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# INSECTICIDAL ACTIVITY OF *Citrus sinensis* (L.) and *Parkia biglobosa* (Jacq.) EXTRACTS AGAINST *Trogoderma granarium* Everts

## SUMMARY

In this study, ethanolic extracts of *Citrus sinensis* (L.) (leaves/peels) and *Parkia biglobosa* (Jacq.) (leaves/seeds) were screened for insecticidal activity against 4th larval instar and adults of khapra beetle, *Trogoderma granarium* Everts (Coleoptera:Dermestidae). Also examined were percentage seed damage, seed weight loss and viability of the seeds. Decorticated seeds of groundnut were treated with different concentrations (1, 3 and 5%) of the extracts against the adults while 5% concentration was tested against the larvae. The bioassay showed that the extracts had larvicidal and adulticidal activities which increased with increase in concentration and exposure time. The seed damage data were used to estimate the Beetle Perforation Index (BPI) indicating that P. biglobosa seed extract was the most effective with a BPI of 20.14%. The results of this study showed that *C. sinensis* and *P. biglobosa* extracts possess some insecticidal activity which may be associated with the presence of secondary metabolites (saponins, alkaloids, steroids, tannins, cardiac glycosides, flavonoids and phenols in them, This report shows that *P. biglobosa* seed extract could be recommended as a source of insecticidal agent in a pest management technique.

**Keywords:** *Trogoderma granarium*, plant extracts, insecticidal activity, biopesticides.

## INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is known by many names including peanut, earthnut, goober peas, monkey peas, pig nuts and pygmy nuts (Alabi *et al*., 2013) and belongs in the family Fabaceae, sub-family Papillionaceae. In Nigeria, it is an important oil, food and forage crop grown by the small-holder farmers in the northern part of the country.

Kernel weight damage in storage has been estimated to range between 10 and 25% under short-term storage period in Nigeria, but it could be higher under long-term storage situations (Musa, 2007). There is no compensation for seed damage caused by stored-product pests as it is the case for damage induced by field pests. In Nigeria, the profitable preservation of groundnut seeds is greatly constrained by *Trogoderma granarium* Everts, thereby creating a challenge of sustainable preservation of the seeds meant for future consumption, planting and

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even trade. *Trogoderma granarium* is a dermestid that can cover the surface of stored grains with crawling larvae capable of turning the grains into powder (Ellis and Hodges, 2007).

The current method of controlling *T. granarium* involves the application of synthetic, broad-spectrum pesticides and fumigants. However, for fumigants to be effective against the pest, high concentration of the chemical must be maintained over the fumigation period to allow penetration into cracks and crevices (Harris, 2006). The use of methyl bromide and phosphine has led to consequent repercussions on the farmers and the environment or even the depletion of ozone layer (Musa, 2007). These synthetic insecticides have also been confirmed to be responsible for development of insect resistance, toxic residues in food, workers' safety and high cost of procurement (Asawalam and Onu, 2014). These problems have necessitated their ban and current research on the use of biopesticides from either plants or micro-organisms that are safe to the farmers and the environment. This study therefore, attempts to contribute to the existing information on the possible effects of ethanolic extracts of plants on *T. granarium*. The objective of the study was to examine the insecticidal activity of sweet orange, *Citrus sinensis* L. and African locust bean, *Parkia biglobosa* (Jacq.) Benth against khapra beetle, *T. granarium,* in stored groundnut.

## MATERIAL AND METHODS

### Culture of *Trogoderma granarium*

The strain of khapra beetle, *T. granarium,* used for this study was obtained from the culture maintained in the laboratory of the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria. The dermestid was raised on groundnut seeds in 250 ml kilner jars at ambient temperature of $28\pm3^0$C and relative humidity of 68±3%. Thirty adults of mixed sexes were picked from the existing culture with the aid of pooter (an aspirator), and used to infest fresh and susceptible seeds. The insects were sieved out after two weeks when they would have mated and laid eggs. Fourth larval instar and freshly emerged adults (1-2 days old) were used in this study.

### Seed disinfestations

Groundnut seeds (SAMNUT 7) obtained from the Institute for Agricultural Research, Samaru, Nigeria were properly sorted, winnowed, wrapped in a polyethylene bag and then placed in a freezer compartment of a refrigerator for 2 weeks to kill all developmental stages of insect pests. The seeds were certified fit for this study after 2 weeks of equilibration. The moisture content determined on a wet basis using oven dry method was 8.7%.

### Plant materials

Fresh leaves of sweet orange, *C. sinensis* were collected from the Park and Gardens of the University of Ilorin, Ilorin, Nigeria while the peels were collected from orange sellers who often regard the peels as waste. The leaves and seeds of African locust bean tree, *P. biglobosa*, were obtained from the tree growing naturally on the campus. These plant parts were washed thoroughly under running water, air-dried, ground and sieved to obtain fine uniform particle size. The powders were kept separately in airtight plastic containers at prevailing temperature and relative humidity.

**Preparation of plant extract**

The powdered mass of 50 g each of the *C. sinensis* leaves/peels and *P. biglobosa* leaves/seeds, was extracted by pouring it in 500 ml ethanol to produce a concentration of 10.0%. The mixture of the powder and ethanol was thoroughly shaken and allowed to stand for 48 h before it was filtered through muslin to get the stock solution. The filtrates were transferred to separate beakers and evaporated over a steam bath at $50^0$ C to obtain the pasty extracts. Different concentrations of 1, 3 and 5% were prepared following filtration process and a 2 mm of each concentration was employed in the bioassay.

**Experimental procedure**

Bioassays were carried out in the Crop Protection Laboratory of the University of Ilorin, Ilorin, at prevailing ambient temperature and relative humidity of $28\pm3^0$C and 68±3% respectively.

**Effect of the extract on larval mortality**

The different extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) were applied at 5% concentration to decorticated groundnut seeds in plastic containers (5.0 cm × 8.0 cm) by means of hypodermic syringe. Each container was gently shaken to ensure coating of the seeds with extracts and then allowed to dry for 1 h before infestation. Ten $4^{th}$ larval instar of *T. granarium* were introduced into each treatment and then covered with muslin fastened with rubber band. Each treatment was replicated three times, including the controls which were seeds either left untreated or treated with 5% ethanol. Dead larvae were counted every 24 h for a total of 96 hours after treatment (HAT).

**Effect of the extract on adult mortality**

In this investigation, the different extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) were applied at 1, 3 and 5% concentration to decorticated groundnut seeds in plastic containers (5.0 cm × 8.0 cm) by means of hypodermic syringe. Each container was gently shaken to ensure coating of the seeds with the extracts and allowed to dry for 1 h before infestation. Three females and two males of freshly emerged adults (1-2 days old) of *T. granarium* were used to infest the seeds. Dead insects were counted every 24 h for a total of 96 HAT.

**Seed weight loss**

Percentage seed weight loss was calculated for larvae-infested groundnut at 30 days intervals using the formula:

$$\text{Seed Weight Loss} = Initial\ Weight - Final\ Weight\ /\ Initial\ Weight \times 100$$

**Seed damage assessment**

Damage assessment was carried out on the larvae-infested seeds by separating the seeds into damaged (seeds with holes) and undamaged (seeds without holes) groups. Therefore, percentage seed damage was based on differences between the total number of seeds and number of seeds perforated divided by total number of seeds expressed as percentage.

*%Seed Damage=Total No.of Seeds-No.of Seeds Perforated/Total No.of Seeds ×100*

**Beetle Perforation Index**

Beetle Perforation Index was determined for each treatment concentration for comparison of seed protectant effects using the procedure of Fatope *et al*., (1995) as shown below:

*BPI=% of treated seeds perforated×100/ % of control seeds perforated+% of treated seeds perforated*

**Seed viability test**

The floatation method described by Ehiagbonare and Enabulele (2007) was modified to check the viability of the seeds after treatment with a concentration of each of the extracts. Twenty seeds per replicate were sprayed with 5 ml solution of the plant extracts. Percent viability was recorded at 2 h after treatment. All treatments were conducted in three replicates and the mean values were calculated using the formula:

$$Perforated\ Viability = (S - Sf/S) \times 100$$

where

S = number of treated seeds used/replicate

$S_f$ = number of floating seeds/replicate

**Data analysis**

Data were subjected to one-way analysis of variance to determine critical difference among treatments. Significantly different (p=0.05) means were partitioned using Duncan`s multiple range test.

## RESULTS AND DISCUSSION

**Effect of ethanolic extracts on *T. granarium* adults**

The effect of ethanolic extracts of C. sinensis (leaves/peels) and P. biglobosa (leaves/seeds) on T. granarium adults is presented in Table 1. The plant extracts applied at various concentrations had no significant difference (p>0.05) on the mortality of *T. granarium* at 24 HAT. However, the extract of *C. sinensis* leaves applied at 5% concentration and *P. biglobosa* seeds applied at 3 and 5% concentrations caused highest mortality which was not significantly different (p>0.05) from the mortality in other treatments at 48 HAT. There was no mortality in 1% concentration of *C. sinensis* peels or *P. biglobosa* seeds and the control which was significantly different from the mortality in other treatments.

The extract of *P. biglobosa* seeds applied at 3 and 5% concentrations caused highest mortality of *T. granarium* which was not significantly different (p>0.05) from the mortality in 3 and 5% concentrations of *C. sinensis* leaves or *P. biglobosa* leaves at 72 HAT. The least mortality was observed in 1% concentration of *C. sinensis* peels, *P. biglobosa* leaves or *P. biglobosa* seeds which was not significantly different (p>0.05) from no mortality in control at 72 HAT.At 96 HAT, the extract of *P. biglobosa* seeds applied at 3 and 5% concentrations again caused highest mortality which was the same as mortality in 5% concentrations of *C. sinensis* leaves. The least mortality was observed in 1% concentration of *P. biglobosa* leaves or *P. biglobosa* seeds which was not significantly different (p>0.05) from no mortality in control at 96 HAT.

Table 1: Effect of ethanolic extracts on mortality of Trogoderma granarium adults

| Extracts | Concentration (%) | Mortality of *T. granarium* adults (HAT) | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| *C. sinensis* leaves | 1 | 0.00(0.00) | 0.33bc (6.6) | 1.00bcd (20.0) | 1.33bc (26.6) |
| | 3 | 0.67 (13.4) | 1.00abc (20.0) | 2.00ab (40.0) | 2.33ab (46.6) |
| | 5 | 1.00 (20.0) | 1.67ab (33.4) | 2.00ab (40.0) | 2.67a (53.4) |
| *C. sinensis* peels | 1 | 0.00 (0.00) | 0.00c (0.00) | 0.33cd (6.6) | 1.33bc (26.6) |
| | 3 | 0.33(6.6) | 0.33bc (6.6) | 1.00bcd (20.0) | 1.33bc (26.6) |
| | 5 | 0.67(13.4) | 0.67abc (13.4) | 1.00bcd (20.0) | 1.33bc (26.6) |
| *P. biglobossa* leaves | 1 | 0.33 (6.6) | 0.33bc (6.6) | 0.33cd (6.6) | 0.67d (13.4) |
| | 3 | 0.00 (0.00) | 0.67abc (13.4) | 1.33abc (26.6) | 1.67abc (33.4) |
| | 5 | 0.67 (13.4) | 1.00abc (20.0) | 1.67ab (33.4) | 2.00ab (40.0) |
| *P. biglobossa* seeds | 1 | 0.00 (0.00) | 0.00c (0.00) | 0.33cd (6.6) | 0.67d (13.4) |
| | 3 | 1.00 (20.0) | 1.33ab (26.6) | 2.33a (46.6) | 2.67a (53.4) |
| | 5 | 0.33 (6.6) | 1.33ab (26.6) | 2.33a (46.6) | 2.67a (53.4) |
| No extract | 0 | 0.00 (0.00) | 0.00c (0.00) | 0.00d (0.00) | 0.00d (0.00) |
| SE± | | 0.32 | 0.32 | 0.29 | 0.35 |
| CV (%) | | 82.0 | 47.5 | 24.4 | 22.1 |

Values with the same superscript(s) in the same column are not significantly different at 5% level of probability using DMRT.
Figures in parentheses are in percentages
KEY: HAT = Hours After Treatment; CV = Coefficient of Variation; SE = Standard error

**Effect of ethanolic extracts on *T. granarium* larvae**

The effect of ethanolic extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) on *T. granarium* larvae is presented in Table 2. The *P. biglobosa* seeds caused significantly higher (P<0.05) mortality of *T. granarium* larvae than the mortality in other treatments at 24 HAT. The subsequent mortality values in *P. biglobosa* seeds were not significantly different (p>0.05) from mortality in *P. biglobosa* leaves from 48 to 96 HAT. Ethanol-treated groundnut showed increase on larval mortality with increase in exposure period. The ethanol-free treatment had no mortality only at 24 HAT while extract-free treatment had no mortality throughout the exposure period. *C. sinensis* leaves consistently had the least larval mortality during the exposure period.

Table 2: Effect of ethanolic extracts on mortality of *Trogoderma granarium* larvae.

| Extracts | Mortality of *T. granarium* larvae (HAT) | | | | |
|---|---|---|---|---|---|
| | Concentration (%) | 24 | 48 | 72 | 96 |
| *C. sinensis* leaves | 5 | 0.33b (3.3) | 0.67c (6.7) | 1.67cd (16.7) | 3.00c (30.0) |
| *C. sinensis* peels | 5 | 0.67ab (6.7) | 1.33c (13.3) | 2.67bc (26.7) | 4.67bc (46.7) |
| *P. biglobossa* leaves | 5 | 1.33a (13.3) | 4.00ab (40.0) | 5.00ab (50.0) | 6.67ab (66.7) |
| *P. biglobossa* seeds | 5 | 3.67c (36.7) | 5.33a (53.3) | 6.33a (63.3) | 7.00a (70.0) |
| Ethanol only | 5 | 0.00b (0.0) | 2.00b (20.0) | 3.00bc (30.0) | 4.33c (43.3) |
| No extract | 0 | 0.00b (0.0) | 0.00c (0.0) | 0.00d (0.0) | 0.00d (0.0) |
| SE± | | 0.22 | 0.67 | 0.71 | 0.42 |
| CV(%) | | 22.2 | 27.0 | 22.9 | 9.7 |

Values with the same superscript(s) in the same column are not significantly different at 5% level of probability using DMRT.

Figures in parentheses are in percentages

KEY

HAT = Hours After Treatment; CV = Coefficient of Variation; SE = Standard error

### Seed damage and beetle perforation index

The effect of ethanolic extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) on percentage seed damage caused by *T. granarium* larvae and percentage perforation index of the plant extracts is presented in Table 3. The *P. biglobosa* seed extract caused the least percentage seed damage which was significantly lower (p<0.05) than the percentage seed damage in *C. sinensis* leaves at 120 DAI. The untreated seeds (control) had significantly higher (p<0.05) percentage seed damage than the seeds treated with the plant extracts. Beetle perforation index for each treatment was proportional to the percentage seed damage recorded. This implies that the seeds treated with the plant extracts had lower damage than the untreated seeds.

Table 3: Percentage seed damage and beetle perforation index of *Trogoderma granarium* larvae.

| | | | |
|---|---|---|---|
| Extract | % Seed damage (120 DAT) | | Beetle Perforation Index (%) |
| *C. sinensis* leaves | 11.53[b] | | 38.07 |
| *C. sinensis* peels | 8.19[bc] | | 30.39 |
| *P.biglobosa* leaves | 5.70[bc] | | 23.28 |
| *P.biglobosa* seeds | 4.72[c] | | 20.14 |
| No extract<br>SE±<br>1.58<br>CV(%) 16.18 | 18.75[a] | | 50.00 |

SE = Standard error; CV = Coefficient of variation; DAT = Days after treatment

Values with the same letter in the same column are not significantly different at p=0.05 using Duncan`s multiple range test.

**Seed weight loss and viability test**

The effect of ethanolic extracts of C. sinensis (leaves/peels) and P. biglobosa (leaves/seeds) on percentage seed weight loss caused by T. granarium larvae and seed viability is presented in Table 2. The P. biglobosa seed extract had the least percentage seed weight loss which was significantly lower ($p<0.05$) than the percentage seed weight loss in other treatments at 30 and 60 DAT; but not significantly ($p>0.05$) different from the percentage seed weight loss in C. sinensis peels at 90 DAT. Of all the seeds treated with the extracts, P. biglobosa had percentage seed weight loss which was significantly different from the control at 60 DAT. The seed weight loss increased with increase in exposure time. The results also showed that P. biglobosa seed extract enhanced better viability of the groundnut seeds than other extracts and the control. It was observed that seeds treated with P. biglobosa seed extract had highest percentage viability followed by P. biglobosa leaf and C. sinensis peel extracts.

Table 4: Percentage seed weight loss caused by *Trogoderma granarium* larvae

| Extract | % seed weight loss (DAT) | | | |
|---|---|---|---|---|
| | 30 | 60 | 90 | % viability test |
| *C. sinensis* leaves | 2.36[a] | 6.23[a] | 10.57[a] | 96.7 |
| *C. sinensis* peels | 2.15[b] | 6.10[a] | 10.12[c] | 98.3 |
| *P.biglobosa* leaves | 2.13[b] | 6.08[a] | 10.22[b] | 98.3 |
| *P.biglobosa* seeds | 1.94[c] | 6.02[b] | 10.13[c] | 100.0 |
| No extract | 3.34[a] | 6.27[a] | 10.61[a] | 71.7 |
| SE± | 0.18 | 0.12 | 0.15 | |
| CV(%) | 7.50 | 1.89 | 1.41 | |

Values with the same superscript(s) in the same column are not significantly different at 5% level of probability using DMRT

SE = Standard error; CV = Coefficient of variation; DAT = Days after treatment

The four plant parts from *C. sinensis* and *P. biglobosa* were evaluated and found to be effective in causing the mortality of *T. granarium* adults with increase in both concentration and exposure time. The *Parkia biglobosa* seed extract applied at 5% was the most effective in significantly causing mortality of *T. granarium* adults. Barbehenn and Martin (1994) and Barbehenn *et al*. (1996) have stated that tannins can enter the haemolymph of the insect through the peritrophic envelope of the gut. Osarumwense *et al*. (2013) reported the presence of saponins, cardiac glycosides, tannins and flavonoids but they reported the absence of alkaloids in phytochemical analysis of *C. sinensis* peels. *Trogoderma granarium,* a phytophagous beetle, requires protein and other nutrients present in groundnut for optimum development. Shi *et al*., (2004) had earlier reported that saponins have been extensively used as pesticide.

This study shows that the whole but untreated seeds were severely attacked by *T. granarium* indicating that the insect is a major storage pest of groundnut. Seeds treated with *P. biglobosa* seed extract were the least attacked in terms of having the least BPI of 20.14% and therefore the least damage. It was found that the various concentrations of ethanolic extracts tested were effective in suppressing the damage caused by an introduced insect population. In this study, percentage seed weight loss caused by *T. granarium* in the absence of extracts caused 10.61% within 90 days. The untreated seeds had the least viability in storage compared with 100% viability in the *P. biglobosa* seed extract. The varying degrees of damage recorded in this study are capable of predicting acceptability and marketability of the seeds. It could be reported that when *T. granarium* was left undisrupted it caused significant grain weight loss and damage in the store accompanied by reduction in seed viability.

Also, all treatments caused an increase in the mean number of *T. granarium* larvae that died following application. Extract of *P. biglobosa* seeds was the most effective in causing larval mortality. It caused significantly higher mortality than other extracts from 24 h to 96 HAT. Similarly, larval mortality of *T. granarium* increased with time for all the treatments. Femi-Ola *et al.*, (2008) had earlier reported that aqueous and acetone extracts of *P. biglobosa* caused total mortality within 40-110 min. after application. This study is an index of possible inclusion of plant products in biopesticide formulation against stored-product insects. The extracts have affected $4^{th}$ larval instar survival, although the rate of mortality varied with extracts. One possible hypothesis that has been given to explain the larvicidal and adulticidal activities of the extracts is the direct toxic effect of some oil constituents (Lienard *et al*., 1993). The present results indicated that the extracts were effective in reducing damage to groundnut seeds from *T. granarium*.

In the present investigation, the ethanolic extracts of *C. sinensis* (leaf/peel) and *P. biglobosa* (leaf/seed) were capable of suppressing larval and adult populations of *T. granarium*. It was also found that the plant products were effective in reducing losses and damage of stored groundnut seeds caused by an introduced larval population of *T. granarium.* This result might have to be confirmed or invalidated as the presence of chemical constituents responsible for this activity depends on growing conditions, extraction method, type of solvent and volatilization. It is possible that these factors are responsible for the potential role of these extracts in seed protection. From the results, it was evident that the groundnut seeds suffered potential increase in weight loss and damage as a result of insect pest infestation.

## CONCLUSION

The preservation of groundnut seeds is hampered by the attack of *T. granarium.* The study has revealed the appreciable insecticidal activity of *C. sinensis* and *P. biglobosa* extracts against *T. granarium*. Thus there is now empirical validation for the use of plant extracts for the control of the stored-product insect pests. However, *P. biglobosa* seed extract proved to be a more promising control agent for further development as botanical formulation for use against *T. granarium* The use of *P. biglobosa* seed extract should be encouraged for resource- poor farmers in stored-product protection.

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