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HIGH-SENSITIVITY TESTING OF EFFECTIVENESS OF CITRUS LIMON, VITIS VINIFERA AND CITRUS SINENSIS IN THE POSTHARVEST CONTROL OF CALLOSOBRUCHUS MACULATUS FABRICIUS (*Coleoptera: Chrysomelidae*) INFESTATION OF COWPEA SEEDS

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ABSTRACT

Traditional control tactics are commonly employed against stored-product insects and mites in developing countries. Therefore, at economically justified dose of 1.0% (w/w), the biological activities of dry peel powders of three citrus species were evaluated in laboratory conditions (30°C, 77.8% r.h.) against *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae) infesting cowpea, *Vigna unguiculata* (L.) Walp seeds in storage. The dry peel powder of the three citrus fruit species (the lemon, *Citrus limon*; the common grape vine, *Vitis vinifera*; sweet orange, *Citrus sinensis*) had contact toxicity on the *C. maculatus* insect pest and affected its biological traits in a trial that used ten varieties of cowpea. *Citrus limon* caused the highest adult mortality at 24 h high-sensitivity exposure time and this differed significantly from mortalities caused by the other citrus species. The same results were obtained for oviposition, adult emergence and seed damage. The pooled mean toxic effect of *C. limon* on the store-product pest was comparable to cypermethrin standard insecticide in 24 h. Trials using pure compounds have shown that limonene is responsible for *C. limon* toxicity. Powders of the citrus materials were effective in this order lemon > grape vine > sweet orange; the effect reduced by 50% across the test materials. *Citrus limon* dry peel powder should be used solely to protect seeds of cowpea in stores against infestation and damage by *C. maculatus*. It should also be incorporated into the integrated pest management system for the control of *C. maculatus* infestation in stored cowpea.

Contribution/Originality: The paper's primary contribution is finding that the toxic effect of *Citrus limon* on *C. maculatus* infesting cowpea seeds in storage was comparable to cypermethrin standard insecticide under high-sensitivity test condition.

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp is an important food legume in the tropical countries of the world, particularly in West African nations [1]. The global use of cowpea for human nutrition has been reported especially in developing countries where they constitute the main sources of protein. Furthermore, cowpea is a source of livestock feed and revenue in the tropics [2] and this adds value to the economy. Nigeria contributes a substantial amount of world cowpea production with about four million hectares cultivated annually [3] thus fostering drive towards global food security.

Several workers have reported that the cowpea chrysomelid, *C. maculatus* Fab., causes severe losses in stored cowpea seeds in Africa [1, 4, 5]. Unfortunately, the huge losses resulting from *C. maculatus* infestation in cowpea during seed storage expose farmers and other users to different degrees of turbulence. As a principal insect pest of stored cowpea seeds, *C. maculatus* infestations have been reported to cause substantial reduction in quality and quantity of affected seeds -within three to five months of storage Ofuya [4]; Ileke, et al. [6]. Ashamo [7] stated that the biggest challenge facing Nigerian farmers is post-harvest losses associated with insect pest infestation and *C. maculatus* infestation is not excluded. Preservation of seeds of cowpea for planting during the next cropping season is a major target of the farmer and this is often hampered by *C. maculatus* which reduces the viability of seeds through its biological activities.

Use of resistant varieties, botanical powders, hermetic storage, biological control, freezing whole storage area and use of chemical insecticides are options available for the post-harvest control of *C. maculatus* [2, 5, 8-13]. Currently, the application of synthetic insecticides is the major means of controlling chrysomelid infestations in stored legume seeds [2]. It could be in form of fumigating with phosphine or carbon disulphide and or dusting with carbaryl, pirimiphos methyl or permethrin [6]. However, consequent upon reported ozone depletion by methyl bromide and carcinogenic concerns with phosphine, conventional fumigation technology is under surveillance in the developed countries Adedire, et al. [14]. Ileke, et al. [6] further highlighted problems associated with the use of synthetic insecticides to include high mammalian toxicity, high level of persistence in the environment, poor application knowledge, exorbitant cost prices, pest resurgence, genetic resistance by the insect pest and deleterious effects on non-target organisms. They suggested that one possible way to overcome the problems of synthetic insecticide is to replace synthetic insecticides with naturally-occurring botanicals that have insecticidal properties which can affect pest infestations. Plant products are neither scarce nor expensive; they are humanly friendly and ecologically safer than conventional synthetic chemicals [15]. Replacement with ecologically safe materials is currently advocated and this is part of the motivation of the present study. Most previous studies did not base plant material evaluations on high-sensitivity scaling and this has been considered in this study.

2. MATERIALS AND METHODS

This study was conducted in the Crop Protection Laboratory, Department of Crop and Soil Science, University of Port Harcourt, Rivers State, Nigeria. The mean room temperature and relative humidity of the site were 30 °C and 77.8%, respectively. The ten cowpea varieties and their sources are presented in Table 1. The samples were cleaned and standardized [16] before they were used for the experiment.

Callosobruchus maculatus Fab. adults used to start the insect culture came from naturally infested cowpea seeds procured at Choba market, Port Harcourt, Rivers State, Nigeria. The *C. maculatus* adults were reared on a susceptible cowpea variety, Ife brown provided by International Institute for Tropical Agriculture, IITA, Ibadan, Nigeria. Rearing was achieved in 1.5-litre glass bottles at laboratory ambient temperature and relative humidity of 30 °C and 77.8%, respectively. Newly-emerged adults (1 to 3 days old) were used for the experiments.

The three plant materials used for the study are the lemon (*Citrus limon* L.), the common grape vine (*Vitis vinifera* L.) and sweet orange (*Citrus sinensis* L.) peels. The three citrus materials were harvested from the neighborhood of University of Port Harcourt. The peels were removed with sharp clean knife and air-dried in the laboratory for 8 weeks before it was weighed. Hundred grams (100 g) of peels of each citrus were pulverized separately and sieved (pore: 1 mm^2) to produce fine powders, which were used immediately for the experiment.

The 24-hour high-sensitivity test against storage insects recommended by Nwosu [17] was used. A quantity of 0.2 g powder of each of the plant materials (the lemon, *Citrus limon*; the common grape vine, *Vitis vinifera*; sweet orange, *Citrus sinensis*) was added separately to ten plastic jars (11 cm diameter and 4.5 cm height) containing 20 g of the cowpea seeds to give 1.0 % w/w. Comparable rate of cypermethrin (standard insecticide) was added into a different set of ten containers containing 20 g of cowpea seeds. An untreated control was set up with the same amount of containers and seeds. The citrus powders and intact seeds were mixed thoroughly to enhance coating of the seeds. Cypermethrin powder and seeds were also thoroughly mixed. Then, five pairs of newly-emerged (1 to 3 days old) *C. maculatus* adults were introduced into each jar which was covered with a muslin cloth and held firmly with a rubber band. The treatments were replicated four times and arranged in a Completely Randomized Design on a workbench in the laboratory. Mortality was observed for 24 h and not extended to maintain high level test sensitivity suggested particularly for *C. maculatus* [2] and data were collected and recorded. Insect was considered dead if it did not respond to a probe with a pin. Percent mortality was calculated using the standard formula:

$$\frac{\text{Number of dead } C.maculatus \text{ adults } X\ 100}{\text{Total number of } C.maculatus \text{ adults}}$$

All dead and live *C. maculatus* adults were sieved out immediately after mortality count to ensure that the emerging adults were direct consequence of the number of eggs oviposited within 24 h. The eggs laid on the cowpea seeds after 24 h in a no-choice protocol were counted and the average number was calculated per treatment. The examined seeds were returned to their respective containers and left under the same experimental conditions for another 43 days, giving a total of 50 days under storage and emerged adults from seeds of different treatments were counted and recorded. Percent seed damage after 50 days of infestation was also recorded.

The mean effects of the different citrus insecticides were computed and their pooled mean effects were calculated. Consequent upon this, the performance rank of the citrus insecticides were established after comparing with a standard insecticide and an untreated control.

Table-1. Ten cowpea varieties used for the study.

Serial number	Varieties	Source
1	99K-573-2-1	IITA, Ibadan, Nigeria
2	07K-292-10	IITA, Ibadan, Nigeria
3	96D-10	IITA, Ibadan, Nigeria
4	IFEBPC	IITA, Ibadan, Nigeria
5	97K-499-35	IITA, Ibadan, Nigeria
6	IFE98-12	IITA, Ibadan, Nigeria
7	99K-573-1-1	IITA, Ibadan, Nigeria
8	ITO7K292-10	IITA, Ibadan, Nigeria
9	Banjaur	Rural farmers in Jos, Nigeria
10	Farin Wake	Rural farmers in Jos, Nigeria

Note: IITA: International institute for tropical agriculture.

3. RESULTS AND DISCUSSION

The effects of botanical insecticides on the life parameters of *C. maculatus* are fairly well-documented with varying success rates [1, 3, 6-8, 14]. In this communication, effects of the citrus botanical insecticides on mortality of *C. maculatus* adults, oviposition deterrence, emergence of new adults and seed protection are summarized in Tables 2 to 5. Percent mortality varied significantly ($P < 0.05$) among the treatments. All the test materials caused certain degree of mortality to the insect after 24 h of exposure, reaching pooled average of 23.6% in lemon, 7.8% in grape vine and 4.2% in sweet orange and 19.0% in cypermethrin standard insecticide. Lemon (*Citrus limon*) caused highest adult mortality at the exposure period and this differed significantly from mortalities caused by the rest of the materials. The same results were obtained for oviposition, adult emergence and seed damage Tables 3, 4 and 5.

This investigation revealed that powder of *C. limon* (lemon) had the highest insecticidal activity against *C. maculatus*. Its pooled average toxic effect on the insect was as good as cypermethrin in 24 h. *Vitis vinifera* (the common grape vine) powder was second while powder of *C. sinensis* (sweet orange) came third in killing *C. maculatus* adults. It was a striking observation that these citrus materials consistently reduced in effect (lemon > grape vine > sweet orange) by 50% in terms of insect mortality. The same result was obtained for oviposition deterrence, suppression of adult emergence and seed protection. Even though *C. limon* (active ingredient limonene) showed good potentials for controlling *C. maculatus* infestations in stored cowpea seeds under the influence of high-sensitivity test, efforts should be intensified in the search for natural material that will kill at least 50 % of the insect in 24 h. Any natural material that will not considerably kill *C. maculatus* in 24 h is not sustainable [2]. This is because the insect has the capacity to lay large number of eggs within 24 h.

In conclusion, the effects of citrus-derived insecticides on the bionomics of *C. maculatus* in stored ten varieties of cowpea were investigated. Powders of the citrus materials were effective in this order lemon > grape vine > sweet orange and the effectiveness reduced by 50% across the test materials. Lemon (*Citrus limon*) powder was most effective at causing insect mortality, oviposition deterrence and suppression of adult emergence and seed perforations. Trials using pure compounds have shown that limonene is responsible for *C. limon* toxicity. Therefore, *C. limon* powder should be used to protect seeds of cowpea in storage against infestation and damage by *C. maculatus*. It should also be incorporated into the integrated pest management system for the control of *C. maculatus* infestation in stored cowpea. The present study has provided new insights for *C. maculatus* management using commonly available and humanly safe citrus materials.

Table-2. High-sensitivity effect of the citrus powders [rate 1.0% (w/w)] on the percent (%) mortality of adult *C. maculatus*.

Varieties	Lemon (*Citrus limon*)	Grape vine (*Vitis vinifera*)	Sweet orange (*Citrus sinensis*)	Control (Untreated)	Cypermethrin (Synthetic)
99K-573-2-1	14.00	6.00	6.00	0.00	20.00
07K-292-10	34.00	26.00	6.00	0.00	20.00
96D-10	14.00	14.00	6.00	0.00	10.00
IFEBPC	60.00	14.00	0.00	0.00	20.00
97K-499-35	46.00	0.00	0.00	0.00	20.00
IFE98-12	1400	6.00	0.00	0.00	20.00
99K-573-1-1	14.00	6.00	6.00	0.00	20.00
ITO7K292-10	0.00	6.00	6.00	0.00	30.00
Banjaur	34.00	0.00	6.00	0.00	30.00
Farin Wake	6.00	0.00	6.00	0.00	0.00
Pooled mean effect	23.6	7.8	4.2	0.00	19
Performance rank	**1st**	**2nd**	**3rd**	**Control**	**Control**

Table-3. High-sensitivity impact of the citrus powders [rate 1.0% (w/w)] on oviposition by *C. maculatus* females.

Varieties	Lemon (*Citrus limon*)	Grape vine (*Vitis vinifera*)	Sweet orange (*Citrus sinensis*)	Control (Untreated)	Cypermethrin (Synthetic)
99K-573-2-1	0.00	1.75	2.00	2.25	0.00
07K-292-10	0.00	1.00	2.00	1.75	0.00
96D-10	0.00	0.75	2.00	2.00	0.00
IFEBPC	0.00	2.00	2.50	2.50	0.00
97K-499-35	1.00	0.75	0.75	0.75	0.00
IFE98-12	0.00	1.00	1.00	1.75	0.00
99K-573-1-1	0.75	1.00	2.00	2.75	0.00
ITO7K292-10	0.00	2.00	2.00	2.75	0.00
Banjaur	0.00	0.75	1.75	1.75	0.00
Farin Wake	0.00	0.00	0.00	1.00	0.00
Pooled mean effect	0.18	1.10	1.60	1.93	0.00
Performance rank	**1st**	**2nd**	**3rd**	**Control**	**Control**

Table-4. High-sensitivity impact of the citrus powders [rate 1.0% (w/w)] on emergence of adult *C. maculatus*.

Varieties	Lemon (*Citrus limon*)	Grape vine (*Vitis vinifera*)	Sweet orange (*Citrus sinensis*)	Control (Untreated)	Cypermethrin (Synthetic)
99K-573-2-1	0.00	1.00	2.00	2.00	0.00
07K-292-10	0.00	1.00	2.00	2.00	0.00
96D-10	0.00	0.00	2.00	2.00	0.00
IFEBPC	0.00	1.00	3.00	2.50	0.00
97K-499-35	0.00	1.00	0.75	0.75	0.00
IFE98-12	0.00	0.00	1.00	2.25	0.00
99K-573-1-1	0.00	1.00	1.75	2.75	0.00
ITO7K292-10	0.00	2.00	2.00	3.5	0.00
Banjaur	0.00	0.00	2.00	1.75	0.00
Farin Wake	0.00	0.00	0.00	1.00	0.00
Pooled mean effect	0.00	0.70	1.65	2.05	0.00
Performance rank	**1st**	**2nd**	**3rd**	**Control**	**Control**

Table-5. High-sensitivity impact of the citrus powders [rate 1.0% (w/w)] on percent damage caused by *C. maculatus*

Varieties	Lemon (*Citrus limon*)	Grape vine (*Vitis vinifera*)	Sweet orange (*Citrus sinensis*)	Control (Untreated)	Cypermethrin (Synthetic)
99K-573-2-1	0.00	1.35	2.76	2.50	0.00
07K-292-10	0.00	1.42	2.85	2.85	0.00
96D-10	0.00	0.00	2.75	2.86	0.00
IFEBPC	0.00	1.43	0.50	3.50	0.00
97K-499-35	0.00	1.60	1.40	1.07	0.00
IFE98-12	0.00	0.00	1.43	3.20	0.00
99K-573-1-1	0.00	1.30	2.45	3.40	0.00
ITO7K292-10	0.00	3.15	2. 60	5.00	0.00
Banjaur	0.00	0.00	2.50	1.75	0.00
Farin Wake	0.00	0.00	0.00	1.00	0.00
Pooled mean effect	0.00	1.03	1.92	2.71	0.00
Performance rank	**1st**	**2nd**	**3rd**	**Control**	**Control**

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