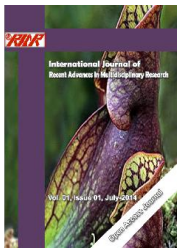

International Journal of Recent Advances in Multidisciplinary Research
Vol. 01, Issue 03, pp.068-071, September, 2014

Full Length Research Article

INSECTICIDAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF GUAVA, *PSIDIUM GUAJAVA* L. LEAF OIL AGAINST KHAPRA BEETLE, *TROGODERMA GRANARIUM* EVERTS (COLEOPTERA: DERMESTIDAE) ON STORED GROUNDNUT

***Musa, A. K. and Olaniran, R. O.**

Department of Crop Protection, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

ARTICLE INFO

Article History:
Received 17th June, 2014
Received in revised form
30th July, 2014
Accepted 07th August, 2014
Published online 30th September, 2014

Keywords:
Khapra beetle,
Groundnut, mortality,
Plant oil, seed losses

ABSTRACT

Leaf oil of Psidium guajava L.obtained from Soxhlet extraction was tested for insecticidal effect and phytochemical screening against khapra beetle, Trogoderma granarium Everts (Coleoptera: Dermestidae). In the laboratory study, groundnut seeds treated with the oil significantly performed better than the control in terms of reduced population and emergence of T. granarium larvae and adults. Percentage mortality of T. granarium in groundnut seeds treated with the oil increased with rate of treatment. The application of the oil at the rate of 0.5 ml gave significantly ($p<0.05$) higher percentage mortality than the control. Percentage seed weight loss caused by the larvae in 0.5 ml was significantly lower than 30 and 45% observed in 0.3 ml at 30 and 60 DAT respectively. The presence of flavonoids, alkaloids, steroids, triterpenoids, cynogenic glycosides, in various amounts is an indication of insecticidal properties of the oil. Lipids and carbohydrates offered reliable nutritional components of the guava oil. Oxalates, tannins, saponins and phenols were absent in the studied leaf oil. Guava leaf oil could form a reliable component of pest management programme against T. granarium in small scale storage of groundnut seeds.

INTRODUCTION

Beetles (Coleoptera) constitute the largest order in the class Insecta and of course in the animal kingdom. They are most frequently encountered at home, in the field and in storage and are particularly known for their economic importance in stored plant and animal products. In Nigeria, most noticeable species include maize weevil, *Sitophilus zeamais* Motschulsky, rice weevil, *S. oryzae* L., bean beetle, *Callosobruchus maculatus* (F.), flour beetles, *Tribolium castaneum* Herbst and Khapra beetle, *Trogoderma granarium* Everts. Ofuya and Lale (2001) demonstrated the various levels of damage caused by these insect pests on cereal grains and their flour, dried pulses, nuts and oil seeds. Toxic synthetic insecticides being applied in solid and liquid forms against these insect pests are generally accepted as effective but carcinogenic, hazardous to non-target organisms and the environment. There is therefore, the growing interest in adapting naturally existing plant and animal materials in crop protection to stem the trend of food shortage as a result of insect infestation. Plant secondary compounds have been subjected to thorough investigation in the past few decades to unravel those components that have insecticidal properties (Akhtar and Isman, 2004) and do not accumulate in the environment (Rozman *et al*., 2007). Guava, *Psidium guajava* L. (Myrtaceae), is cultivated in the country for its nutritional value, unique flavour, taste and a number of beneficial substances contained in the leaves, including antioxidants like vitamin C and flavonoids. The availability of the plant and its constituents will make it a reliable substitute to the repeated use of synthetic insecticides if found to be effective against stored product insect pests. The present study was undertaken to investigate the insecticidal effects and phytochemical screening of guava, *P.guajava,* leaf oil against khapra beetle, *T. granarium* in groundnut.

Corresponding author: Musa, A. K.
Department of Crop Protection, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

MATERIALS AND METHODS

The study on the potential of guava leaf oil to preserve groundnut seeds against *T. granarium* was examined under ambient conditions (26±3^{0}C and 68±5% relative humidity). The study was carried out in the laboratory of Crop Protection, University of Ilorin between November 2013 and March 2014.

Culture of *T. granarium*

Heterogeneous populations of *T. granarium* were maintained on edible groundnut seeds in 250 ml Kilner jars under

laboratory conditions. Freshly emerged larvae (1st instar) and adults (1-2 days old) from this culture were used for this study.

Disinfestations of groundnut seeds

Decorticated groundnut seeds (variety SAMNUT 17) obtained from Institute of Agricultural Research, Samaru, were sorted and winnowed before they were wrapped in a polythene bag and disinfested in a freezer for 7 days to ward off any developing immature stages of insects. After the 7 days of disinfestation in cold treatment, the seeds were air-dried for another 7 days to sustain the seeds in the course of study.

Preparation of *Psidium guajava* oil

Guava, *P. guajava,* leaves were plucked from the trees growing in the University of Ilorin Senior Staff Quarters. The leaves were thoroughly washed with distilled water and dried under shade for 14 days. The dried leaves were ground using an electric blender and passed through 90 micron mesh sieve to obtain uniform powder. The leaf powder (160 g) was extracted for 5 h with 300 ml of petroleum spirit (b.p. 60-80^{0}C) in a Soxhlet extractor. The leaf oil obtained was allowed to dry over a water bath set at 40^{0}C. The oil was stored in a glass vial at -4^{0}C until required.

Larvicidal and adulticidal effects of *P. guajava* oil against *T. granarium*

Groundnut seeds (25 g) were treated with 0.0, 0.2, 0.3, 0.4 and 0.5 ml of *P. guajava* oil separately in five plastic containers (8.5 cm diameter) and covered with muslin to allow aeration but prevent insect escape. The oil was applied to the seed sample with a micro-syringe. Five (5) freshly emerged first instar larvae of *T. granarium* were placed in the containers. There were three replicates of each treatment including the control to examine the larvicidal effects of the oil. Similar experimental set-up was prepared to determine adulticidal effects of *P. guajava* oil against *T. granarium*, larval emergence and seed weight loss after the storage period of 30, 60 and 90 days after treatment.

Phytochemical screening of the oil

The procedure of Farnsworth (1985) was adopted in detecting the various components of *P.guajava* leaf oil. The various components were quantitatively determined by adopting the procedure described by Adeniyi *et al*., (2009).

Test for alkaloids

10 ml of hydrochloric acid was tested with 30 ml of the leaf oil. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was then tested with 2 drops of Wagner`s reagent. A positive reaction was recorded if brown precipitate or turbidity was observed.

Test for flavonoids

3 ml of a mixture of hydrochloric acid and water 1:1:1 and a few magnesium turnings were added to 3 ml of the filtrate. A positive reaction was recorded if a red/pink colour was observed.

Test for saponins

The froth which persists after shaking of the filtrate for at least 10 seconds was indicative of the presence of saponin.

Test for tannins

The filtrate of the oil was mixed with 2 ml of 0.1 ml FeCl3 in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. Tannin concentration was then extrapolated from a calibration curve.

Data collection

Data were subjected to analysis of variance and Least Significant Difference statistic was used to separate significantly different treatment means at 5% level of probability.

RESULTS

Larvicidal and adulticidal effects of *P. guajava* oil against *T. granarium*

The effects of varying levels of *P. guajava* leaf oil on mean percentage mortality of *T. granarium* larvae and adults are shown in Table 1. No dead larvae and adults of the insect were found in the controls at 1 and 2 DAT. It was observed that low dosage (0.2 ml) of guava leaf oil had no insecticidal effect on larvae at 1 and 2 DAT. However, a highly significant difference ($p<0.05$) in larval mortality was noticed between the lowest and other rates of treatment. The seeds treated with the oil had varied mean percentage mortality of both larvae and adults which differed significantly ($p<0.05$) when compared with the mortality recorded in the lowest rate of treatment. The seeds treated with 0.4 and 0.5 ml of the oil had significantly higher ($p<0.05$) larval mortality (40.0%) than 0.3 ml (20.0%) at 1 DAT. The groundnut seeds treated with 0.5 ml of the oil had significantly higher ($p<0.05$) larval mortality (86.67%) than 0.3 and 0.4 ml with 26.67 and 48.67% larval mortality at 2 DAT respectively. However, significantly higher mortality was recorded at 0.4 ml when compared to 0.3 ml at 2 DAT. The results also showed that 0.5 ml/25 g seeds had a remarkable improvement in its insecticidal potential over 0.4 ml/25 g from 2 to 5 DAT. Groundnut seeds treated with 0.5 ml of the oil had significantly higher ($p<0.05$) adult mortality of 80.0% when compared with lower rates treatment having13.4 to 20.0% at 1 DAT. At 2 DAT, groundnut had significantly higher adult mortality of 86.6% when compared with lower rates of treatment having the same adult mortality of 26.6%.

At 3 DAT, the seeds treated with 0.5 ml had significantly higher ($p<0.05$) larval mortality (93.3%) than lower rates of treatment with larval mortality which ranges from 6.67 to 46.7% while the control had no mortality. Groundnut seeds treated with 0.5 ml of the oil had significantly higher ($p<0.05$) adult mortality of 93.4% when compared with lower rates having adult mortality ranging from 33.4-66.0% while 6.6% adult mortality was recorded in the oil-free control at 3 DAT. Groundnut seeds treated with 0.3, 0.4 and 0.5 ml rates of guava leaf oil had significantly higher larval mortality of *T. granarium* (53.3, 60.0 and 93.3%) than 0.2 ml with 6.67% mortality at 4 DAT. Groundnut seeds treated with 0.4 and 0.5 ml of the oil maintained adult mortality of 66.0 and 93.4% respectively which were significantly higher ($p<0.05$) than mortality recorded in lowest rate of treatment (40.0%) and the control 4 DAT. At 5 DAT the seeds treated with 0.5 ml had 100.0% mean percentage larval mortality which was significantly higher ($p<0.05$) than lower rates of treatment. T

he results however, established that at 0.5 ml, higher larval mortality was recorded than lower rates, especially 0.4 ml/25 g rate of treatment which also had a substantial larval mortality recorded. At 5 DAT, groundnut seeds treated with 0.5 ml of the oil had significantly higher ($p<0.05$) adult mortality of 93.4% when compared with lower rates treatment at 5 DAT. There was also adult mortality of 53.4% at 0.2 and 0.3 ml which were not significantly different from the mortality recorded at 0.4 ml/25 g seeds.

Comparison of larvae and adult mortality in seeds treated with *P. guajava* leaf oil

When the mortality of larvae and adults of *T. granarium* due to *P. guajava* leaf oil were compared (Table 2), 80.0% adult mortality was obtained in 0.5 ml at 1 DAT. at 2 DAT, 86.6% adult mortality was obtained in 0.5 ml rate of treatment, showing that the oil extract had no contact toxicity on the insect, since 100% mortality of both larvae and adults was not recorded during the exposure period.

of treatment. Larvae emerged among the seeds treated with varying rates of treatment, but the seeds with the lowest rate of treatment and the control had the highest mean number of emerged larvae at 60 and 90 DAT. Results showed a difference of larval emergence in 0.4 ml which was not significantly different from highest rate of treatment. There was however, no significant difference in mean percentage larval emergence recorded among the treatments at 30 and 90 DAT. Results obtained from the analysed data revealed that all the treated seeds performed better than the seeds in the control. Table 3 also shows the mean percentage weight loss from the infested groundnut seeds as a result of the feeding activities of the *T. granarium.* All rates of treatment did not differ significantly ($p>0.05$) in mean percentage weight loss at 30, 60 and 90 DAT.

DISCUSSION

Extracts from different plants have been known to possess insecticidal properties against a wide range of insect pests (Abdullah and Muhammad, 2004). Other authors opined that

Table 1. Influence of *Psidium guajava* leaf oil on larval and adult mortality of *Trogoderma granarium* in stored groundnut

Treatment (ml/25 g seeds)	Percentage larval mortality of *Trogoderma granarium* (DAT)					Percentage adult mortality of *Trogoderma granarium* (DAT)				
	1	2	3	4	5	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	6.67	13.3	20.0
0.2	0.0	0.0	6.7	6.7	20.0	13.3	26.7	33.3	40.0	53.3
0.3	20.0	26.7	40.0	53.3	53.3	20.0	26.7	40.0	40.0	53.3
0.4	40.0	46.7	46.7	60.0	66.7	20.0	26.7	60.0	66.7	73.3
0.5	40.0	86.7	93.3	93.3	100.0	80.0	86.7	93.3	93.3	93.3
F-Value	12.30	32.30	15.66	15.32	8.57	15.34	28.41	19.58	10.04	8.31
$LSD_{(0.05)}$	0.64	0.72	1.07	1.13	1.30	1.24	0.95	1.15	1.49	1.49

DAT = Days after treatment LSD = Least Significant Difference at 5% level of probability

Table 2. Comparison of percentage mortality of *Trogoderma granarium* larvae and adults in groundnut seeds exposed to *Psidium guajava* leaf oil

Stage of *T. granarium*	DAY ONE				
	0.0	0.2	0.3	0.4	0.5
Larvae	0.0	0.0	15.0	30.0	30.0
Adults	0.0	13.4	20.0	20.0	80.0
	DAY TWO				
Larvae	0.0	0.0	20.0	35.0	65.0
Adults	0.0	26.6	26.6	26.6	86.6
	DAY THREE				
Larvae	0.0	5.0	30.0	35.0	70.0
Adults	6.6	33.4	40.0	66.0	93.4
	DAY FOUR				
Larvae	0.0	5.0	40.0	45.0	70.0
Adults	13.4	40.0	46.0	66.0	93.4
	DAY FIVE				
Larvae	5.0	15.0	40.0	50.0	75.0
Adults	20.0	53.4	53.4	73.4	93.4

Effect of *P. guajava* oil on larval emergence of *T. granarium* and seed weight loss

Table 3 shows the mean percentage larval emergence of *T. granarium* in groundnut seeds treated with *P. guajava* leaf oil. The analysis of variance revealed that there was significant difference ($p<0.05$) in the mean percentage of *T. granarium* larvae that emerged from groundnut seeds treated with the oil at 30, 60 and 90 DAT. At 30 DAT, the seeds treated with 0.3 ml of *P. guajava* oil had more larval emergence (30.0%) when compared with those treated with 0.2 ml (45.0%), though the difference between the treatments was not significantly different ($p>0.05$). Similarly, the seeds treated with 0.3 ml of *P. guajava* oil had significantly lower mean percentage larval emergence when compared with those treated with higher rates

plants offer a cheaper sustainable alternative to synthetic insecticides, store design, fumigation and thermal distribution methods (Mukanga *et al*., 2010). In the laboratory study, it was found that *P. guajava* leaf oil had faster and greater insecticidal action against adults than larvae of *T. granarium.* The adult mortality recorded in the treated seeds at varying levels of treatment was significantly better than the control. This study also shows that there was an improvement in the potency of rates of treatment with increase in exposure period of the infested seeds to the oil. The results obtained showed that the use of *P. guajava* leaf oil in storing groundnut against *T. granarium* was effective as significant difference occurred between the treatments and the control. The oil had effect on the larvae since there was reduction in seed weight loss of treated groundnut caused by the immature insect.

Table 3. Influence of *Psidium guajava* leaf oil on mean percentage larval emergence and seed weight loss

Treatment (ml/25 g seeds)	Mean percentage larval emergence (DAT)			Mean percentage seed weight loss (DAT)		
	30	60	90	30	60	90
0.0	45.0	75.0	75.0	14.78	15.16	17.06
0.2	45.0	75.0	75.0	13.52	14.73	15.82
0.3	30.0	45.0	45.0	12.93	13.64	15.56
0.4	5.0	40.0	45.0	12.15	12.44	14.31
0.5	5.0	20.0	25.0	11.97	12.38	14.27
F-Value	4.24	5.26	3.98	2.68	2.72	3.01
$LSD_{(0.05)}$	1.57	1.57	1.64	NS	NS	NS

Table 4. Qualitative and quantitative analysis of phytochemical components of *Psidium guajava* leaf oil extract

Component	Quality	Quantity (mg/100 g)
Flavonoids	++	2.50
Alkaloids	+	0.74
Steroids	++	2.05
Triterpenoids	++	2.18
Cynogenic glycosides	+++	4.15
Lipids	+++	4.70
Carbohydrates	+++	64.01
Tannins	-	-
Saponins	-	-
Oxalate	-	-
Phenols	-	-

+ = Present - = Absent

The insecticidal properties of the guava leaf oil might be connected with the phytochemical constituents such as alkaloids, flavonoids, steroids and triterpenoids. Morales *et al.* (1994) reported that the leaf extract of *P. guajava* contained flavonoids, mainly quercetin derivatives. These components may be having the role of biochemical defences or protection against *T. granarium*. Chen and Yen (2007); Ogunwale *et al*. (2003) confirmed the presence of β-caryophyllene, and α-pipene and 1,8-cineole as major compounds in guava leaf essential oil. In a related investigation, Sami and Shakoori (2007) reported the presence of tannins in leaf extract of guava tested against several insects. The high mortality rate of adults (73.4%) in 0.4 ml rate of treatment revealed that the leaf oil has potential for short-term storage of groundnut seeds against *T. granarium* adults. This consequently had effect on reproductive ability of the insect thus reducing larval emergence.

The feeding ability of the larvae is depicted by the seed weight loss recorded despite the treatment. Seed weight loss could be associated with the larvae of *T. granarium.* Results from this study showed that *P. guajava* leaf oil had high mortality effect against larvae and adults of *T. granarium*. In previous researches it was reported that seed oils were faster in action than powder (Anene, 2005). Efforts are therefore, being made to test the effect of *P. guajava* seed oil on the insect. Infestation of the seeds by the beetle resulted in characteristic holes and tunnels leading to qualitative and quantitative seed losses. Groundnut seeds infested with *T. granarium* larvae caused 11.97% seed weight loss irrespective of rate of treatment and exposure time. It was observed that the immature stage exerted its influence on the rich nutritional components and soft kernel coat of the seeds. Groundnut is an important source of plant protein and cooking oil in Nigeria, besides being a vital requirement in animal feed formulation. The feeding activities of the larvae caused a reduced weight loss in seeds treated with higher than lower rate of the guava leaf oil and control even though there was no significant difference among the various treatments and the control. It is therefore imperative to prevent insect infestation to achieve sustainable preservation of agricultural produce.

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