



Efficacy of *Citrus sinensis* and *Citrus limon* Peels Extracts in the Control of *Callosobruchus maculatus* on Stored Cowpea, *Vigna unguiculata*

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Abstract

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) is the most destructive pest of stored cowpea worldwide. The use of synthetic pesticides against *C. maculatus* have proven effective but with great concerns on its resulting effects such as pest resistance and residual toxicity threatening food security. The toxicity effect of *Citrus sinensis* and *Citrus limon* were evaluated against the adult *C. maculatus*. Four concentrations of *C. sinensis* (20%, 15%, 10%, and 5%) and *C. limon* (5%, 15%, 10%, and 20%) including a control of only acetone used as standard comparative to treat the pest. It was set up in completely randomized design. Results were recorded at 12 hourly intervals for 3 days. At highest concentration of 20% for *Citrus sinensis*, mortality recorded was 87% and at lowest concentration, it recorded 50%, while the LC50 and LC90 were 5.8% and 30.7% respectively, LT50 and LT90 were 27.2 and 45.3 hours respectively. Similarly, *C. limon* at highest concentration recorded 76% and at lowest concentration recorded 43% while the LC50 and LC90 were 8.4% and 57.7% respectively, LT50 and LT90 were 12.2 and 43.1 hours respectively. It was observed from the study that mortality was concentration and time dependent, therefore, *C. sinensis* and *C. limon* could be used as good alternatives to synthetic pesticides which has adverse effects on man and food crops.

Keywords: *Callosobruchus Maculatus*; *Citrus Sinensis*; *Citrus Limon*; Peels Extracts; *Vigna Unguiculata*

Introduction

Cowpea (*Vigna unguiculata* L.) is a pulse crop that can be grown successfully in extreme environments such as high temperature, low rainfall and poor soils with few inputs [1]. It is one of the most nutritionally important indigenous African grain legumes produced throughout the tropical and subtropical areas of the world. Nigeria is its largest producer and consumer, accounting for about 45 percent of its world's production. Cowpea seed pods and leaves are consumed in fresh form as green vegetables in some African countries [2], while the rest of the cowpea plant after the pods have been harvested serves as a nutritious fodder for livestock [3] and also a source of cash income [1]. The seed is high in protein contents and can be consumed directly, make flour, sprouts, wean-

ing food for young children and thus ameliorating malnourishment and stunting. The crude protein from the seed and leaves ranges, respectively between 23% and 32%. It can be referred to as "protein source for all" because it is affordable for both the rich and poor citizens [4]. The production and storage of cowpea has faced so many constraints, through West Africa such as diseases and limited use of fertilizers and irrigation inputs but the major constraint is the insect pest known as *Callosobruchus maculatus* [5]. *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), also known as the cowpea beetle, is a major cosmopolitan pest of a variety of legume crops. This insect pest infests cowpea in storage, thereby reducing the quality and quantity of the seeds. They have two distinct morphs: a flightless, inactive, normal or sedentary morph and

a flight or active morph [5]. *Callosobruchus maculatus* is very destructive on account of its short life cycle. Cowpea weevil infests cowpea before harvest and the level of damage is directly proportional to the infestation levels of the seeds in storage. Damage of cowpea seeds occur in form of emergence holes and weight loss which leads to loss of economic value. Infestations on stored grains may reach 50% within 3-4 months of storage [6]. *Callosobruchus maculatus* attacks cowpea before and after harvest consequently leading to loss of economic value.

To reduce storage losses due to insect pests, synthetic pesticides have been recommended. It has been widely known as the control measure of insect pest of cowpea. However, the use of synthetic insecticides has led to several problems including environmental pollution and increase in health related issues like cancer and immune system disorders. Hence it is imperative that alternative measures are developed and put into general use as soon as possible [7]. The increasing public concern over pesticide safety and possible damage to the environment has resulted in increasing attention being given to natural products for the control of stored insect pests [2]. The pesticidal properties of many plants have been known for a long time and natural pesticides based on plant extracts such as rotenone, nicotine and pyrethrum have been commonly used during the earlier half of this century. However, after the world war II, they lost their importance with the introduction of synthetic chemicals which were concentrated products with a high knock down effect on pest organism [5]. Some of this knowledge has been neglected over the past decades. In Nigeria traditional grain storage, *Aframomum melegueta* seed, *Capsicum nigrum* seed, *Allium sativum* bulb, *Zingiber officinale* rhizome, *Azadirachta indica* leaves and *Ocimum gratissimum* leaves, Citrus peels in which *Citrus sinensis* and *Citrus limon* peels are often employed. One potential solution lies in the utilization of natural plant extracts with insecticidal properties [2]. *Citrus sinensis* (sweet orange) and *Citrus limon* (lemon) are two citrus fruits widely consumed and known for their various health benefits. The peels of these fruits contain bioactive compounds such as limonoids, flavonoids, and essential oils, which have been reported to possess insecticidal properties against a range of pests [6].

The efficacy of *Citrus sinensis* and *Citrus limon* peels extracts in the control of *Callosobruchus maculatus* on stored cowpea has been the subject of several studies. These studies have investigated the potential of these extracts as natural alternatives to synthetic insecticides for cowpea weevil management. One study conducted by [8] evaluated the insecticidal activity of *Citrus sinensis* peel extract against cowpea weevils. The researchers found that the extract exhibited significant toxicity against both adults and larvae of *Callosobruchus maculatus*. The mortality rates increased with increasing concentrations of the extract, indicating its potential as an effective biopesticide [8]. Similarly, another study by [1] investigated the efficacy of *Citrus limon* peel extract against cowpea weevils. The researchers observed that the extract showed strong insecticidal activity, causing high mortality rates in both adults and larvae of *Callosobruchus maculatus*. The study also revealed that the extract had a repellent effect, reducing the oviposition and emergence of new weevils [1]. Furthermore, a study by [9] compared the efficacy of *Citrus sinensis* and *Citrus limon* peel extracts against cowpea weevils. The researchers found that both extracts exhibited significant insecticidal activity, causing high mortality rates in the weevils. However, *Citrus limon* extract showed slightly higher efficacy compared to *Citrus sinensis* extract [9]. These studies collectively suggest that *Citrus sinensis* and *Citrus limon* peels extracts have potential as natural insecticides for the control of *Callosobruchus maculatus* on stored cowpea. The bioactive compounds present in these extracts act as toxicants, affecting the survival, development, and reproduction of the weevils. Additionally, the repellent effect of these extracts can help prevent re-infestation and reduce crop damage [8]. The use of citrus peel extracts as biopesticides offers several advantages over synthetic insecticides. Firstly, they are derived from natural sources and are therefore considered safer for human health and the environment. Secondly, they are readily available as waste products from citrus processing industries, making them cost-effective and sustainable alternatives. Lastly, their use can contribute to the reduction of chemical residues in food crops, promoting food safety [8]. Therefore the efficacy of *Citrus sinensis* and *Citrus limon* peels extracts in the control of *Callo-*

sobruchus maculatus on stored cowpea has been demonstrated in various studies. These natural plant extracts offer a promising alternative to synthetic insecticides for cowpea weevil management. Further research is needed to optimize their application methods and determine their long-term effects on non-target organisms. Nonetheless, their utilization can contribute to sustainable agriculture practices and reduce the reliance on harmful chemical pesticides.

Materials and Methods

Study area

The study was conducted in the Department of Parasitology and Entomology laboratory of Nnamdi Azikiwe University, Awka. Awka lies within the geographical coordinates 6°12'N and 7°04'E in the tropical Zone of Nigeria. It is situated about 25 miles (40km) Northeast Onitsha and 45 miles (72km) Southwest of Enugu, sited on the Awka-Orlu uplands with available land and patched forests and watered by a few springs and streams. The laboratory of the Department of Parasitology and Entomology is located in Science Village in Nnamdi Azikiwe University, Ifite-Awka.

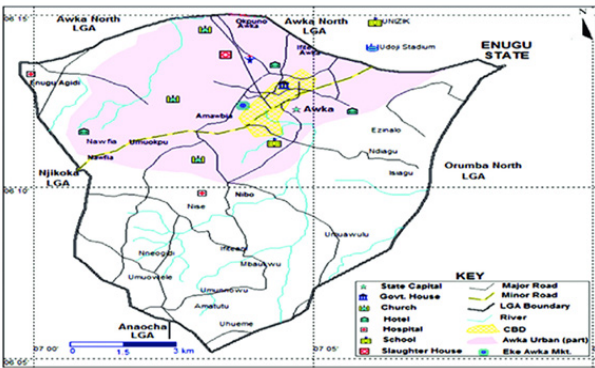


Figure 1: Map of Awka Metropolis (Source: Nwankwo, 2009).

Procurement of experimental Bean grains and insect culture

Infested beans grains were procured from Eke-Awka Market, Awka and adult *C. maculatus* used in this study were obtained from it. The weevils were transferred into a transparent plastic container for culturing, under optimal temperature. The lid was removed and replaced with a fine mesh net, this was done to enable aeration in the containers and prevent the weevils from escaping, also, preventing other insects from entering. The culture was left undisturbed for several weeks under optimal temperature, to allow for growth and oviposition of the adult insect, which enables the emergence of new adult weevils before being utilised for experiment.

Collection and procurement of *citrus sinensis* and *citrus limon*

Fresh *Citrus sinensis* and *Citrus limon* were purchased from the Eke-awka Market open market in Awka, Anambra state and were taken to the Botany Laboratory of Nnamdi Azikiwe University for identification and authentication by a taxonomist in the Laboratory.

Extraction of *citrus sinensis* and *citrus limon* essential oils

The fruits were washed and peeled respectively, and the peels were cut into smaller pieces of enable it fit into the round bottom flask. The cut peels were weighed, 100g each and were housed in

the still round bottom flask and 200ml of water was measured with a beaker and introduced into the round bottom flask and was placed in the Clevenger apparatus. The peels were immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapour and finally collected by decantation [10].

Experimental Set-Up and Formulation of extract

The experiment was set up in a Completely Randomized Block pattern. The essential oils were diluted serially using acetone according to each concentrations which were: 20%, 15%, 10%, 5% respectively. 20% concentration was equivalent to 2ml of the essential oils diluted with 8ml of acetone to give 10ml of treatment mixture. 15% concentration was equivalent to 1.5ml of the essential oil diluted with 8.5ml of acetone to give 10ml of treatment mixture. 10% concentration was equivalent to 1ml of essential oil diluted with 8.5ml of acetone to give 10ml of mixture treatment and 5ml concentration is equivalent to 0.5ml of essential oil diluted with 9.5ml of acetone to give 10ml of treatment mixture. These were for the four replicates of each concentrations, for the control, 1ml of acetone was used.

Treatment of the adult *C. maculatus*

Four different 10ml syringes were used to collect treatment mixture of each concentrations respectively. These were introduced into the experimental vials containing filter paper, each treatment was replicated four times and was allowed to air dry for a period of 30 minutes. A 2ml syringe was used to collect 1ml of acetone which served as control. Five adult weevils were then introduced into each of the four separate experimental vials, including the control respectively. Each vials were covered with its lid with was perforated at the centre and sealed with a fine net mesh to prevent insect pests from escaping. Afterwards, each vials was exposed to treatment for 3 days, records of mortality was taken after every 0-12 hours, 0-24 hours, 0-48 hours.

Data collection and analysis

Data was generated and recorded from mortality count of adult *C. maculatus* for three days and were used to determine the most

efficacious concentration for both treatment mixtures respectively. *C. maculatus* were considered dead when they showed no form of movement when probed with a blunt probe. Dead weevils were counted and discarded after every count. Data collected on mortality of the weevils due to the efficacy of the treatment mixtures were subjected to Analysis of Variance (ANOVA) using SPSS computer software package (Version 25) at 0.05 significant levels. Log-probit-regression analysis was carried out to determine the LD50 and LD90 as well as the LT50 and LT90 of the treatments.

Results

Mortality rate of *C. maculatus* using different concentrations of *Citrus sinensis* and *Citrus limon*, hence the LD 50 and LD 90

Mortality rate of *Callosobruchus maculatus* after treatment with *Citrus sinensis*

Table 1 shows the mortality response of *C. maculatus* exposed to the toxicity of *Citrus sinensis* at 12 hourly intervals for 3 days. The result shows that there was concentration dependent mortality response to the toxicant. At the highest concentration of 20%; mortality was 87%, while at the lowest concentration of 5%; mortality was 50%. This shows increase in mortality with increase in concentration. Figure 1 shows the results of LC50 and LC90 of *Citrus sinensis* and *Citrus limon*, where the values for *Citrus sinensis* are 5.8% and 30.7% respectively. Furthermore, the analysis of variance of the concentrations showed the mortality as a result of the concentrations was significantly different ($P < 0.05$, $P = 0.096$).

Mortality rate of *Callosobruchus maculatus* after treatment with *Citrus limon*

Table 2 shows the mortality response of *C. maculatus* exposed to the toxicity of *Citrus limon* at 12 hourly intervals for 3 days. The result shows that there was concentration dependent mortality response to the toxicant. At the highest concentration of 20%; mortality was 50%, while at the lowest concentration of 5%; mortality was 43%. This shows increase in mortality with increase in concentration. Figure 2 shows the results of LC50 and LC90 of *Citrus sinensis* and *Citrus limon*, where the values for *Citrus limon* are 8.4% and 57.7% respectively. Furthermore, the analysis of variance of the concentrations showed the mortality as a result of the concentrations was significantly different ($P < 0.05$, $P = 0.103$).

Conc (%)	12hrs	24hrs	48hrs	Mean ± s.e	% Mortality	Probit
20	4.3	4.7	8.7	5.90±1.40	87	6.09
15	2.3	3.3	7.3	4.30 ± 1.52	73	5.56
10	2.0	3.0	6.7	3.90 ± 1.42	67	5.39
5	1.7	2.7	5.0	3.13 ± 0.97	50	4.93
Mean ± s.e	2.57 ± 0.58	3.42 ± 0.44	6.92 ± 0.76	-	-	
% Mortality	25.7	34.3	69.3	-	-	
Control	0.0	0.5	1	0.5	5	
Probit	4.21	4.49	5.45			

Table 1: Mortality response of *C. maculatus* exposed to residual application of *Citrus sinensis* oil extract at 12 hourly intervals.
Mean of the three replicates (±s.e), Pv = 0.096; Pv = 0.002

Conc (%)	12hrs	24hrs	48hrs	Mean ± s.e	% Mortality	Probit
20	3.3	3.7	7.6	4.86 ± 1.37	76	5.66
15	2.3	3.0	6.7	4.00 ± 1.36	67	5.39
10	2.0	2.7	5.0	3.23 ± 0.90	50	4.93
5	1.7	2.3	4.3	2.76 ± 0.78	43	4.74
Mean ± s.e	2.33 ± 0.34	2.92 ± 0.29	5.90 ± 0.75	-	-	
% Mortality	23.2	29.3	59.0	-	-	
Control	0.0	0.5	1	0.5	5	
Probit	4.16	4.34	5.17			

Table 2: Mortality response of *C. maculatus* exposed to residual application of *C. lemon* oil extract at 12 hourly intervals.
Mean of the three replicates (±s.e), Pv = 0.103; Pv = 0.002.

Probit against Log Conc. for *C. sinensis* and *C. limon*

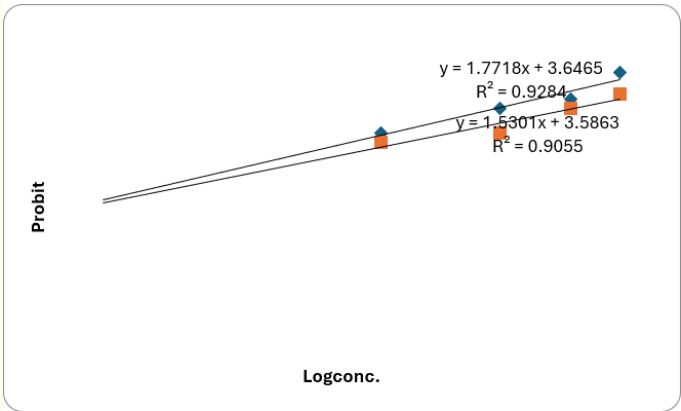


Figure 2: Plot of Probit against Logconc. of *C. sinensis* and *C. limon*.
C. sinensis, $y = 1.7718x + 3.6465$, $R^2 = 0.9284$, LC50=5.8%, LC90= 30.7%,
C. limon, $y = 1.5301x + 3.5863$, $R^2 = 0.9055$, LC50=8.4%, LC90=57.7%

Effect of exposure time of *C. sinensis* and *C. limon* to *C. maculatus*

The result of three days exposure to *Citrus sinensis* and *Citrus limon* is shown in Table 1 and 2 respectively. The result showed increased mortality with increase in exposure time. At 12 hours, mortality after treatment with *Citrus sinensis* and *Citrus limon* was 25.7% and 23.2% respectively while at 48 hours after treatment mortality was 69.3% and 59.0% respectively. Significant differ-

ence ($P < 0.005$, $P = 0.002$) was observed for *Citrus sinensis* time. Significant difference ($P < 0.005$, $P = 0.002$) was observed for *Citrus limon* time. Figure 3 shows that the LT 50 and LT 90 were 27.2 hours and 45.3 hours respectively, and Figure 3 also shows that the LT 50 AND LT 90 of *Citrus limon* were 12.2 hours and 43.1 hours respectively.

Probit against LogTime for *C. sinensis* and *C. limon*

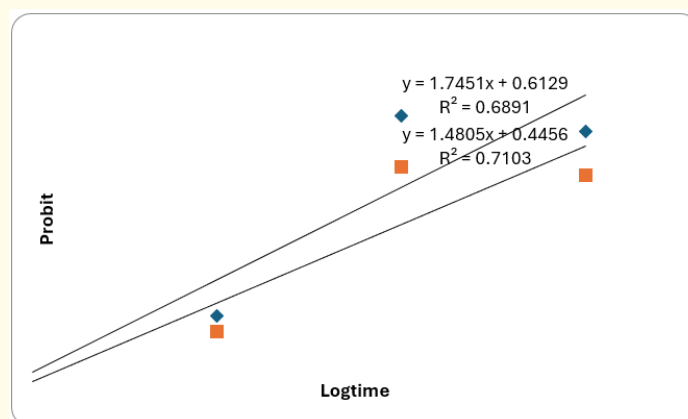


Figure 3: Plot of Probit against Logtime of *C. sinensis* and *C. limon*.
C. sinensis, $y = 1.7451x + 0.6129$, $R^2 = 0.6891$, LT50= 27.2 hours, LT90= 45.3 hours,
C. limon, $y = 1.4805x + 0.4456$, $R^2 = 0.7103$, LT50=12.2 hours, LT90=43.1 hours

Discussion

This study focused on exploring the efficacy of plant-based insecticides, specifically those derived from *C. sinensis* and *C. limon*, in controlling the population of *Callosobruchus maculatus*. The results revealed that these treatment mixtures influenced the survival rate of the insect, suggesting their potential as environmentally friendly and human-safe substitutes for conventional insecticides. The results of the toxicity study indicated that the essential oil extracts effectively eliminated *C. maculatus*. This is consistent with the findings of [11], who utilized *Ocimum* leaf powder to control *Sitophilus zeamais* infestation in stored maize grains. Furthermore, [7] demonstrated the efficacy of *Moringa oleifera* and *Annona muricata* against *Aedes aegypti* mosquito larvae. This suggests that the essential oils of *Moringa oleifera* and *Annona muricata* leaves may contain toxic substances similar to those found in *C. sinensis* and

C. limon peels. Hence, the result obtained from the toxicity study demonstrated the active potential of the peels essential oil extracts in causing mortality in the population of the weevils. This is line with [12] who reported 7.1% mortality of *T. castenum* adult with 0.5g powder of *P. guineese*.

The protectants significantly reduced adult mortality of *C. maculatus* in all the samples, relative to the control at ($p < 0.05$) with increasing concentrations of the treatments, mortality showed proportional increase as seen in tables 1 and 2. Apart from being directly toxic to the insects it affected, it is also possible that the treatments have slight insect deterrence quality. [13] reported that the neem seed extract was very effective and caused 82.5% mortality of *C. maculatus* at concentration of 200UI/20g seeds of Cowpea. The result in this study demonstrated the highest concentration of

C. sinensis and *C. limon* at 20% had percentage mortality of 87% and 76% respectively while the lowest concentration at 5% had percentage mortality of 50% and 43% respectively. The result of the mortality was concentration dependent. Similarly, [14] reported that the efficacy of root powder of *L. cyanescens* on adult *S. zeamais* was concentration dependent [14].

The study result showed that the mortality was time dependent, as increase in exposure time caused an increase in mortality. At 12 hours after treatment with *Citrus sinensis* and *Citrus limon*, mortality was 25.7% and 23.2% respectively, while at 48 hours after treatment, mortality was 69.3% and 59.0% respectively. This however showed that with long post exposure periods, the direct effect of these insecticides increased, leading to *C. maculatus* mortality. This also showed that they are long lasting insecticides and may be used for pre-infestation treatments before most fields to store pests colonises the stored products. In this present study, higher mortalities of *C. maculatus* occurred at the last hours of exposure. Timing of spray applications is important in any pest management operations. A study carried out by [15] reported 65-100% mortality of Cowpea weevil treated with neem seed powder at 12 and 120 hours of exposure. This present study also suggested that *C. sinensis* and *C. limon* as a long lasting insecticide is time dependent and will require few repeated applications to bring about mortality of insect pests.

Conclusion

This research work has demonstrated the great potential of botanical insecticides. It has been suggested that botanical insecticides may improve the effectiveness of conventional insecticides and make it possible to lessen side effects. The use of botanical products prevents several insect pests from infesting stored food products. The study of the efficacy of the essential oils extracted from the peels of *Citrus sinensis* and *Citrus limon* shows that they can be used as botanical insecticides. This shows they can serve as alternative to synthetic chemicals used in insect pest control in storage which may accumulate to damage man's health and the environment. The study revealed the importance of exposure time and dose of the bioinsecticide on mortality of the *C. maculatus*.

This implies that when treating cowpea with *Citrus sinensis* and *Citrus limon* both directly or as a fumigant, a longer period of time and a calculated dose should be applied for a more desirable result. Both *Citrus sinensis* and *Citrus limon* are common fruits found in west tropical Africa, which make them easily available and affordable by farmers in the region. On this basis, local farmers are advised to use the essential oils extracted from the peels of these citrus fruits tested to protect cowpea seeds in storage against weevil infestation.

Author Contributions

UCU and IJE wrote the first draft, developed the protocol and did the experimental design. OEO edited the manuscript and UCU oversaw study implementation while OIE did the statistical analysis.

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Competing Interest

The authors declare that they do not have any clashes of interest.

Data Availability

The data used to support the findings of this study are available upon astute request.

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