



## Comparative Efficacy of Aqueous Extracts of Lime (*Citrus aurantifolia*) and Grape (*Citrus paradisi*) Peel in the Control of *Callosobruchus maculatus* (F.)

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### Abstract

A laboratory study was conducted in the Department of Crop Protection, University of Ilorin, to evaluate the single and mixed insecticidal action of aqueous extracts of grape (*Citrus paradisi*) and lime (*Citrus aurantifolia*) peel in the control of cowpea beetle, *Callosobruchus maculatus*. The experiment was laid out in a completely randomized design. There were four treatments with three replicates per treatment. The treatments were applied at the following rates, 0.5ml, 1.0ml, and 1.5ml per 100g of cowpea seeds. The following parameters were used to assess the efficacy of the treatments; adult mortality, oviposition, number of larvae, number of pupae and F<sub>1</sub> progeny emergence. The findings of this experiment showed that 1.0 ml of aqueous peel extract of grape + lime had significantly higher female mortality compared to control at 3<sup>rd</sup> and 5<sup>th</sup> DAT. Aqueous peel extract of lime caused a significantly lower (70.67 + 4.3) oviposition rate compared to control (141.33 + 7.4) at 2 DAT with 1.5 ml being the most effective rate of application at 4 DAT. Aqueous peel extracts of lime was most effective in significantly reducing larval emergence (1.33 + 0.3) compared to control (8.33 + 0.5). The trend was similar for pupal emergence. Aqueous peel extracts of grape was most effective in reducing male F<sub>1</sub> progeny emergence while lime was most effective in reducing female F<sub>1</sub> progeny of *C. maculatus*. Aqueous peel extract of lime could be included in control measures used by local farmers in storage of their grains for two months

**Keywords:** Botanicals, cowpea, damage, storage, weevil.

### INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp is a legume belonging to the Order Fabales and family Fabaceae (Gumede *et al.*, 2022). It is one of several species of the widely cultivated genus *Vigna*. Members of the Leguminosae include many of the

economically important warm season grains and oilseed legumes, such as soyabean (*Glycine max*), common bean (*Phaseolus vulgaris*) and mung bean (*Vigna radiata*) (Guterres *et al.*, 2022). It is a significant dietary source of protein that nutritionally supports staple low-protein cereal and tuber crops and is essential in the lives of millions

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of people in Africa and other developing countries (Carneiro da Silva *et al.*, 2019). It is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carneiro da Silva *et al.*, 2019).

Dry grain for human consumption is the most important product of cowpea plants (Gomes *et al.*, 2020) in Africa, however the domestic production is in the hands of small scale farmers who obtain low yields of 200-350kg/ha and in some cases zero yield due to lack of improved technologies (Kamara *et al.*, 2018) and insect pests infestation. Cowpea production is affected by insect pests and disease infestations which lead to economic losses (Tiroesele *et al.*, 2015). Insect damage is the major constraint to cowpea grain production in most cowpea producing nations (Hornet *et al.*, 2022). Souleymane *et al.*, (2013) reported that every stage in the life cycle of the crop has at least one major insect pest.

The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is classified as the primary post-harvest pest of cowpea in the tropics and is a global field to store pest (Tiroesele *et al.*, 2015) causing significant qualitative and quantitative losses, including perforated seeds and decreases in seed weight, market value, and seed germination. (Akinkurolere, 2012). Infestation on stored grains may reach 50% within 3-4 months of storage (Pascual-vilalobus and Bellesta-Acosta, 2003), resulting in economic losses to the farmer (Singh *et al.*, 2003). Sometimes yield reductions caused by insects can reach as high as 95%, depending on location, year, and cultivar (Ekoja *et al.*, 2022).

In order to reduce serious losses experienced during storage, various techniques and control methods have been developed and more are still being developed

(Tiroesele *et al.*, 2015). Ezeaku *et al.*, (2017) stated that the cowpea yield in Nigeria can be improved and raised tenfold, when insects are controlled with insecticides. Approaches aimed at controlling attack by insect pests have relied heavily on the use of synthetic insecticides. Various synthetic insecticides have been developed over the years for the control of *C. maculatus*. Despite the effectiveness, many problems are associated with these chemicals such as the development of insect resistance, the cost of purchase, toxic residues in food, workers' safety and high cost of procurement. Insecticides also have negative impact on the environment, humans and non-target organisms (Tiroesele *et al.*, 2015), residual effect, health hazard to grain handlers and the widespread development of resistance in insect pests are still issues of great concern (Dzemo *et al.*, 2010).

It is in the light of the above problems that the need to develop alternative control strategies that will be affordable and eco-friendly becomes expedient. These problems have necessitated research on the use of alternative eco-friendly insect pest control strategies like the use of botanical insecticides. The use of botanical pesticides to protect plants from pest is very promising (Ivbijaro, 2012). The use of plant oils in stored product protection has been researched upon (Papanikolaou *et al.*, 2022). Geraldin *et al.*, (2020) reported that botanical pesticides are generally much safer than conventionally used synthetic pesticides. They usually do not pose any adverse effect on the ecosystem. Furthermore, plant-derived insecticides are cheaper. Some plants have more than one chemical as an active agent responsible for their biological properties. This work aims at investigating the efficacy of aqueous extracts of lime and grape peel in the control of *Callosobruchus maculatus* on cowpea in storage.

## MATERIALS AND METHODS

### Insect Culture

The initial culture of *Callosobruchus maculatus* was obtained from Nigerian Stored Products Research Institute (NSPRI), along Asa Dam road, Ilorin (latitude and longitude 8.4799° N, 4.5418° E), Nigeria. This stock culture was used to establish more cultures in the laboratory of the Department of Crop Protection, Faculty of Agriculture, University of Ilorin. The cultures were maintained under ambient temperature of 25-30°C and relative humidity of 70-75% in the laboratory.

### Seed Type and Source

The cowpea seed variety (Ife-brown) used for the experiment was obtained from a local agro allied store in Ilorin. The seeds were sterilized by storing them in the refrigerator for 7 days at 5°C prior to use. This was done to kill existing insects and any developing stages of *C. maculatus*.

### Preparation of Test Materials

The natural materials used were fresh green peels of grape (*Citrus paradisi*) and lime (*Citrus aurantifolia*). Healthy grape fruits were sourced from the Lower Niger River Basin Development Authority's orchard at Ilorin, Nigeria, while lime fruits were obtained from Ipata Market Ilorin, Kwara State, Nigeria.

To obtain the single application of the aqueous extract, 450g of green peels each of *C. paradisi* and *C. aurantifolia* were soaked in 1L of water separately in plastic containers (24 cm x 23 cm) and left for 24-h. The mixture was then stirred after 24 hours and the aqueous contents were sieved using a muslin cloth to obtain the filtrate. To obtain the mixed application, 225g peels each of *C. paradisi* and *C. aurantifolia* (making 450g) were soaked in 1 L of water. This was sieved

as described above to obtain the mixed aqueous extract.

### Experimental Setup

One hundred grams (100g) of the cowpea seeds were weighed and placed in transparent plastic containers (5cm x 9cm dimension). Aqueous peel extracts of *C. paradisi*, *C. aurantifolia* and their mixture in equal proportion were applied at the rate of 0.5, 1.0 and 1.5 ml/100 g cowpea seed in an experiment set up as completely randomized design, with the treatments replicated three times. A control treatment without aqueous peel addition was also set along with the treatments.

The treatments were applied to the seeds using a 5 ml hypodermic syringe and mixed properly with the seeds to ensure uniform coating of the seeds with the extracts. Seeds were allowed to air dry for 10 mins. Thereafter, six newly emerged adults of *C. maculatus* (1-2 days old) randomly selected from the culture were then introduced into each of the replicates. The containers were then covered with muslin cloth to prevent the escape of the insects and to allow for proper aeration.

The following data were collected and used to assess the efficacy of the treatments. Adult mortality rate was assessed by counting the number of dead *C. maculatus* adults on the 1st, 3rd, 5th, 7th and 9th day after treatment (DAT). The seeds were very carefully broken open with bare hands by pressing gently until broken and the developmental stages observed. Seeds were checked at 19th and 23rd DAT for larvae and pupae emergence respectively. At 29 DAT, newly emerged adults of *C. maculatus* (F<sub>1</sub> progeny) were counted, recorded and discarded.

### Data Analysis

Data collected were subjected to Analysis of Variance and the treatment means were separated using the Least Significant Difference (LSD). The statistical analysis was done using the SPSS software, Version 21.

### RESULTS

Though all treatments increased mortality of *C. maculatus* on stored cowpea when compared to the control, they were not significantly different ( $P > 0.05$ ) except on the 5<sup>th</sup> day after treatment (DAT) (Table 1) for male *C. maculatus*. On the 5<sup>th</sup> day, aqueous peel extract of lime had the highest increase in mortality of *C. maculatus* and

though not significantly different ( $P > 0.05$ ) from other treatments (Table 1), it was significantly different ( $P < 0.05$ ) compared to control. There was no significant difference ( $P > 0.05$ ) between the rates of treatment application in male *C. maculatus* (Table 1).

The trend was slightly different in adult female *C. maculatus*, in which mixed application of grape/lime had the highest mortality on the 3<sup>rd</sup> and 5<sup>th</sup> day and it was significantly different ( $P < 0.05$ ) from the control but not significantly different from the other treatments. The rate of treatment application was significantly different on the 3<sup>rd</sup> DAT for female *C. maculatus* (Table 1) with 1.0ml having the highest increase in mortality followed by 1.5ml.

**Table 1:** Effect of aqueous extract on mortality of adult male and female *C. maculatus*

Treatment	DAT (Days after treatment)				
	1	2	3	4	5
<b>Male (Mean ± SE)</b>					
AE Grape	0.44±0.18	0.77±0.24	0.44±0.18	0.22±0.17	0.11±0.12 <sup>b</sup>
AE Lime	0.33±0.18	0.44±0.24	0.55±0.18	0.44±0.17	0.22±0.12 <sup>b</sup>
AE G/L	0.11±0.18	0.88±0.24	0.66±0.18	0.33±0.17	0.00±0.12 <sup>b</sup>
Control	0.00±0.31	0.00±0.41	0.33±0.31	0.33±0.30	0.66±0.21 <sup>a</sup>
	ns	ns	ns	ns	
<b>Rate</b>					
0.5	0.25±0.18	0.49±0.24	0.46±0.18	0.40±0.17	0.28±0.12
1.0	0.25±0.18	0.39±0.24	0.46±0.18	0.30±0.17	0.28±0.12
1.5	0.15±0.18	0.69±0.24	0.46±0.18	0.30±0.17	0.18±0.12
	ns	ns	ns	ns	ns
<b>Female (Mean ± SE)</b>					
AE Grape	0.44±0.15	0.55±0.24	0.66±0.21 <sup>ab</sup>	0.22±0.19	0.00±0.08 <sup>b</sup>
AE Lime	0.11±0.15	0.66±0.24	0.55±0.21 <sup>ab</sup>	0.66±0.19	0.00±0.08 <sup>b</sup>
AE G/L	0.00±0.15	0.55±0.24	1.00±0.21 <sup>a</sup>	0.33±0.19	0.11±0.08 <sup>ab</sup>
Control	0.00±0.27	0.00±0.41	0.00±0.37 <sup>b</sup>	0.33±0.33	0.33±0.14 <sup>a</sup>
	ns	ns		ns	
<b>Rate</b>					
0.5	0.17±0.15	0.61±0.23	0.18±0.21 <sup>b</sup>	0.48±0.19	0.14±0.08
1.0	0.07±0.15	0.31±0.23	0.88±0.21 <sup>a</sup>	0.28±0.19	0.14±0.08
1.5	0.17±0.15	0.41±0.23	0.58±0.21 <sup>ab</sup>	0.38±0.19	0.04±0.08
	ns	ns		ns	ns

Values followed by the same superscript in the same column are not significantly different ( $P > 0.05$ )

**Key:** AE – Aqueous extract, G/L – Grape/Lime mixture, ns – Not significant

There was a significant difference ( $P<0.05$ ) between treatments and control for oviposition by *C. maculatus* (Table 2). Aqueous lime peel extract had the lowest oviposition rate for the 3 days observed and though not significantly different ( $P>0.05$ ) from the other treatments was significantly

different ( $P<0.05$ ) compared to the control (Table 2).

Rate of treatment application were not significantly different ( $P>0.05$ ) at all levels except for the 4th DAT, where 1.5ml resulted in the lowest oviposition rate followed by 0.5ml and 1.0ml respectively.

**Table 2:** Effect of aqueous extracts on oviposition of *C. maculatus*

Treatment	DAT (Days after treatment)		
	2	3	4
AE Grape	79.33±4.3 <sup>a</sup>	3.67±1.3 <sup>a</sup>	0.22±0.51 <sup>a</sup>
AE Lime	70.67±4.3 <sup>a</sup>	2.44±1.3 <sup>a</sup>	0.00±0.51 <sup>a</sup>
AE G/L	76.56±4.3 <sup>a</sup>	2.89±1.3 <sup>a</sup>	1.56±0.51 <sup>a</sup>
Control	141.33±7.4 <sup>b</sup>	21.00±2.3 <sup>b</sup>	16.33±0.9 <sup>b</sup>
<b>Rate</b>			
0.5	94.47±4.2	7.20±1.34	56±0.50
1.0	90.07±4.2	7.30±1.35	36±0.50
1.5	91.37±4.2	8.00±1.33	66±0.50
	ns	ns	

Values followed by the same superscript in the same column are not significantly different ( $P>0.05$ )

**Key:** AE – Aqueous extract, G/L – Grape/Lime mixture, ns – Not significant.

All treatments significantly ( $P<0.05$ ) reduced larval and pupal emergence compared to control (Table 3). Though aqueous peel extract of lime had the lowest larval and pupal emergence on the 19<sup>th</sup> and 23<sup>rd</sup> DAT

respectively, it was not significantly different ( $P>0.05$ ) from the other treatments (Table 3). The different rates of application for the larval and pupal emergence were not significantly different ( $P>0.05$ ) (Table 3).

**Table 3:** Effect of aqueous extracts on larval and pupal emergence of *C. maculatus*

Treatments	DAT (Days after treatment)	
	19	23
	Larval	Pupal
AE Grape	1.78±0.3 <sup>a</sup>	1.56±0.31 <sup>a</sup>
AE Lime	1.33±0.3 <sup>a</sup>	1.33±0.31 <sup>a</sup>
AE G/L	2.22±0.3 <sup>a</sup>	1.89±0.31 <sup>a</sup>
Control	8.33±0.5 <sup>b</sup>	7.67±0.54 <sup>b</sup>
<b>Rate</b>		
0.5	3.48±0.3	2.91±0.31
1.0	3.68±0.3	3.31±0.31
1.5	3.08±0.3	3.11±0.31
	ns	ns

Values followed by the same superscript in the same column are not significantly different ( $P < 0.05$ )

Values are means±SE. **Key,** AE – Aqueous extract, G/L – Grape/Lime mixture, ns – Not significant

The treatments significantly ( $P < 0.05$ ) reduced  $F_1$  progeny emergence compared to the control (Table 4). It is interesting to note that though treatments were not significantly different ( $P > 0.05$ ) from each other, aqueous peel extract of grape appeared to be most

effective in reducing the male  $F_1$  progeny emergence while aqueous peel extract of lime was most effective in reducing female  $F_1$  progeny emergence of *C. maculatus* (Table 4).

**Table 4:** Effects of extracts on  $F_1$  progeny emergence

Treatment	Male (Mean $\pm$ SE)							
	DAT (Days after treatment)							
	29	30	31	32	33	34	35	36
AE Grape	0.33 $\pm$ 2.2 <sup>a</sup>	0.33 $\pm$ 0.4 <sup>a</sup>	0.56 $\pm$ 0.3 <sup>a</sup>	1.00 $\pm$ 0.3 <sup>a</sup>	0.89 $\pm$ 0.3 <sup>a</sup>	0.78 $\pm$ 0.3 <sup>a</sup>	0.11 $\pm$ 0.1 <sup>a</sup>	0.11 $\pm$ 0.1 <sup>a</sup>
AE Lime	0.33 $\pm$ 0.2 <sup>a</sup>	0.78 $\pm$ 0.4 <sup>a</sup>	0.67 $\pm$ 0.3 <sup>a</sup>	1.11 $\pm$ 0.3 <sup>a</sup>	1.00 $\pm$ 0.3 <sup>a</sup>	0.67 $\pm$ 0.3 <sup>a</sup>	0.00 $\pm$ 0.1 <sup>a</sup>	0.00 $\pm$ 0.1 <sup>a</sup>
AE G/L		0.33 $\pm$ 0.2 <sup>a</sup>	0.78 $\pm$ 0.4 <sup>a</sup>	1.44 $\pm$ 0.3 <sup>a</sup>	1.56 $\pm$ 0.3 <sup>a</sup>	1.22 $\pm$ 0.3 <sup>a</sup>	0.56 $\pm$ 0.3 <sup>a</sup>	0.22 $\pm$ 0.1 <sup>a</sup>
Control	0.00 $\pm$ 0.1 <sup>a</sup> 3.67 $\pm$ 0.4 <sup>b</sup>	5.67 $\pm$ 0.6 <sup>b</sup>	5.67 $\pm$ 0.4 <sup>b</sup>	5.67 $\pm$ 0.5 <sup>b</sup>	5.67 $\pm$ 0.5 <sup>b</sup>	5.33 $\pm$ 0.5 <sup>b</sup>	3.00 $\pm$ 0.2 <sup>b</sup>	0.67 $\pm$ 0.2 <sup>b</sup>
Treatment	Female (Mean $\pm$ SE)							
	DAT (Days after treatment)							
	29	30	31	32	33	34	35	36
AE Grape	0.33 $\pm$ 0.3 <sup>a</sup>	1.33 $\pm$ 0.4 <sup>a</sup>	1.00 $\pm$ 0.3 <sup>a</sup>	0.89 $\pm$ 0.2 <sup>a</sup>	1.22 $\pm$ 0.3 <sup>a</sup>	1.22 $\pm$ 0.3 <sup>a</sup>	0.22 $\pm$ 0.2 <sup>a</sup>	0.11 $\pm$ 0.1 <sup>a</sup>
AE Lime	0.22 $\pm$ 0.3 <sup>a</sup>	1.33 $\pm$ 0.4 <sup>a</sup>	0.89 $\pm$ 0.3 <sup>a</sup>	0.78 $\pm$ 0.2 <sup>a</sup>	1.00 $\pm$ 0.3 <sup>a</sup>	0.67 $\pm$ 0.3 <sup>a</sup>	0.44 $\pm$ 0.2 <sup>a</sup>	0.00 $\pm$ 0.1 <sup>a</sup>
AE G/L		0.33 $\pm$ 0.3 <sup>a</sup>	1.44 $\pm$ 0.4 <sup>a</sup>	1.33 $\pm$ 0.3 <sup>a</sup>	1.11 $\pm$ 0.2 <sup>a</sup>	1.33 $\pm$ 0.3 <sup>a</sup>	0.78 $\pm$ 0.3 <sup>a</sup>	0.44 $\pm$ 0.2 <sup>a</sup>
Control	0.11 $\pm$ 0.1 <sup>a</sup> 4.33 $\pm$ 0.5 <sup>b</sup>	6.00 $\pm$ 0.7 <sup>b</sup>	6.67 $\pm$ 0.6 <sup>b</sup>	5.67 $\pm$ 0.4 <sup>b</sup>	4.33 $\pm$ 0.5 <sup>b</sup>	6.33 $\pm$ 0.6 <sup>b</sup>	3.33 $\pm$ 0.3 <sup>b</sup>	0.67 $\pm$ 0.2 <sup>b</sup>

Values followed by the same superscript in the same column are not significantly different ( $P > 0.05$ ).  
**Key:** AE – Aqueous extract, G/L – Grape/Lime mixture.

## DISCUSSION

The result of the study showed that aqueous peel extracts of lime and grape were effective in controlling *C. maculatus* on stored cowpea. There was a reduction in oviposition rate, larval emergence, pupal emergence and  $F_1$  progeny emergence. These findings support Ahmed and Gazzy, (2011) who stated that efficacy of plant extracts against *Callosobruchus maculatus* in causing adult mortality, oviposition inhibition, progeny reduction and repellence have been reported. In this study, aqueous peel extract of lime was the most effective in controlling *C. maculatus* and this corroborates the work of Musa and Sulyman (2014) who reported that application of lime peel extract was relatively more toxic to *C. maculatus* as all insects died 72 post treatment and confirmed its superiority over grapefruit extract. Khanikor *et al.*, (2021) also reported that the

fruit peel of some citrus species has insecticidal properties against insects. Even though aqueous peel extract of grape and their mixture was not as effective as lime, they were able to protect the cowpea seeds compared to untreated seeds. The reduction in oviposition rate, larval and pupal emergence might be due to toxicity of the botanicals to *C. maculatus* rather than an increase in adult mortality of *C. maculatus*. This is due to the fact that though the botanicals caused adult mortality of *C. maculatus*, its effect was not pronounced. It should be noted that though the botanicals reduced oviposition rate, larval, pupal and  $F_1$  progeny emergence, they did not eliminate *C. maculatus* presence or their effect on seeds but rather reduced the deleterious effects of *C. maculatus* on treated seeds when compared to unprotected seeds.

Wahedi *et al.*, (2015) and Sani *et al.*, (2020) reported that essential oil of citrus peel can be used to reduce oviposition or larvae emergence through parental adult mortality as a result of limited contact of *C. maculatus* with the treated seed thereby inhibiting the emergence of F1 progeny. In this study, oviposition, larvae and pupae emergence were reduced though it did not appear to be through parental adult mortality. The mechanism for the reduction observed in this study is not known. Some plant extracts especially oil, have been reported to have inhibitory effect on development of eggs of *C. maculatus* (Dwivedi and Kumari, 2000; Nisar *et al.*, 2022). Ovicidal effect of oils had been attributed to its ability to penetrate the chorion of the eggs which creates hypercarbic condition within the eggs (Alamuoye, 2022).

Asawalam *et al.* (2007) reported that insecticidal activity of any plant extract depends on the active constituents of the plant extract. Bozkurt *et al.*, (2017) reported the major active component of citrus oil as d-limonene. Ware and Whitacre (2004) reported that limonene mode of action is similar to that of pyrethrum. It affects the sensory nerves of the peripheral nervous system. Phat *et al.*, (2022) reported that *Citrus paradisi* contains limonene (91.5%),  $\beta$ -pinene (0.8%), linalool (1.1%) and  $\alpha$ -terpinene (0.7%).

According to reports by Erdogan and Mustafa (2021) lesser grain borer is known to be adversely affected by essential oils made from orange peel in terms of toxicity, feeding deterrence, and poor development. Rice weevil *Sitophilus oryzae* (L.), red flour beetle *Tribolium castaneum* and *Rhyzopertha dominica* (F.). The peel oil was also reported to have toxicity towards the cowpea weevil, *C. maculatus* (Nisar *et al.*, 2022).

The extracts could be incorporated into the control measures used by resource poor

farmers in developing countries in place of the expensive synthetic pesticides.

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