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RESEARCH PAPER

ORANGE PEEL POWDER AND ITS EXTRACTS AS PRESERVATIVE AGAINST *CALLOSOBRUCHUS MACULATUS (COLEOPTERA:BRUCHIDAE)* ATTACK IN COWPEA.

Nwaehujor, I. U.[1] and Olatunji, G. A.[2]
[1]*Nigerian Stored Products Research Institute, Km3, Asa -Dam Road, P.M.B. 1489, Ilorin, Nigeria.*
[2] *Chemistry Department, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria*

ABSTRACT
Ethanolic crude extract, volatile oil and the peel powder of sweet orange (Citrus sinensis) were evaluated for their efficacy against the bean weevil (Callosobruchus maculatus Fab.). The peel powder was evaluated at concentrations of 5.0 g, 7.5 g, and 10.0 g /50 g of cowpea seeds. Ethanolic crude extract and volatile oil were evaluated at concentrations of 0.1 ml, 0.2 ml and 0.3 ml / 50 g of cowpea seeds. Mortality count was recorded at 24, 48, and 72 hours post treatment. F_1 adult emergence was also counted after 4 weeks of storage. All treatments exhibited significantly ($p < 0.05$) higher mortalities and lower F_1 adult emergence than the untreated control. Ethanolic crude extract gave the least F_1 adult emergence compared with other treatments and control. At the concentration of 0.3 ml/ 50 g of Ethanolic crude extract, there was no F_1 adult emergence. Ethanolic crude extract at this concentration gave adequate protection to stored cowpea seeds.

Keywords: *Callosobruchus maculatus, citrus, cowpea*

INTRODUCTION

Cowpea is a widely cultivated crop on the Africa continent. It is a major source of protein in the daily diets of many people in the sub-Saharan Africa. A major problem in cowpea seed storage is its susceptibility to insect pests. Several bruchid species attack cereals and pulse in storage. The most important genera of Bruchidae known to be associated with pulse include *Acanthoscelides*, *Callosobruchus*, *Caryedon* and *Zabrotes*. However, species of *Callosobruchus* are the most important bruchid pests infesting cowpeas (Kabeh and Lale, 2004).

Synthetic chemical insecticides have been an important part of pest management for many years. Interest is however growing fast in the possible role of plants and minerals as traditional protectants of stored products and as alternatives to the use of highly persistent synthetic chemicals with their attendant dangers and high cost. The use of natural products, which are cheap, effective, less toxic to mam-

mals, easy to adopt and environmentally friendly have been investigated worldwide (Ivbijaro, 1983; Dike and Mbah, 1992; Onu and Aliyu, 1995; Oparaeke and Dike, 2005; Oparaeke and Amodu, 2000).

Over 120 plants and plants products have been shown to have insecticidal or deterrent activity against stored product pests (Dale, 1996). Currently, farmers in parts of Africa and Asia use some of these botanicals to protect their legumes from attack by bruchids, with varying degrees of success (Don-pedro, 1990; Singh, 1990; Dharmasena *et al.,* 1998). However the number and quality of plants used by farmers is often limited by availability (Dharmasena, 1995).

Onu and Sulyman (1997) reported that powder peels of citrus fruits were effective in reducing the damage caused by *Callosobruchus maculatus* to cowpea seeds. Dungum *et al.,* (2005) also reported that orange peel was effective in insect pest management. Lale (2003) reported that oily acetone extract of *Piper guineense* was more toxic to maize weevil *Sitophilus zeamais* than the powdered preparation. Also Okonkwo and Okoye (2002) reported that the crude extract of *Piper guineense* gave adequate protection to stored cowpea against *Callosobruchus maculatus*. Limonene, a monoterpene constituent in the extracts of citrus fruit peel was reported as a safe, natural pesticide for insect pests (Hilling, 2005).

The efficacy of orange peel has already been established (Don-pedro, 1985; Taylor, 1978; Su *et al.,* 1972). The aim of this research work was to compare simultaneously the efficacy of the volatile oil, ethanolic crude extract and the peel powder as preservatives against *Callosobruchus maculatus* in stored cowpea.

MATERIALS AND METHODS

The experiment was conducted at the University of Ilorin, Ilorin, Nigeria during the rainy season of 2008.

Preparation of orange peel sample

Ripe orange fruits (*Citrus sinensis*) were purchased from a market in Ilorin. The orange fruits were washed with water and sorted to ensure that only the wholesome ones were used for the experiment. The peels were removed with a clean knife. Then one hundred grammes of the fresh peel were taken out for steam distillation. Three hundred grammes of the fresh peels were spread on trays and dried for 10 days at room temperature ($28 \pm 3\ ^0$C and 53.73 ± 2.7 % r.h).The dried peels were blended into powder using a moulinex ^{R} blender. The powdered peel was stored in an airtight container in a refrigerator until it was used.

Preparation of cowpea seeds

Cowpea seed (*Vigna unguiculata* L) was purchased from a market in Ilorin, Nigeria. The cowpea seeds were sorted and seeds showing signs of damage in the form of emergence holes and cracks were discarded. Wholesome cowpea seeds were disinfested by refrigerating for 24 hours at 10 ^{0}C and then dried under the sun to ensure that it was free from insect infestation. The seeds were then packed in polythene bags and later used for the experiment.

Source and culturing of test insects

The test bruchid (*C. maculatus*) adults were collected from previously infested cowpea seeds purchased from a market in Ilorin. They were brought to the laboratory and cultured on a white cowpea variety, Kananado. One hundred adult *C. maculatus* were introduced into two 4 litre jars containing 1 kg of the cowpea seed each. The jars were covered with fine muslin cloth and perforated covers. The insect culture was kept in the laboratory at room temperature ($26 - 30\ ^0$C and 70.41 % r.h). The parents stocks were sieved out after 7 days. The seeds containing the eggs were left in the jar. The subsequent F_1 progenies from the cultures were used for the experiment.

Oil extraction

Ethanolic crude extract of orange peel was obtained using Soxhlet extraction method (Anon,

1984). Fifty grammes of the dried powdered sample was used for the extraction. The crude Ethanolic extract was concentrated on a water bath to remove the solvent. It was further concentrated in vacuum to give 28.75 g (57. 51%) which was then transferred into a sample bottle and stored inside a refrigerator.

Extraction of the volatile oil was done using steam distillation method (Vogel, 1989). Hundred grammes of the fresh peel was used for steam distillation. The extract was weighed (3.33 g, 3.33%) and transferred into a sample bottle and stored inside a refrigerator.

Bioassay of the different extracts

Fifty grammes of undamaged cowpea seeds were weighed into 250 ml kilner jars. The peel powder was tested for its efficacy against *C. maculatus* at 5.0 g, 7.5 g, and 10.0 g per 50 g of seeds. The concentrations used to test crude ethanolic extract and the volatile oil were 0.1 ml, 0.2 ml, and 0.3 ml per 50 g of seeds. The peel powder was thoroughly admixed manually with the cowpea seeds in each kilner jar by shaking for one minute. The crude ethanolic extract was taken using a micro syringe into a 5 ml beaker and dissolved in 2 ml of n-hexane and then mixed thoroughly with the cowpea seeds in each jar after which the seeds were allowed to dry before the introduction of the test insects. The volatile oil was measured with a micro syringe and dissolved first in n-hexane before admixed with cowpea seeds. Twenty highly mobile adult *C. maculatus* were placed in each jar and covered with muslin cloth, the jars were then capped. Holes were created on the cap of the jars for aeration. Control treatments were similarly set up with 20 highly mobile adult insects exposed to 50 g of cowpea seeds. Each treatment including the untreated control was set up in three replicates. The jars were placed on the laboratory bench in a completely randomized design (CRD) at room temperature (26 - 30 ^{0}C and 57.2 ±2.2% r .h.). The contents of each jar were emptied into a clean glass Petri dish after 24, 48, and 72 hours and the numbers of dead *C. maculatus* were counted and recorded. The dead insects were assessed using a blunt probe (Dyte and Forster, 1972). Dead insects were discarded while live insects were placed back in their respective jars. After 14 days all the test insects were discarded. The set-ups were allowed to stand for four weeks after which the F_1 adult emergence was recorded. The insects in each jar were sieved out and counted manually.

Data obtained were subjected to analysis of variance and Duncan's Multiple Range Test to determine whether there were significant differences between treated and untreated means.

RESULTS

Effects of extract on mortality of *C. Maculatus*

The effect of the different extracts of orange peel on mortality of *C. maculatus* is shown in Table 1. All treatments recorded higher mortality than untreated control. Cumulative mortality obtained indicated that at 24 hours post treatment (PT), the ethanolic crude extract produced the highest mortality of *C. maculatus* whilst at 48 and 72 hours post treatment, the peel powder showed highest mortality of *C. maculatus.* Ethanolic crude extract at 0.3 ml / 50 g of seeds recorded the highest mortality at 24 h PT. The peel powder at 7.5 g / 50 g seed recorded higher mortality than at 10.0 g / 50 g seed at 48 and 72 hours PT. Cumulatively, ethanolic crude extract recorded the highest mortality followed by the volatile oil then the powder. The control recorded least mortality. The peel powder and the extracts recorded significantly higher mortality than the control ($p < 0.05$).

Effects of extracts on the emergence of F_1 generation of *C. Maculatus*

Table 2 summarizes the effects of the different extracts of orange peel on emergence of F_1 generation of *C. maculatus* after 4 weeks of treatment. Cumulatively, ethanolic crude extract recorded the least F_1 emergence compared with other treatments and control. The peel powder and extracts recorded significantly ($p < 0.05$) lower F_1 emergence than the control. Orange

peel powder recorded highest F_1 emergence compared with other treatments. Ethanolic crude extract at the concentration of 0.3 ml /50 g of seeds recorded no adult emergence. Ethanolic crude extract showed lower F_1 emergence than the other treatments and the control.

Table 1: Effect of the different extracts of orange peel on mortality of *Callosobruchus* maculatus.

Treatments/50 g of cowpea seeds	Mean No. of dead adults per jar Hours of post treatment			Mean mortality per concentration ± S.E
Volatile extract (ml)	24	48	72	
0.1	0.7	2.0	3.7	2.1 ± 0.87^{a}
0.2	2.0	1.7	2.3	2.0 ± 0.19^{a}
0.3	3.3	2.7	3.7	3.2 ± 0.29^{ab}
Ethanolic crude extract (ml)				
0.1	1.0	2.0	0.7	1.2 ± 0.40^{a}
0.2	3.0	1.7	1.3	2.0 ± 0.51^{a}
0.3	7.0	1.7	6.7	5.1 ± 1.72^{b}
Powder (g)				
5.0	0.7	1.0	3.3	1.7 ± 0.84^{a}
7.5	1.0	4.3	4.0	3.1 ± 1.06^{ab}
10.0	1.0	2.3	3.0	2.1 ± 0.59^{a}
Control	0.0	1.0	2.0	1.0 ± 0.58^{a}

Means in each column followed by the same letter are not significantly different ($p < 0.05$). (S.E = Standard error.)

Table 2: Effect of the different extracts of orange peel on emergence of F_1 generation of *Callosobruchus maculatus.*

Treatments/50 g of cowpea seeds	Mean No. of F_1 generation at 4 weeks PT ± S.E
Volatile extract (ml)	
0.1	91.3 ± 16.58^{c}
0.2	31.0 ± 12.10^{ab}
0.3	3.3 ± 0.88^{a}
Ethanolic crude extract (ml)	
0.1	20.3 ± 12.14^{ab}
0.2	0.7 ± 0.33^{a}
0.3	0.0 ± 0.00^{a}
Powder (g)	
5.0	97.3 ± 7.26^{c}
7.5	47.3 ± 8.45^{b}
10.0	42.3 ± 12.03^{b}
Control	121.3 ± 24.11^{c}

Means in each column followed by the same letter (s) are not significantly different at ($p < 0.05$).

DISCUSSION

The results indicated that ethanolic crude extract of orange peel recorded significantly ($p < 0.05$) higher mortality and lower F_1 adult emergence of *C. maculatus* than other treatments and the control. Ethanolic crude extract and volatile oil were found to reduce the damage of cowpea seeds by *C. maculatus* than orange peel powder. This is consistent with the observation of Lale (2003) who reported that oily preparations were more effective than powdered preparations of some botanicals in stored product pest management. He suggested that the efficacy of the oil was due to the solubility of the constituent active ingredients. The volatile oil at 0.3 ml / 50 g of seeds recorded high mortality of *C. maculatus* and suppressed emergence of F_1 offspring. The volatile oil at this concentration afforded protection to stored cowpea against *C. maculatus* infestation. This is in agreement with Moravvej and Abber (2008) who reported that the volatile component of citrus peels has high toxicity against cowpea beetles, *C. maculatus*.

The peel powder recorded lowest mortality and significantly ($p < 0.05$) higher F_1 adult emergence compared with other treatments. In practise, it might be difficult to use orange peel powder by farmers because of large quantities required and low activity compared with other preparations. Volatile oil was found to perform better than the powder by causing higher mortality and low F_1 adult emergence. The activity of the volatile oil was comparable to that of ethanolic crude extract, but the low yield (3.33%) might make it uneconomical for use by local farmers because large quantities of materials have to be processed in order to obtain the volatile oils in quantities sufficient for commercial scale tests. Ethanolic crude extract was found to be most effective in causing high mortality and suppressing the emergence of F_1 offspring. Also considering the yield (57.5%), it is cost effective to consider Ethanolic crude extract as having insecticidal effect on pest of stored products.

The advantages of using citrus peel extract are: it can be easily extracted from the peel, is not toxic to mammals since citrus oil is one of the popular flavourings and is consumed by people in various parts of the world. It is cost effective and its application is easy.

CONCLUSION

In conclusion, ethanolic crude extract is recommended for use as preservative for cowpea seeds in storage. It is suggested that small scale industry for the extraction of this oil should be established at the village level in Nigeria.

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