

Attempts made to reduce the menace of *C. muculatus* led to the acquisition of improved traditional storage suggested [21]. Other storage methods include underground pits, drums, bags and pots. 30 percent of these traditionally stored cowpeas may face attack after three months, and 50 - 60% after six months [5]. [3] suggested drying and storage of cowpea on small scale with ashes in airtight containers.

Conventional methods of protecting stored cowpeas is known to depend on the use of synthetic chemical insecticides [12]. Synthetic insecticides have proved very effective in the control of the beetle [13]. Control of

cowpea pests using chemical pesticides is however being discouraged because of health hazards to humans and environmental concerns amongst others [14, 24]. An alternative approach for the reduction of bruchid attack is the use of natural products of plants origin. The low cost and safety of botanical extract is gaining more importance in controlling cowpea pests [7, 23, 20, 16, 31].

Oil extracts from various aromatic plants have been widely investigated and their effect on stored insect pest has been of special interest in recent years [18, 11, 19, 1, 2]. This study aim at assessing the effectiveness of citrus peel essential oils as cowpea seed protectant against damage by the cowpea bruchid, *Callosobruchus maculatus* F.

MATERIALS AND METHODS

Experimental Cowpea Seeds

Cowpea seed (*Vigna ungiculata*) used for this experiment were purchased from the central Oba Market in Benin, Southern Nigeria. The seeds were handicapped to remove infested seeds and debris and examined under a light microscope to make sure there were no visible signs of weevil attack or damages and kept by deep freezing for 2 weeks as recommended [20]. The seeds were thereafter bulked and transferred into IL Kilner jar covered with fine mesh and left for 24 hours under ambient condition of 30 ± 2^0C and 70 - 80% RH.

Insect Culture

The test insect (*Callosobrucus maculatus* Fabricius) were obtained from previously infested cowpea seeds and establish in four Bama bottles covered with fine mesh netting and kept under laboratory conditions (30 ± 2^0C, RH 70 ± 5% and 12h photoperiod). Twenty pairs of male and female *C. maculatus* adults were isolated and introduced into pots containing cowpea seeds [27] to allow for mating and oviposition. The pots were covered with nylon netting held in place by mean of rubber band to prevent the escape stock were sieved out. The subsequent F_1 progenies that emerged were used for the experiment.

Plant Material and Extraction of Essential Oil

Four species of citrus fruit (*Citrus aurantifolia* Chistm., *C. Limonium* Risso., *C. Sinensis* Osbeck and *C. paradisi* Macf.) purchased from central Oba market, Benin, Southern Nigeria were used for the study. The peels of the fresh fruits were sum-dried for seven days with 8 hours of sunlight; the dried peels were grounded into fine powder using laboratory pestle and mortar and placed in conical flasks. The hydrodistillation procedure [6] was employed in extracting essential oils from the powdered material. Extracted oils were stored in a refrigerator at 5^0C until commencement of tests.

Experimental Procedure

The experiment was carried out in small plastic containers (12cm diameters). Fifty seeds of cowpea were placed in the containers and the oil extract at concentrations of 2.75 and 5.5ml was applied, the range of concentration hade been chosen based on a number of preliminary tails. The oil extracts was thoroughly mixed with the aid of glass rod to ensure that the seeds were uniformly coated and allowed to dry. No oil was applied to the control set up. Five normal males and five females of newly emerged *C. maculatus* were then introduced into the dishes and allowed to oviposit till no live bruchid was left, twenty-one days after oviposition when emergence started; the progenies were removed daily till the seizure of emergence. The experiments, which were in four replications, were observed daily for 7 weeks under room temperature of 30 ± 2^0C and 65 ± 5% relative humility. The extent of bruchid damage to seeds was evaluated by counting the exit holes.

Data Analysis

The data obtained from the experiments were subject to one-way analysis of variance (ANOVA) of the (SPSS) version 6.0 statistical difference between the means was separated using the Least Significance Difference (LSD) test. Significance difference was set at P > 0.05 level. Percentage damage (PD) and weevil Perforation Index (WPI) was calculated according to the methods of [10].

$$PD = \left[\frac{\text{Total No of treatment grains perforated}}{\text{Total No of grains}} \right] X \ 100$$

$$WPI = \left[\frac{\% \text{ of treatment grains perforated}}{\% \text{ of control grains perforated} + \% \text{ of treated grains perforated}} \right] X\ 100$$

Percent Protectant Ability (PPA) = 100 – WPI

RESULTS

The effects of citrus essential oil on cowpea seed damage due to infestation by the cowpea weevil, *Callosobruchus maculatus* is presented in Table 1. The percentage cowpea seed damage in the citrus oil treatments ranged between 0.5 to 2.50 as against 23.00 in the control. Percent damage was highest in *Citrus luminium* 2.00 - 2.50, followed by *C. aurantifolia* (1.50 - 2.00), it was lowest in *C. sinensis* and *C. paradisi* (0.05).

In *C. aurantifolia* and *C. limonium* oil treatments, the 5.5ml concentration gave a higher defense against damage (1.5 - 2) than the lower concentration of 2.75ml (2.0 – 2.5). In *C. sinensis* and *C. paradisi* however, the two series of concentrations were similar in their action (0.50).

TABLE 1: Effects of Citrus Essential Oils on Cowpea Seed Damage

Citrus species	Concentration (ml per 50 seeds)	Total No of seeds	No of seeds perforated	% seed damage	Weevil Perforation Index (WPI)
C. aurantifiola	2.75 5.50	200 200	4 3	2.00 1.50	8.00 6.13
C. limonium	2.75 5.50	200 200	5 4	2.50 2.00	9.81 8.00
C. sinensis	2.75 5.50	200 200	1 1	0.50 0.50	2.13 2.13
C. paradisi	2.75 5.50	200 200	1 1	0.50 0.50	2.13 2.13
Control	0.00	200	46	23.00	66.67

**Weevil Perforation Index (WPI) above 50 is an indication of negative protectant ability.*

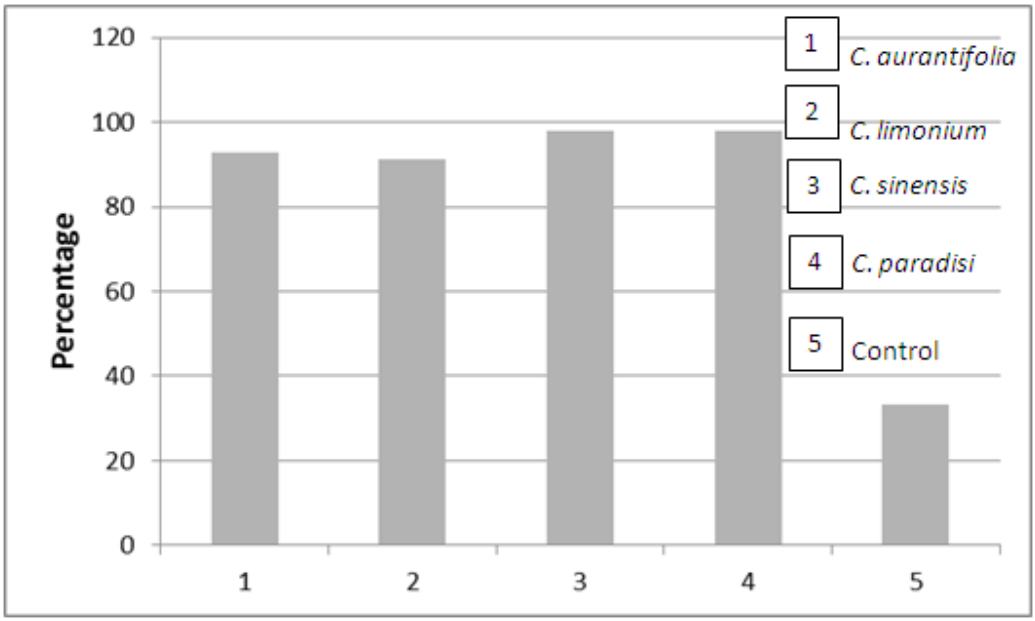

Fig. 1: Graph showing percent protectant ability of citrus essential oils against *C. maculatus*

The Weevil Perforation Index which indicate the ability of the essential oils in protecting the cowpea seeds ranged in values from 2.13 to 9.81 compared to the control (66.67) values of this index above 50 indicate negative protectant ability.

The effectiveness of the citrus oils as protectant against damage by *C. maculatus* represented as percent protectant ability showed that oil concentrations of 2.57 and 5.5ml resulted in 90.19 - 92% ability in *C. lumanium*, 92 - 93.87% in *C. aurantifolia*, and 97.8% in *C. senensis* and *C. paradisi.* The average percent protectant ability is represented graphically in fig. 1.

DISCUSSION

Varying degrees of success have been recorded by many farmers in the tropics in the use of botanicals to protect their legumes [29, 17]. Among the numerous plant parts, the essential oils from the fruit peel of citrus (Rotaceae) appear to have promising level of control over pulse pests. Citrus fruits are cultivated widely in the tropics and therefore offer opportunity for developing their products as alternatives to hazardous pesticides to protect stored cowpeas from pest damage. [17] had discussed some advantages of using citrus peel oils as grains protectant and showed that it can be easily extracted from peels by water steam distillation, it may have very low toxicity to mammals since citrus oil is one of the popular food flavourings; it is also cost effective and its application is easy.

The results of the four citrus essential oils treated on cowpea seeds to evaluate damage due to infestation by *Callosobruhus maculatus* in this study showed that the effectiveness of the essential oil was relatively ideal. The results showed that all the citrus essential oils proved effective in reducing damage to seeds, lowering the weevil perforation index and increasing protectant ability.

Damage to cowpea seeds in this study was very low due to protection of the seeds by the citrus oils.

The percentage damage to seed ranged between 0.05 to 2.50. Percentage seed damage in descending order of the oil treatments was *C. limonium, C. aurantifolia,* and *C. sinensis / C. paradisi. C. sinensis* and *C. paradisi* oils showed higher effectiveness at preventing damage.

A similar trend of citrus essential oil activity in preventing grain damage to cowpea by *C. maculatus* was observed [15]. In their findings citrus oil was able to suppress grains damage to about 4.16%. Percentage damage in untreated seeds in this study was about 23%, this value is relatively higher that that of the oil treated experiments. [26] also attributed loss of seed material as considerable – each adult *Callosobruchus* emerging from a cowpea would have consumed about 25% of the seed from which it emerged.

Weevil perforation index, which indicate the protectant ability, were significantly lower in the citrus oil treated experiments that the non oil treated control, the value recorded in the control was higher than 50. Values above 50 is usually an indication of negative protectant ability [15]; this study recorded a value of 66.67 in the control compared to 2.13 - 9.81 recorded in the citrus oil treatments, this low values is an indication of the high protectant ability of the citrus oils.

The effectiveness of the citrus oils as cowpea seed protectant against *C. maculatus* manifested by percentage protectant ability indicated that the levels of protection were all above 91% compared to the control. Seed protection was highest in treatments with *C. sinensis* and *C. paradisi* essential oils where an averaged 97.8% protection was achieved. The high effectiveness could be due to coating of the seed by the oil extracts. [22] and [25] had previously shown that oil coating is effective in controlling *C. maculatus.* The protective ability of essential oils could be attributed to interspecific insect responses to oil constituents [8]. The active component of citrus oils is limonene [17, 1]. Insecticidal activity of limonene has been successfully applied for the control of insects [32].

This study reveals that the essentials oils of *C. aurantifolia, C. lumonium, C. sinensis* and *C. paradisi* are highly effective as biopesticide for protecting cowpea seeds from *C. macualtus* infection and damage.

CONCLUSION

The results obtained in this study revel as that the essential oils of *C. aurantifolia, C. lumonium, C. sinensis* and *C. paradisi* have strong effect in protecting cowpea seeds from *C. maculatus* damage. Citrus oils may therefore be incorporated and adopted for the control of pulse pests, this could further reduce the use of synthetic chemical pesticides.

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