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Control of *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae) using Ethanolic Extracts of Peels from Five *Citrus* Species

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Abstract

Ethanolic extracts of peels from grape (*Citrus paradisi*), lemon (*C. limon*), lime (*C. aurantifolia*), sweet orange (*C. sinensis*) and tangerine (*C. reticulata*) were investigated for their insecticidal efficacy against *Callosobruchus maculatus* Fabricius, a cosmopolitan field-to-storage insect pest of cowpea. The insect was exposed to 3% of each extract admixed with 50 g of cowpea. The setup was a Completely Randomized Design in four replications. Data collected were subjected to analysis of variance and where *F*-test was significant, means were separated using Student Newman Keul's test ($\alpha = 0.05$). Pearson's correlation analysis was also carried out to show the association between *C. maculatus* mortality, infestation, seed damage and germination variables. Bioassay results showed significant insecticidal activity of all the tested *Citrus* peel extracts against *C. maculatus* life stages and a significant reduction in cowpea seed damage. Adult bruchid mortality caused by the extracts exceeded 90% at 120h post-treatment, with *C. limon* and *C. aurantifolia* extracts causing 100% of adult bruchid mortality in the same period. About 62.3-76.4% reduction in the insect's egg production was observed with the use of the extracts. The perforation index obtained from all the treated seeds was below 50%, and it showed a positive protective potential of the extracts against *C. maculatus*. The loss in seed weight was significantly lower (<10%) in treated seed compared with the control (>20%). Cowpea seed germination was not impaired by any of the extracts, rather germination increased significantly and seeds treated with *C. limon* and *C. aurantifolia* had higher viability (>45.5%) compared with the control. Therefore, ethanolic extracts of peels from the five *Citrus* species could be a potent pest control option against *C. maculatus* infestation.

Keywords: Cowpea, Callosobruchus maculatus, Citrus peel, Crop damage, Pest control, Seed viability, Storage pest

Introduction

The production and storage of cowpea (*Vigna unguiculata* (L.) Walp) in Africa have faced a lot of constraints, *viz*: insect pests, diseases, weeds, drought, limited farm inputs, lack of market etc (Sobda *et al*., 2018). Among these factors, insect pests occupy a prominent position and depending on location, year, and cultivar, the losses they cause to cowpea in fields and storage could be as high as 95% of the total yield (Carlos, 2000; Ilesanmi and Gungula, 2010). In Africa, the key insect pest responsible for 10-100% losses in stored cowpea is the seed bruchids, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae) (Sanon *et al*., 2018; Ekoja and Ogah, 2020; Ekoja *et al*., 2022). *C. maculatus* infestation could begin in the field or during storage when the female insect lay eggs on the seed testa. Emergence holes on seed are the major visible damage characteristics attributed to *C. maculatus* larvae, and it has been the major factor responsible for loss in cowpea seed weight, impairment of germination and reduction in the economic value of the crop. The insect is holometabolous with a relatively short lifecycle (3-4 weeks), and there could be several overlapping generations of progenies if preventive or artificial control measures are not employed (Sanon *et al*., 2018; Ekoja and Ogah, 2020).

Most farmers, grain merchants, warehouse managers and seed producers in Africa have relied on the use of synthetic insecticides, such as phosphine, dichlorvos, pirimiphos-methyl, spinosad and pyrethroids to control *C. maculatus* infestation (Ekoja *et al*., 2021). But the dependence on these chemicals often leads to the development of genetic resistance, a resurgence of the treated population, residual toxicity, environmental hazards and other problems such as direct toxicity to predators, pollinators, aquatic animals and man (poison cases, sudden deaths, blindness, and skin irritation) (Magaji *et al.*, 2005; Owolabi *et al*., 2012). Besides, some of the synthetic insecticides used against *C.*

maculatus are too expensive for most subsistent farmers in Africa (Mabbet, 2004; Shazia *et al*., 2006). Some farmers have opted for the use of different concentrations/ levels of natural products including plant powders (Shazia *et al*., 2006), botanical extracts (Fotso *et al*., 2019), essential/ fixed vegetable oils (Law-Ogbomo and Egharevba, 2006; Ekoja and Ogah 2020), ashes (Wolfson *et al*., 1999; Tiroesele *et al*. 2019), minerals (Stoll, 2000; Shazia *et al*., 2006), cow dung (Suleiman and Haruna, 2020) and triglycerides from animal sources (Ekoja *et al*., 2021) with varying degrees of efficacy against *C. maculatus*.

Among the plant products, the efficacy of extracts from several *Citrus* species against *C. maculatus* has been investigated. Both oil and organic solvent extracts have been reported to cause adult bruchid mortality (Moravvej and Abbbar, 2008; Abdullahi *et al*., 2019), reduce female oviposition (Rotimi and Evbuomwan, 2012), suppress their adult emergence (Nwaehujor and Olatunji, 2011) and reduce the seed damage rate of stored cowpea (Rotimi and Ekperusi, 2012). Because of their high volatility, extracts from *Citrus* species provide a potential biodegradable alternative to synthetic pesticides. The contact and fumigant insecticidal activity of extracts from *Citrus* peels have also been demonstrated against *C. maculatus* (Don-Pedro, 1996; Saeidi *et al*., 2014; Siskos, 2014).

The current public interest in botanicals for the control of storage pests is part of a new trend aimed at minimizing the adverse effects associated with the use of synthetic chemicals in a food storage environment. Even though the peels of *Citrus* species have been reported to demonstrate bioactivity against *C. maculatus*, information on the *Citrus* type with the most effective peel extract is still scanty. The phytochemical profile of the *Citrus* peels used in some of the previous studies is not also reported. It is therefore imperative to direct more research to these areas so as to provide stakeholders in the cowpea production/ storage sector with adequate empirical information that will enable them to take effective pest management decisions. Therefore, this experiment was set up to test the efficacy of ethanolic extracts of peels from grape (*Citrus paradisi* L.), lemon (*C. limon* L. Burm. F.), lime (*C. aurantifolia* Christm.), sweet orange (*C. sinensis* L. Osbeck) and tangerine (*Citrus reticulata* Blanco) as cowpea seed protectants against infestation and damage caused by *C. maculatus*. The phytochemical component of the extracts and their effect on seed germination were also investigated.

Materials and Methods

Study Environment

The experiment was conducted at the Crop and Environmental Protection Laboratory of the Federal University of Agriculture, Makurdi (FUAM), Benue State, Nigeria (NG) (Coordinates: Latitude 7°47'45.0"N, Longitude 8°36'56.8"E). The mean temperature and relative humidity of the environment were 28.8 ± 3°C and 75 ± 2% respectively.

Preparation of Cowpea Seeds

Ife brown variety of cowpea (*Vigna unguiculata* L.) was obtained from the Institute of Agricultural Research and Training (IAR&T), multiplied at the crop production unit of the Teaching and Research Farms of FUAM, NG and used in the study. It has a brown and rough seed coat. Specks of dirt and damaged seeds were sorted and discarded. Undamaged and clean seeds selected were disinfested by refrigerating them for 25 days at -4°C and then dried under the sun for one week to ensure that the minimum moisture content for storage was not exceeded. All the life stages of bruchids, particularly the eggs, are reported to be sensitive to cold treatment (Koehler, 2003). A seed moisture content value of 11.50% was obtained using the procedure described by Fotso *et al*. (2019). The seeds were packed in airtight jars until they were needed for the experiment.

Preparation of Ethanolic Crude Extracts from Citrus Peels

Ripe fruits of C. *paradisi* (L.), *C. limon* (L.) Burm. F., *C. aurantifolia* (Christm.), *C. sinensis* (L.) Osbeck and *C. reticulata* (Blanco) were purchased from Makurdi fruit market. They were washed with water and sorted to ensure that only the wholesome fruits are used for the extraction. The fresh peels (exocarp) from each *Citrus* species were crushed using an electric blender (Binatone, BLG403) prior to extraction. About 150g of each sample was soaked in ethanol (500mL) in 1000mL glass beakers for 72h and stirred at 20min. intervals with a glass rod to ensure uniformity. The mixture was filtered using a double layer of Whatman No. 1 filter papers and the filtrate was subjected to a rotary evaporator at 30 to 40°C with a speed of 3 to 6 rpm for 8 h to separate the solvent from the extract. The resulting extract from each *Citrus* species was poured into a beaker and then air-dried to remove traces of the solvent (Murugan *et al*., 2012). The extracts were stored in labelled amber bottles in a refrigerator (-4°C) until they were required for the experiment.

Phytochemical Screening of Extracts from Peels of the Citrus Species

A stock solution of each extract (1 mg/ml) was prepared and used for the screening. The qualitative phytochemical analysis to determine the presence/ absence of secondary metabolites (such as tannins, saponins, flavonoids, steroids, alkaloids and terpenoids) was carried out in the laboratory using the procedure described by Oikeh *et al*. (2013).

Insect Culture

The stock of bruchid (*C. maculatus*) was collected from previously infested cowpea seed, purchased from the North Bank Cereal Market in Makurdi, Nigeria. They were reared on undamaged Ife brown variety of cowpea at the Crop and Environmental Protection Laboratory of FUAM, Nigeria. One hundred unsexed adult *C. maculatus* were introduced into two 4-litre transparent plastic jars containing 2kg of cowpea seed each. The top of each jar was covered with a muslin cloth to allow air into the container for bruchid respiration. The parent's stocks were removed after mating for 7 days. The culture was maintained in the laboratory for 35 days after infestation under ambient laboratory conditions at 27 ± 3°C and 75 ± 4% relative humidity (RH). The emerging F_1 progenies ((≤ 24 h old) from the cultures were used for the experiment.

Toxic Effect of Ethanolic Extracts from Peels of Five Citrus Species on Adult Callosobruchus maculatus Fab

Treatments comprised of crude extracts from peels of *C. paradisi* (L.), *C. limon* (L.) Burm. F., *C. aurantifolia* (Christm.), *C. sinensis* (L.) Osbeck, *C. reticulata* (Blanco) admixed with 50g of Ife brown variety of cowpea at 3% concentration inside 250ml plastic jars. This concentration was achieved by diluting 0.3ml of the stock solution (extracts) in 9.7ml of solvent (Ashamo and Akinnawonu, 2012). Untreated seed served as the control for each *Citrus* extract. Ten copulating pairs (1:1 male: female) of adult *C. maculatus* (from the laboratory culture) were introduced into each jar containing treated and untreated cowpea seeds. The set-up was in a completely randomized design with four replicates. All treatments were maintained under ambient laboratory conditions at 26 ± 3°C and 76 ± 4% RH. Bruchid mortality was recorded at 24h intervals for 7 days. A 32mm long pin was used to probe the status (alive or dead) of the insects in each jar. The percentage of adult bruchid mortality (%ABM) was calculated using:

$$\%\text{ABM} = \left(\frac{\text{Number dead bruchids}}{\text{Total number of bruchids introduced}}\right) \times 100$$

After 7 days of mortality counts, all the insects (dead or alive) in both treated and untreated jars were removed and the mean number of eggs laid was recorded (n = 10 randomly selected seeds per jar). At 12 weeks post-treatment, the number of seeds damaged (perforated) by *C. maculatus* larvae was counted and expressed as a proportion of the total number of seeds examined (n = 100 seeds). The perforation index (PI) was also calculated using the formula reported by Ileke *et al*. (2020):

$$\text{PI} = \left(\frac{\%\text{ treated seeds perforated}}{\%\text{ control seeds perforated}}\right) \times 100$$

Extracts with PI-values below 50% are considered to be positive protectants, while those above 50% are regarded as negative protectants (i.e. they cannot significantly suppress bruchid-induced seed damage). Furthermore, the content of each jar was sifted and sieved to remove dust, insects, and frass before taking the final gain weight data. The percentage seed weight loss (%SWL) was calculated using:

$$\%\text{SWL} = \left(\frac{(W_i - W_f)}{W_i}\right) \times 100$$

Where: W_i and W_f are the initial and final weight of cowpea seeds respectively (Ekoja *et al*., 2021).

Seed Viability Test

This trial was carried out at 12 weeks (90 days) after seed treatment with the extracts. Twenty seeds [treated and untreated (control)] were randomly selected from each jar and used for this test. They were placed in Whatman No. 1 filter paper in sterile Petri dishes (90 × 15mm) and moistened daily with distilled, de-ionized water to determine the effect of bruchid control with *Citrus* peel crude extracts on cowpea seed viability. Germination in each dish was counted and recorded at the 7^{th} day and expressed as a proportion of the total number of seeds as described by Ekoja *et al*. (2021).

Statistical Analyses

In order to meet the assumptions of normality and homogeneity of variance, the mortality counts (%) and seed weight loss (%) were transformed to arcsine values, while the number of eggs and F_1 progeny were transformed using the square root model [$(x + 0.5)^{½}$]. The data were subjected to analysis of variance using SAS/STAT® 9.2 software by SAS Institute (2009). Where the F-statistics were significant, means were separated using Student Newman Keul's test (SNK) (α = 0.05). Pearson's correlation analysis was also carried out to show the association between *C. maculatus* mortality, the number of eggs laid, induced seed damage and cowpea seed germination (%).

Results and Discussion

Results

The results of the phytochemical analysis showed various secondary plant metabolites such as tannins, flavonoids, steroids, alkaloids, and terpenes in the *Citrus* extracts. However, saponins were was present only in the ethanolic extracts of *C. sinensis* (L.) Osbeck and *C. reticulata* (Blanco) (Table 1). All the *Citrus* peel extracts showed significant toxic effect against *C. maculatus* when compared with the control throughout the period of adult mortality assessment (24h: $F_{5,12}$ = 255.53, $P < 0.0001$; 48h: $F_{5,12}$ = 117.20, $P < 0.0001$; 72h: $F_{5,12}$ = 105.00, $P < 0.0001$; 96h: $F_{5,12}$=296.57, $P < 0.0001$; 120h: $F_{5,12}$ =306.12, $P < 0.0001$) (Table 2). Bruchid mortality exceeded 50% at 24 hours after exposure (HAE) to the extracts. At 120 HAE, over 90% of the bruchid introduced into each jar were killed by the extracts with extracts from *C. aurantifolia* (Christm.) and *C. limon* (L.) Burm. F. causing 100% mortality in *C. maculatus* at this period. All the insects in the control jars were alive throughout the adult mortality assessment period of the experiment (16 h). Bruchid eggs production was significantly lower ($F_{5,12}$ = 68.39, P < 0.0001) on *C. aurantifolia* (Christm.) and *C. limon* (L.) Burm. F.-treated seed compared with those on *Citrus paradisi* L. treated seeds and the control (Figure 1). However, there was no significant (P > 0.05) difference between the number of eggs laid on *C. reticulata* (Blanco) and *C. sinensis* (L.) Osbeck-treated seeds. Bruchid perforation index ranged from 16.2 to 32.6 among treated seeds. Those treated with *C. aurantifolia* (Christm.) were the least damaged, but it was not significantly different from the number of perforations observed on seeds treated with *C. limon*

(L.) Burm F. The number of untreated seeds that were damaged was significantly higher ($F_{5, 12}$ = 247.42, P < 0.0001) compared with seed treated. The damage done to the seed resulted in a significant reduction in the weight of the cowpea. The lowest loss in seed weight was observed in seeds treated with *C. aurantifolia* (Christm.) and *C. limon* (L.) Burm. F. and they differed significantly ($F_{5, 12}$ = 74.14, P < 0.0001) from other treated seeds and the control. A double-digit loss (>20%) in seed weight occurred in the untreated seeds (Table 3). The *Citrus* peel extracts significantly ($F_{5, 12}$ = 28.42, P < 0.0001) increased cowpea seed germination when compared with the control (Figure 2). Seeds treated with *C. aurantifolia* (Christm.) had the highest percentage germination, but it was not significantly (P > 0.05) different from that of seeds treated with *C. limon* (L.) Burm. F. Germination of untreated seeds was the lowest. Correlation results showed a strong positive association between seed germination and adult bruchid mortality (r = 0.878; n = 24; P < 0.0001) (Table 4). However, the number of eggs laid (r = -0.904; n = 24; P < 0.0001), seed perforation (r = -0.907; n = 24; P < 0.0001) and loss in cowpea seed weight (r = -0.909; n = 24; P < 0.0001) were negatively correlated with seed germination.

Discussion

The presence of tannins, flavonoids, alkaloids, terpenes and steroids in the phytochemical profile of the *Citrus* peels was consistent with the findings of Lawal *et al*. (2013), Mathew *et al*. (2013) and Raymond *et al*. (2017), who also reported similar phytochemicals in peels of some species of the plant. Even though secondary plant metabolites synthesized by plants are for protective purposes, some of these compounds have been identified and categorised as substances that are toxic to insects. It is also important to note that the absence or presence of these bioactive compound groups depends on the polarity of the solvent used as reported by Mahmoudi *et al*. (2013) and Fotso *et al*., (2019). However, the presence of saponin in *C. sinensis* (L.) Osbeck and *C. reticulata* Blanco could be associated with the sweet flavour inherent in these two *Citrus* species. Saponins have been reported to confer sweet flavour on fruits (Raymond *et al*., 2017). Terpenes are the major organic compounds in the phytochemical profile of *Citrus* and it is composed mainly of mono and sesquiterpenes that accumulate especially in oil glands present in the epicarp (Takita *et al*., 2007). These phytochemicals identified in this study may have exerted insecticidal actions via disruption of the physiological, biochemical and enzymatic processes of the insects leading to the significant bioactivity observed.

The significant insecticidal activity of all the extracts against *C. maculatus* was consistent with previous reports on the insecticidal potentials of the botanical extract (Moravvej and Abbbar, 2008; Nwaehujor and Olatunji, 2011; Rotimi and Evbuomwan, 2012; Rotimi and Ekperusi, 2012; Raymond *et al*., 2017; Abdullahi *et al*., 2019). However, the extracts from *C. aurantifolia* (Christm.) and *C. limon* (L.) Burm. F. seems to be more effective in mitigating the infestation and damage caused by the insect. The efficacy of the extracts may be due to the preponderance of d-limonene (a cyclic monoterpene) in the phytochemical profile of this *Citrus* spp. The amount of d-limonene in *Citrus* spp. has been reported to range from 60 to 90% of the total organic compounds in extracts of their peels (Ladaniya, 2008). D-limonene was also reported to be an effective natural pesticide for the control of some insect pests (Hollingsworth, 2005; Mursiti *et al*., 2019). A study by Karr and Coats (1988) showed that terpenoids possess repellent, ovicidal and larvicidal properties. Their report also showed contact, fumigant and oral toxicities of d-limonene on some insect species. Don-Pedro (1996) and Moravvej and Abbar (2008) attributed mortality induced by *Citrus* peel-treated cowpea seed on *C. maculatus* to the fumigant activity of the vapour released by oil from the peels.

Over 50% of adult *C. maculatus* mortality was observed after 24h of exposure to the ethanolic extract of the *Citrus* peels. This outcome was similar to the findings of Ojebode *et al*. (2016) who observed >50% mortality in *C. maculatus* after 24 h of exposure to oil extract from *C. sinensis* (L.) Osbeck peels. Musa and Sulyman (2014), Ojebode *et al*. (2016) and Abdullahi *et al*. (2019) observed 100% mortality of adult *C. maculatus* at 72h using extracts of *Citrus* peels, but the highest mortality achieved in the study at the same time was 83.3% using extracts from *C. aurantifolia* (Christm.) peels. Egg production by the bruchids was also significantly suppressed by all the *Citrus* extracts. According to Abouo *et al*. (2010), the reduction in egg-laying could be due to the fumigant actions of the oil components of extracts as they act as chemosterilisants causing ovarian changes and reduction in egg-laying potentials of the female insects.

The untreated cowpea seeds suffered severe damage (seed perforations) because of *C. maculatus* infestation. This further showed the huge negative effect of the insect on stored cowpea if preventive or artificial control measures are not employed as earlier reported by Sanon *et al*. (2018), Ekoja and Ogah (2020) and Ekoja *et al*. (2022). However, the perforation index showed that the *Citrus* peel extracts provided positive protection against the bruchids. The significant reduction in cowpea seed perforation and weight loss that was observed in this study could also be attributed to the higher adult mortality, oviposition deterrence, ovicidal, antifeedant, larvicidal and reproduction inhibitory properties of the organic compounds in the *Citrus* peel extracts as suggested by Isman (2006), Ileke *et al*. (2020) and Ekoja *et al*. (2021).

Depending on the concentration, extracts from peels of some *Citrus* species have been reported to exert allelopathic effects on seed germination (AlSaadawi *et al*., 1985; Fujihara and Shimizu, 2003). But our result showed that the use of the extracts did not impair cowpea germination. The insecticidal effect of the extracts on bruchid mortality, oviposition, progeny production and bruchid-induced seed damage had a significant influence on the viability of treated cowpea seeds as shown by the strong association between these parameters and cowpea seed germination.

Conclusion

The ethanolic extracts of peels from the *Citrus* species tested showed insecticidal properties against *C. maculatus*, but *C. aurantifolia* (Christm.) and *C. limon* (L.) Burm. F. seems to be more toxic against the insect. *Citrus* peel extracts are generally not toxic to man, livestock, and pets. The process of extraction is easy and its cost is relatively cheaper compared with synthetic insecticides. The oil component is also used as a flavouring agent and it is consumed by people in various parts of the world. Therefore, the use of the extracts could be considered a useful tool in an integrated pest management strategy for *C. maculatus* in farmer's cowpea storage facilities. However, further research is required to study the persistence of bioactive compounds in the extracts under different storage conditions.

Acknowledgement

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Table 1: Qualitative analysis of secondary metabolite in peels of five *Citrus* species

Citrus peel extract	Tannins	Saponins	Flavonoids	Steroids	Alkaloids	Terpenoids
Citrus paradisi (L.)	+	-	+	+	+	+
C. limon (L.) Burm. F.	+	-	+	+	+	+
C. aurantifolia (Christm.)	+	-	+	+	+	+
C. sinensis (L.) Osbeck	+	+	+	+	+	+
C. reticulata (Blanco)	+	+	+	+	+	+

+ = Present; - = Absent

Table 2: Effect of ethanolic extracts of peels from five *Citrus* spp. on adult *Callosobruchus maculatus* Fab.

	Mortality count (%)				
Citrus peel extract	24 h	48 h	72 h	96 h	120 h
Citrus paradisi (L.)	57.50 ± 2.50b	67.50 ± 2.50b	70.00 ± 4.08 c	80.00 ± 4.08 b	100.00 ± 0.00a
C. limon (L.) Burm. F.	72.50 ± 2.50a	82.50 ± 4.79a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
C. aurantifolia (Christm.)	70.00 ± 4.08a	85.00 ± 2.89a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
C. sinensis (L.) Osbeck	60.00 ± 0.00b	67.50 ± 2.50b	80.00 ± 4.08 b	87.50 ± 2.50 b	95.00 ± 2.84ab
C. reticulata (Blanco)	57.50 ± 2.50b	67.50 ± 2.50 b	85.00 ± 2.89 ab	87.50 ± 2.50 b	92.50 ± 4.79 b
Control (Untreated)	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
F-value	255.53	117.20	105.00	296.57	306.12
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cv (%)	6.48	9.37	9.75	5.82	12.11

Data on insect mortality count were transformed (arcsine) before the F-test; Mean followed by the same alphabet in a column are not significantly different from each other (SNK: P > 0.05); Cv = Coefficient of variability; P-value = Probability value; % = Percentage

Table 3: Effect of ethanolic extracts of peels from five *Citrus* species on bruchid-induced damage on cowpea seeds

Extract	Number of seeds perforated (*n* = 100)	PI	Seed weight loss (%)
Citrus paradisi (L.)	25.75 ± 1.11 bc	30.3	6.40 ± 0.36 b
C. limon (L.) Burm. F.	15.25 ± 1.03 d	17.9	3.55 ± 0.15 c
C. aurantifolia (Christm.)	13.75 ± 1.44 d	16.2	3.25 ± 0.43 c
C. sinensis (L.) Osbeck	20.75 ± 2.84 c	24.4	5.60 ± 0.67 b
C. reticulata (Blanco)	27.75 ± 0.95 b	32.6	4.55 ± 0.55 bc
Control (Untreated)	85.00 ± 2.04 a	100.0	20.30 ± 1.52 a
F-value	247.42		74.14
P-value	<0.0001		<0.0001
Cv (%)	10.88		20.73

Mean followed by the same alphabet in a column are not significantly different from each other (SNK: P > 0.05); PI = Perforation index; fi PI is <50 = Positive protectability, but when it is >50 = Negative protectability; Cv = Coefficient of variability; P-value = Probability value

Table 4: Pearson's correlation coefficients (*r*) showing the degree of association between cowpea seed germination and bruchid mortality, infestation and damage parameters

Parameters	Seed germination (%)
[a]Adult mortality	0.878**
Number of eggs	-0.904**
Number of perforated seeds	-0.907**
Seed weight loss	-0.909**

*n = 24; [a] = mortality at 120 h after exposure to the extracts; ** = significant (P < 0.0001)*

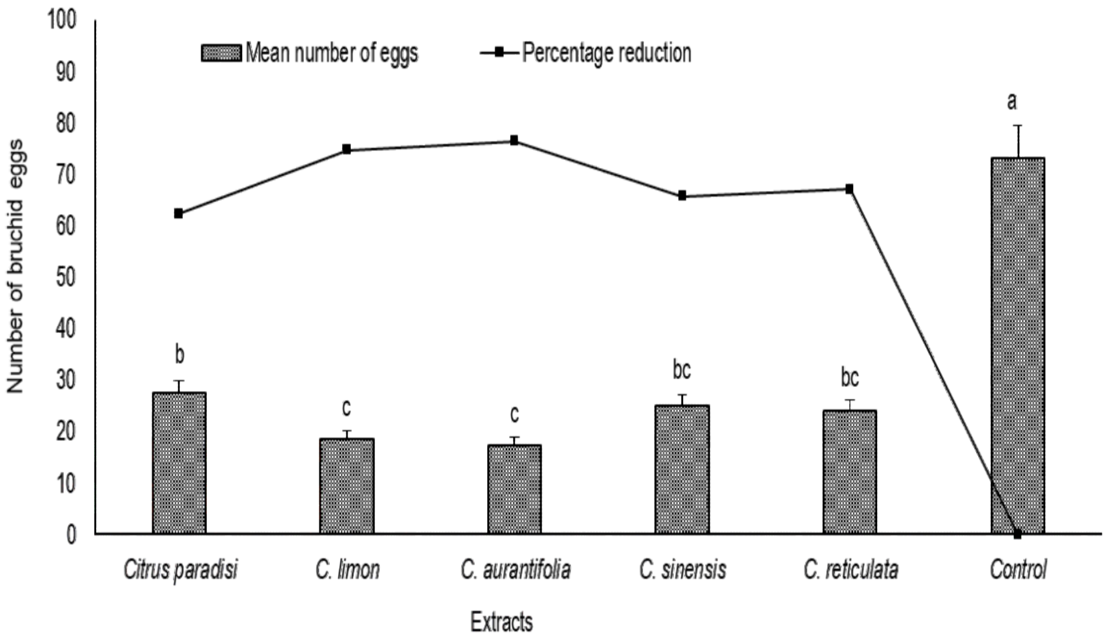

Figure 1: Effect of ethanolic extracts of peels from five Citrus species on the number of eggs laid by Callosobruchus maculatusFab.

Bars with the same alphabet are not significantly different from each other (SNK: P > 0.05)

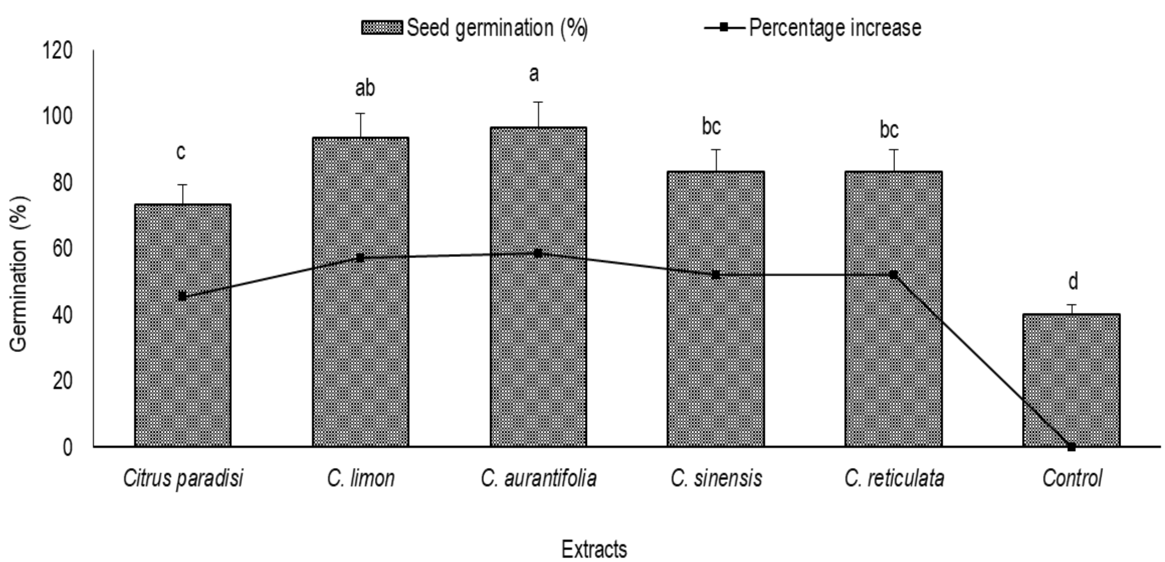

Figure 2: Percentage germination of cowpea seeds after 12 weeks of exposure to ethanol extracts of peels from five *Citrus* spp.

Bars with the same alphabet are not significantly different from each other (SNK: P > 0.05)

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