Potential of Sweet Orange (*Citrus sinensis* L. Osbeck) Peel Extract as Insecticidal Agent against Cowpea Bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract
Laboratory experiments were conducted to examine the insecticidal potentialof C. sinensis peel extract against cowpea bruchid,Callosobruchusmaculatus(F.) (Coleoptera: Chrysomelidae). The effectiveness of C. sinensispeel extract was determined as seed protectant against damage by adult cowpea beetle, C. maculatus at 0, 5, 10 and 15 µl administered on Whatman No. 1 filter paper in 100 µl methanol using micropipette and placed in a 9 cm Petri dish with a cover. Citrus peel oil was extracted using the Soxhlet extraction technique and the oil was concentrated in steam water bath at 40^{0}C. The experiment was arranged in a completely randomized design (CRD) replicated four times. Ten unsexed 1–2 day old adult C. maculatus were introduced on 20 g cowpea seed placed in the Petri dish containing different rates of extracts. Methanol extract of Citrus peel at the highest concentration gave complete mortality 2 days after treatment (DAT). The results also showed that cowpea seeds loss in all the peel extract-treated seeds was significantly (p<0.05) lower (1.17-3.40%) than in the control (3.30-6.33%). The higher concentrations of peel extract caused significant reduction in progeny emergence of the insect. The present study shows that the sweet orange extract has potential to cause mortality of C. maculatus and so can be used against the pest under small scale storage.

Key words: *Bruchid,Callosobruchusmaculatus, plant extracts, sweetorange.*

INTRODUCTION

In Nigeria, the most important pest of stored legume seeds is cowpea bruchid, *Callosobruchusmaculatus*(Coleoptera: Chrysomelidae: Bruchinae). It affects mature cowpea seeds in the field from where it is carried to the store where greater damage is often caused by the beetle. It is therefore regarded as a field-to-store pest. Ofuya*et al*. (2007) reported that damaged cowpea seeds are defaced with egg chorions and riddled with adult exit holes and subsequently have reduced weight, poor marketability and low viability.

Conventionally, farmers control stored product insect pests by means of synthetic insecticides such as pyrethroids, organophosphates and fumigants. In view of the environmental and ecological concerns, human health hazards, and increasing insect resistance, many insecticides have been banned or replaced by newer chemicals (Pimentel *et al.*, 2010; El-Bakry *et al.*, 2016; Rajashekar *et al.* 2016). The current trend includes the continued search for sound and sustainable pest management approaches that are locally available, farmer friendly, affordable and biodegradable.

El-Wakeil (2013) reported that botanical pesticides have long been touted as attractive alternatives to synthetic chemical pesticides for pest management because botanicals reportedly pose little threat to the environment or human health. Several studies revealed that the essential oils of *Citrus* spp. have potential to act as repellents, fumigants, ovicides, larvicides, insecticides and insect growth regulators against various insect species (Mondal and Khalequzzaman, 2009; Nenaah, 2014). Reports also showed that essential oil derived from orange peels is known to have insecticidal activity against beetles, notable among them are lesser grain borer,

ISSN 978-978-54729-6-7
THEME: "Horticulture for Improved Food Security, Sustainable Environment and National Economic Growth
18th – 22nd November, 2018

Rhyzopertha dominica (F.), rice weevils, *Sitophilus oryzae* (L.) and red flour flour beetle, *Tribolium castaneum* (Herbst) (Tripathi *et al*., 2003). Moravvej and Abbar (2008) reported that volatile components of *Citrus paradise* Macf., *C. aurantium* Risso, *C. limonium* Risso and *C. sinensis* peel essential oils had high fumigant activity against cowpea adult bruchid, *Callosobruchus maculatus* (F.). Having considered the availability of sweet orange peels as wastes in the country, methanol extract of the plant part was tested against bean beetle, *C. maculatus*in stored cowpea seeds.

MATERIALS AND METHODS

Study site

The experiment was carried out under laboratory conditions of the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria at ambient temperature of 28±3^{0}C, relative humidity of 68±3% and photoperiod 12:12 (L:D).

Test insect

Fifty unsexed adults of cowpea bruchids collected from a local market were raised on 200 g susceptible cowpea seeds in a 250 ml Kilner jar. The jar was covered with a mesh lid to permit aeration and prevent insect escape. The parent adults were sieved out after being allowed to lay eggs for 3 days. Parent adults were removed to avoid overlap with the freshly emerged adults (1-2 day old) used for the study.

Seed disinfestation

A bag of cowpea seeds already disinfested for 72 h with 2 tablets of Phostoxin® was sorted while 5 kg seeds used for the experiment was thereafter spread on the laboratory desk to allow the gas to dissipate.

Preparation of *C. sinensis* peel extract

Fresh peels of *C. sinensis* fruits were collected from a grocer in a local market in Ilorin. The peels were chopped into pieces using sterilized scalpel and ground using electric blender (Kenwood BL335,12B07). The powder was sieved to obtain uniform particles that passed through 40 mesh sieve. The *C. sinensis* peel powder (20 g) was properly wrapped in tissue paper and placed in the thimble of Soxhlet extractor while 200 ml methanol (solvent) was introduced for extraction continuously for 6 h. The peel oil extracted was concentrated on a steam bath set at 40^{0}C. The extractant was stored at 4^{0}C in a vial for 24 h prior to use.

Experimental procedure

Mortality activity of *C. sinensis* peel extract was tested against adults of *C. maculatus* by contact action. The different concentrationsof 5, 10 and 15 µl extract were applied separately on Whatman No. 1 filter paper in 100 µl methanol using micropipette. The filter paper was dried to dissipate the methanol for 10 min. before transferring into Petri dish (9 cm diameter). Ten adults of *C. maculatus*(mixed sexes of 1-2 days old) were placed with 20 g cowpea seeds in the container (7.4 cm in diameter).Three replications were set for each concentrationincluding the control (seeds treated with methanol alone) using micro-pipettein a completely randomized design. Data for adult mortality was recorded 24 and 48 days after treatment (DAT).

The experimental arrangement was kept for progeny assessment. The treated and untreated seeds were thereafter examined daily for emergence of progeny. Count of emerged adults commenced 28 days after infestation and a daily count was made for 5 consecutive days according to the method adopted by Emeasor*et al*. (2007).

Damage assessment was carried out on treated and untreated seeds. The extent of bruchid damage to seeds was evaluated by

ISSN 978-978-54729-6-7
THEME: "Horticulture for Improved Food Security, Sustainable Environment and National Economic Growth
18th – 22nd November, 2018

counting the exit holes. Damage by the beetle to the seeds was assessed 90 DAT using the number of perforated seeds as the index. Samples of 100 seeds were taken from treated and untreated seeds and the number of damaged (seeds with characteristic holes) and undamaged seeds were counted and weighed. Percentage weight loss was calculated by count and weight method as described in FAO (1985) as follows:

$$\% \text{ weight loss} = \frac{(UaN-(U+D) \times 100}{UaN}$$

Where:

U= wt of undamaged fraction in the sample, N

N=total number of seeds I sample

Ua=average weight of one undamaged seeds

D=weight of damaged fraction in the sample

Data analysis

Data were subjected to one-way analysis of variance and significant differences between means were determined using the Least Significant Difference at p=0.05.

RESULTS AND DISCUSSION

The *C. sinensis* peel oil extracted with methanol caused mortality of test insect at all concentrations that were significantly ($P \leq 0.05$) higher than mortality in control at 1 and 2 DAT (Table 1). Results of this study showed that *C. sinensis* methanol extract caused high mortality of *C. maculatus*and is effective as contact botanical against *C. maculatus*. Peel extract of *C. sinensis* applied at all concentrations showed significant effect. At the highest concentration of 15 µl, total mortality (100%) of *C. maculatus* was recorded 2 DAT. No dead beetles were observed in the control. All concentrations showed significantly higher mortality than the control. It was found that the effectiveness of the plant extract was dependent on both concentration and exposure period. In all the concentrations tested, seed protected with the highest concentration (15 µl) of plant extract gave better protection against the test insect than lower concentration. In this study the insecticidal activity of the extract might be dependent on the ability of the oil to dissolve the lyphophytic fatty tissues of the exoskeleton. The insecticidal activity of *C. sinensis* methanol extract to the test insect may be ascribed to solubility of the active ingredients in the extract. The essential oil of *C. sinensis* showed contact toxicity against *Zabrotessubfasciatus* L. (Zewde*et al.*, 2010). The *C. sinensis*extract applied at various concentrations indicated varying contact action to adults of *C. maculatus*. There was no mortality in the control within 2 days of treatment when adult mortality was achieved in all concentrations. Moravvej and Abbar (2008) had earlier reported that at 12 h exposure, the minimum rates of mortality of 5% in adults were attained by the presence of 148 µl L^{-1} of *C. sinensis*peel oil. In this study, the *C. sinensis*extract recorded 100% mortality against the test insect at 15 µl within 2 days of treatment. The study shows that mortality of the insect increased with exposure time. The emerged *C. maculatus*adults in the *C. sinensis*peel extract concentrations were significantly ($p<0.05$) lower than in the control except for 5 µl at 29, 30 and 31 DAT (Table 1). Conversely, significantly higher mean number of *C. maculatus*adults emerged in the control treatment than in seeds protected with highest concentration of the extract except at 28 DAT. All concentrations of the peel extract caused significant reduction in adult emergence of *C. maculatus* when compared to the control at 32 DAT, but there was no significant ($p>0.05$) difference among the various concentrations of the extract and the control at 28 DAT. *Citrus sinensis*

ISSN 978-978-54729-6-7

extract at the highest concentration of 15 µl completely suppressed *C. maculatus* emergence at 28 DAT. Although, adult emergence was higher in seeds treated with 5 µl, differences in emergence were not significant compared with control at 30 and 31 DAT.

Weight loss assessments of the treated and untreated seeds are shown in Table 2. All the treatments significantly ($p<0.05$) reduced weight loss compared to the untreated control at 1, 2 and 3 months after treatment. However noticeable feeding damage was observed on all treated and untreated seeds. Dawit and Bekelle (2010) reported that essential oil of orange peels effectively reduced the grain damage weight loss.

The *C. sinensis* methanol extract caused reduction in the number of seeds damaged by *C. maculatus*. The number of damaged seeds was significantly ($p<0.05$) lower at the various concentrations and the control except for 5 µl at 1 month after treatment (MAT). However, no significant differences were recorded in the number of damaged seeds in the concentrations of 10 and 15 µl at 2 and 3 MAT (Table 2) suggesting that any of the concentrations could be used to tackle the menace of the insect pest. The study revealed that adopting adult control through the peel extract would reduce damage caused by the insect, thus avoiding insect population build up on the stored seeds. The reduction in rate of adult emergence may have been caused by inhibition of egg laying or hatching by the plant extract. The short comings include the need to collect large quantities of the peels for extraction purposes. Tripathy*et al*. (2003) reported the contact toxicity of d-limonene present in *Citrus* spp. against stored-product insects. In another investigation, Oboh*et al*. (2017) reported that percentage mortality of insects increased with increase in concentration of the essential oil and exposure time. They associated the effectiveness of the essential oil to its inhibitory effects on acetylcholinesterase and $Na+/K+=ATPase$ activity. Although, insecticidal components of *C. sinensis* extract were not examined in this study, insecticidal activity may probably be due to the presence of limonene, myrcene, α-pinene, linalool, octanal and decanal (Njoroge*et al.* 2009).

CONCLUSION AND RECOMMENDATION

The study has shown that *C. sinensis* peel extracted with methanol can be exploited for use as seed protectant. The results of this study have the potential to increase the utilization of *C. sinensis* methanol extract for post-harvest protection of cowpea seeds under small scale storage. Adoption of this botanical control is recommended to reduce environmental pollution attributed to citrus peel wastes. Since the peels are not known to be toxic to human health, they could be added directly to seeds meant to be stored for future use.

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ISSN 978-978-54729-6-7

Table 1. Contact action of *C. sinensis*peel extract against*C. maculatus* and its progeny emergence

Concentration (µl) of *C. sinensis* peel extract	Mean mortality of *C. maculatus*adults (DAT)		Mean adult emergence of *C. maculatus*(DAT)				
	1	**2**	**28**	**29**	**30**	**31**	**32**
0	0.00	0.00	1.0	1.62	2.14	2.87	2.87
5	5.67	9.67	0.67	1.38	1.90	2.37	2.37
10	6.33	9.67	0.33	1.00	1.62	1.80	1.80
15	7.33	10.00	0.0	0.33	1.14	0.41	1.38
LSD$_{(0.05)}$	**3.02**	**0.58**	**NS**	**0.53**	**0.41**	**0.55**	**0.16**

Values are means of three replicates. DAT=Days after treatment NS= Not significant

Table 2. Contact effect of *C. sinensis*peel extract on number of cowpea seeds damaged and percentage seed weight loss by *Callosobruchus maculatus*

Concentration (µl) of *C. sinensis*	Mean No. of damaged seeds (MAT)			% Seed weight loss (MAT)		
	1	**2**	**3**	**1**	**2**	**3**
0	4.58	21.00	28.00	3.30	5.71	6.33
5	3.40	11.67	17.33	1.70	2.43	3.40
10	2.30	5.67	7.33	1.67	2.84	2.87
15	1.71	3.00	5.67	1.17	1.75	2.00
LSD$_{(0.05)}$	**0.70**	**4.06**	**3.02**	**2.19**	**1.13**	**1.57**

Values are means of three replicates.MAT=Month(s) after treatment

ISSN 978-978-54729-6-7
THEME: ''Horticulture for Improved Food Security, Sustainable Environment and National Economic Growth
18th – 22nd November, 2018