



Comparative Study of *Psidium guajava* and *Ocimum gratissimum* Leaf Oils on Adult Maize Weevil (*Sitophilus zeamais* M.)

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Abstract

A laboratory study was conducted to determine the efficacy of *Psidium guajava* and *Ocimum gratissimum* leaves oil for *Sitophilus zeamais* control. The experimental design was a completely randomized design with three replications. The leaf oil was extracted using soxhlet extractor. The leaves oil at four levels of concentration was mixed with 20 g of disinfested maize in 2 by 5 cm container. All the treatments with the leaves oil showed significant level of toxicity to the weevil. The highest concentration (4%) of the leaves oil of the plant material tested induced the highest mortality in the *S. zeamais* after 6days. Grains treated with the leaves oil extract significantly reduced the number of progeny emerged of both F1 and F2 respectively by *S. zeamais*, and evoked low weight loss and seed damage against the weevil on grains treated with the highest dosage of the leaves oil extract. *P. guajava* was found to be most effective recording 100% mortality of *S. zeamais* after 5days, lower LC₅₀ value(1.94µml/l), lower F1 and F2 emergence (0.000±0.000), lower %weight loss (1.00%), lower %seed damage(11%) and higher viability(50%) which compared favourably with that recorded in positive control. Further studies should be conducted to find out the most bioactive ingredient of those plant leave oils.

Keyword: Emergence, Leaf oil, Mortality, *S. zeamais*, Viability, Weight loss.

INTRODUCTION

Maize (*Zea mays* L.) belongs to the family Gramineae (IITA, 2005) and it's presently recognized as one of the most important food crops cultivated in tropical countries (Asawalam and Hassanali, 2006). Maize is a widely adopted crop capable of producing during the appropriate season in almost all parts of the world where farming is done (Kyenpia *et al.*, 2009) and its the third most important food crop in the world surpassed only by two other grains, wheat and rice (Kyenpia *et al.*, 2009; IITA, 2005). It's one of the major staple food crops in West and Central Africa. These sub-regions have the greatest potential such as adequate moisture, abundant sunshine and relatively fertile soils for maize production (Badu-Apraku *et al.*, 2006). Essential oils and individual compounds from medicinal and aromatic plants have been known to exhibit anti-feedant properties against a number of insects (Huang *et al.*, 2000; Koschier *et al.*, 2002). Essential oils have recently attracted particular attention as alternative pest control agents because of their specificity of

action against insect pests, biodegradable nature, and potential for commercial application (Park *et al.*, 2003). Increases in oil concentration from leaves of *Mentha longifolia* L. resulted in an increase in maize weevil mortality and high repellency was reported for the oil at all concentrations tested, thus a potential product for protection of stored products against the maize weevil (Odeyemi, 2003).

The maize weevil, *Sitophilus zeamais* (Curculionidae: Coleoptera) is a primary, field-to-store pest of maize. The adults attack whole grains and larva feeds and develop entirely within grain (Storey, 1987). According to Nukenine *et al.* (2002) and Ngamo *et al.* (2004), the maize weevil, *Sitophilus zeamais* (Motsch.) is the most important post-harvest insect pest causing severe damage to stored maize grain in the tropics and it also result in total damage of the grain kernels (Ileleji *et al.*, 2004). To prevent such losses however, most small holders rely on the use of synthetic insecticides; but the high cost, toxicity to non-target organisms, inherent environmental hazards and the development of resistance by

insect pests have limited their effective use for maize storage (Al- Moajel, 2006). In order to avert the use of these synthetic chemical insecticides, research studies have been focused on a suitable alternative control measures such as plants with a natural insecticidal activity, easy to use, biodegradable alternative that will be safe effective, and safe to human health and the environment (Arannilewa *et al.*, 2006). Based on this, the present study was carried out to evaluate the potentiality of leaves oil from *Psidium guajava* and *Ocimum gratissimum* plant leaves that are readily available within a home, road site, and farms and have been used traditionally by the rural farmer in protecting stored product from insect pest infestation.

MATERIALS AND METHODS

Experimental Site

This study was carried out at Insectory Laboratory, Department of Biological Sciences, Gombe State University, Gombe under ambient condition of temperature (28 ± 2 °C) and relative humidity of $75 \pm 5\%$.

Collection, Identification and Processing of Plant Materials

Psidium guajava leaves were collected in Gombe metropolis along Biu road, and *Ocimum gratissimum* was collected at Gombe State University Staff Quarters Gombe State, Nigeria. They were identified and authenticated at Department of Plant Biology, Bayero University, Kano. The voucher specimen was numbered and kept in the herbarium for further reference. Healthy leaves of the *P. guajava* and *Ocimum gratissimum* that were collected identified and washed properly under running tap water followed by distilled water, the Leaves were then air dried separately in the laboratory for 7-14days under a room temperature. The completely dried leaves of *psidium guajava* was ground with pestle in a mortar and sieved to get a fine powder of the leaves (Epidi *et al.*, 2009). The sieved powder particle was kept in a glass bottle until required for extraction.

Extraction of oil from plants materials

The leaves oil was extracted from the plants powder using soxhlet apparatus described by (Ahmed *et al.*, 2004) with modification. One hundred and sixty(160g) of pulverized sample was wrapped in a plain white sheet of paper and then put in the thimble-holder of the soxhlet apparatus compartment. The condenser was connected to a chiller for the recycling of the cold water and stream during the process. 300 ml

of the solvent (n-Hexane) was added with the aid of a funnel by passing it through the thimble containing the sample to the round bottom flask system of the soxhlet. The process was reiterated flow after flow until target oil extracted inside the soxhlet apparatus is accomplished for 5 hrs at 65-70°C.

Collection and Processing of Maize Seeds

The maize grains variety was obtained from IITA, Kano State (99%EDTV) and damage maize seeds were excluded. The undamaged seeds were placed in a plastic vial and stored temporarily in a deep freezer at -20 °C for 24 hrs to eliminate insipient infestation of insects (Adedire and Ajayi, 2003). The seeds were removed from freezer and kept at room temperature for one hour to equilibrate. The moisture content of the maize samples was determined before each laboratory experiment.

Insect Culture

Hundred (100) unsexed adult *S. zeamais* from the stock were introduced into a plastic container, sealed with a clean fine muslin cloth tight with rubber band containing 500 g of the infested maize grain. The insects were allowed to oviposit for 14 days before they were sieved out and the container was sealed again with the cloth to prevent possible escape and/or re-infestation. The F1 adults that emerged were introduced onto a sample of the test maize (Asawalam, 2006).

Laboratory Investigation

Effect of Leave Oil on Adult Mortality of *Sitophilus zeamais*

Adult mortality was assessed following the procedure described by (Asawalam *et al.*, 2007) and (Akinkurolere *et al.*, 2006) with modification was conducted. Four different concentrations (0.1, 0.2, 0.3 and 0.4 ml) were added to 10ml of n Hexane to give 1.0%, 2.0%, 3.0%, and 4.0% of the leaves Oil extract of *P. guajava* and were separately mixed with 20g of maize grain in 5cm diameter plastic container. The oil concentrations were thoroughly agitated to ensure uniform coating. Five (5) newly emerged unsexed adults *S. zeamais* were introduced into the containers and covered with the lid perforated by a net to allow proper circulation of air (Zapata and Guy, 2010). Untreated and Dichlorvos (1%) experiments were set up as the control experiments. All treatments were replicated three times and arranged in a completely randomized design. Mortality of the insects were observed and recorded at 24 hrs interval for 168 hrs (7days).

The weevils were confirmed dead when there is no response after probing the abdomen with sharp object (Adedire *et al.*, 2011). Percentage adult mortality was corrected using Abbott (1925) formula

$$P_T = \frac{P_o - P_c}{100 - P_o} \times \frac{100}{1}$$

Where PT = corrected mortality (%)
 PO = observed mortality (%)
 PC = control mortality (%)

Effect of Leaves Oil on Progeny Emergence

The experiment one above was observed for adult emergence after removal all the dead and alive insects from 32 days after initial infestation onward. The number of adults that emerged from each replicate were counted and recorded by direct examination of the grain with the aid of a dissecting microscope (Parugrug and Roxas, 2008).

Assessment of Damaged and Weight Loss of the Treated Maize Seeds

Damage assessment was carried out on treated and untreated grains after adult emergence using weighing balance (FAO, 1985). Samples of 50 grains were randomly taken from each container and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed after 42days. Percentage weight loss and percentage damage respectively was calculated using the formulae according to Baba-Tierro (1994).

% Grain weight loss = $\frac{\text{Initial weight of grain} - \text{Final weight of grain}}{\text{Initial weight of grain}} \times 100$

of grain

% Grain damage = $\frac{\text{Number of damaged grains}}{\text{Total number of grains}} \times 100$

Weevil Perforation Index (WPI) used by (Fatope *et al.*, 1995) quoted by (Arannilewa *et al.*, 2006) was adopted for the analysis of damage.

WPI = $\frac{\% \text{ treated maize grains perforated}}{\% \text{ control maize grains perforated}} \times 100$

WPI value exceeding 50 was regarded as enhancement of infestation by the weevil or negative protect ability of the leaves oil tested.

Effect of the plant extracts on the germination of the treated maize seeds

Ten (10) seeds were randomly selected from each treatment after 56days emergence and planted on moistened cotton wool in a petri dish. Germination count was taken on the 5th day according to the normal planting methods (Adedire *et al.*, 2011). Seed germination was thereafter determined and expressed as percentage of total grains planted as follows:
 % Germination = $\frac{\text{Number of grain that germinated}}{\text{Total number of grains planted}} \times 100$

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Statistical Analysis

Two ways Analysis of Variance (ANOVA), at P<0.05; Probit analysis were calculated from the data obtained from the experiment using Open-Stat statistical software (version 08.12.14) and Microsoft Excel 2007 was also used to calculate the percentage mortality and Standard Error (S.E). Least significant difference (LSD) at P<0.05 was used to separate the means.

RESULTS AND DISCUSSION

Effect of Various Plant Leave Oil on Adult Mortality of *S. zeamais*

The table 1 showed the effect of the test leave oil concentrations of *O. gratissimum* and *P. guajava* on mortality of adult *S. zeamais* in maize grains. It revealed that the highest concentration (0.4ml) of both test leave oil had the highest mean mortality of adult *S. zeamais* (80.00 and 100) respectively which was higher than the control (6.66 ± 0.33) after 6days post exposure time. The analysis of variance of the effects of the various test leaves oil concentrations on mortality of adult *S. zeamais* in treated maize grains showed that, there was significant difference between the mortality caused by the two plant leaves at P<0.05. *O. gratissimum* and *P. guajava* also have LC₅₀ values of NIL, and 1.94µml/L, respectively at 96hrs post treatment.

Effect of Leaves Oil on Adult Emergence

All the test oils extracts were observed to have potentials of reducing adult emergence (Table2). No progeny was produced in grains treated with the highest dose (0.4ml) in both plant leaves oil. The effect of these plant leave oil on adult emergence of *S. zeamais* treated on maize grains were significantly (p<0.05) different among the treatments and the control. Adult emergence decreased with increase in the amount of the test leave oil used.

Combined Result of Effect of n-Hexane Leave Oils Extract of *H. suaveolens*, *O. gratissimum* and *P. guajava* Treatment on Grain Weight Loss and Seed Damage

Weight loss caused by *S. zeamais* to treated and untreated grains is shown in table 3. Weight loss was dose dependent. The weight loss of 5.48% and 6.80% in the untreated control was greater than losses in the treated concentration of both the treatment at 28 days and 56 days post emergence respectively and there is no significant difference with the higher concentration (0.4ml) when compared with standard check (+ve control). However treatment at 0.1 ml/ 20 g grain also suffered significant weight loss compared with the grains treated with 0.4 ml of leaf oil extract after F1 Adult emergence. In the untreated grains, 46% damage occurred as revealed by emergent holes of the weevils. Weight loss in untreated control maize grains was significantly higher ($p < 0.05$) than in the treated maize grain and loss in weight is dose dependant two (2) month post treatment. After F2 emergence and Weevil Perforation Index (WPI) indicated that the plant oil used have significant effects as grain protectants (Table 3). Moreover, the efficacy was observed to be directly proportional to the amount of oil applied.

Effect of *O. gratissimum* and *P. guajava* Oil Extracts on the Viability of Treated Maize Grains

The percentage of maize grains that germinated after treatment with 0.4ml of both the oil extracts is presented in Table 4 at the end of 7 days viability period, all the treated grains recorded high viability. The untreated maize grains had the highest percentage germination of 80% for standard check; followed by the grains treated with *P. guajava* (50%). The least percentage germination was recorded in *O. gratissimum* (35%) extracts.

Table1: Percentage Mortality of *S. zeamais* Treated with Different Plant n-Hexane Oil Extract on Maize Grain

	Conc V/V (%)	No of inset used	%mortality In hours ± S.E							LC ₅₀ (µm/l)
			24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs	
<i>O. gratissimum</i>	0.1(1)	5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	13.33±0.33 ^{ab}	20.00±0.00 ^{abc}	53.33±0.66 ^{cd}	60.00±0.57 ^{ab}	NIL
	0.2(2)	5	0.00±0.00 ^c	0.00±0.00 ^a	13.33±0.33 ^{ab}	13.33±0.33 ^{ab}	33.33±0.88 ^{ab}	60.00±0.57 ^{de}	86.66±0.66 ^{bd}	
	0.3(3)	5	13.33±0.00 ^b	20.00±0.57 ^b	26.00±0.88 ^b	33.33±0.33 ^{abc}	40.00±0.57 ^{abc}	66.66±0.33 ^{de}	86.66±1.20 ^{bd}	
	0.4(4)	5	13.33±0.00 ^b	20.00±0.57 ^b	33.33±0.33 ^b	46.66±0.33 ^{bcd}	46.66±0.88 ^{ab}	80.00±1.00 ^{de}	93.33±0.33 ^d	
<i>P. guajava</i>	0.1(1)	5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	6.66±0.33 ^{ab}	13.33±0.33 ^{abc}	26.66±0.33 ^{ab}	1.94
	0.2(2)	5	0.00±0.00 ^a	6.66±0.33 ^{ab}	20.00±0.57 ^{ab}	33.33±0.66 ^{abc}	40.00±0.57 ^{abc}	46.66±0.66 ^c	46.66±0.66 ^{abd}	
	0.3(3)	5	0.00±0.00 ^a	13.33±0.66 ^{ab}	26.66±0.88 ^b	46.66±0.33 ^{bcd}	53.33±0.88 ^{bc}	66.66±0.88 ^{de}	93.33±0.33 ^d	
	0.4(4)	5	6.66±0.33 ^b	13.33±0.33 ^{ab}	26.66±0.33 ^b	53.33±0.66 ^{cd}	93.33±0.57 ^d	100±0.00 ^e		
Control (-Ve)	0.0(0)	5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.66±0.00 ^a	1.66±0.00 ^a	
Control (+Ve)	0.1(1)	5	100±0.00 ^c	-	-	-	-	-	-	
LSD(0.05)			11.18	18.87	25.45	39.30	52.84	49.28	67.25	

%mean mortality± S.E with the same letter are not significantly differ by LSD

Table 2: Effect of n-Hexane Oil Extract on Adult Emergence of *S. zeamais* from Treated Maize Grain

Treatment	Concentration V/V (%)	Weight of maize (g)	No of insect used	Mean Adult emergence	
				F1	F2
<i>O. gratissimum</i>	0.1(1)	20	5	1.33±0.0003 ^d	1.03±0.0005 ^{ab}
	0.2(2)	20	5	1.00±0.0003 ^c	0.50±0.0006 ^a
	0.3(3)	20	5	1.33±0.0005 ^d	1.00±0.0003 ^{ab}
	0.4(4)	20	5	0.00±0.000 ^a	0.67±0.0003 ^a
<i>P. guajava</i>	0.1(1)	20	5	1.33±0.0006 ^d	1.32±0.0006 ^b
	0.2(2)	20	5	0.67±0.0003 ^b	0.33±0.0003 ^a
	0.3(3)	20	5	0.67±0.0006 ^b	0.67±0.000 ^b
	0.4(4)	20	5	0.00±0.000 ^a	0.00±0.000 ^a
Control (-Ve)	0.0(0)	20	5	1.67±0.0003 ^e	5.00±0.001 ^c
Control (+Ve)	0.1(1)	20	5	0.00±0.000 ^a	0.00±0.000 ^a
LSD(0.05)				0.31	1.05

Mean Adult emergence ± S.E with the same letter in the same column are not significantly different by LSD P<0.05

Table 3: Assessment of % Weight Loss of the Treated Maize after F1 Adult Emergence at 28 days Post Treatment

Treatment	Concentration V/V (%)	No of insect used	Mean Initial weight of maize grain(g)	Mean Final weight(g)	Mean weight Loss \pm S.E	% weight loss
<i>O. gratissimum</i>	0.1(1)	5	20	19.67	0.33 \pm 0.08 ^{abc}	1.65
	0.2(2)	5	20	19.80	0.20 \pm 0.05 ^a	1.00
	0.4(4)	5	20	19.95	0.05 \pm 0.03 ^a	0.25
<i>P. guajava</i>	0.1(1)	5	20	19.36	0.64 \pm 0.08 ^{bc}	3.20
	0.2(2)	5	20	19.46	0.54 \pm 0.43 ^{bc}	2.70
	0.3(3)	5	20	19.60	0.40 \pm 0.20 ^{abc}	2.00
	0.4(4)	5	20	19.96	0.04 \pm 0.03 ^a	0.20
Control(-Ve)	0.0(0)	5	20	19.26	0.74 \pm 0.08 ^c	3.70
Control (+Ve)	0.1(1)	5	20	20.00	0.00 \pm 0.00 ^a	0.00
LSD(0.05)					0.52	

Table 4 Assessment of % Weight Loss and Seed Damage of the Treated Maize after F2 Adult Emergence at 56days Post Treatment

Treatment	Concentration V/V (%)	No of insect used	Initial weight of maize grain	Final weight(g)	Mean weight Loss \pm S.E	% weight loss	% damage of grain	WPI
<i>O. gratissimum</i>	0.1(1)	5	20	19.15	0.85 \pm 0.14 ^{cbde}	4.25	36	42.30
	0.2(2)	5	20	19.85	0.15 \pm 0.08 ^a	0.75	29	37.17
	0.3(3)	5	20	19.86	0.14 \pm 0.08 ^a	0.70	14	22.22
	0.4(4)	5	20	19.87	0.13 \pm 0.05 ^a	0.65	11	18.33
<i>P. guajava</i>	0.1(1)	5	20	19.00	1.00 \pm 0.02 ^{de}	5.00	42	47.72
	0.2(2)	5	20	19.20	0.80 \pm 0.21 ^{cbde}	4.00	41	47.12
	0.3(3)	5	20	19.60	0.40 \pm 0.13 ^{ab}	2.00	20	30.33
	0.4(4)	5	20	19.72	0.28 \pm 0.03 ^a	1.00	11	19.29
Control(-Ve)	0.0(0)	5	20	18.64	1.36 \pm 0.39 ^c	6.80	46	50
Control (+Ve)	0.1(1)	5	20	19.84	0.16 \pm 0.03 ^a	0.80	0.00	0.00
LSD(0.05)					0.59			

Mean weight loss \pm S.E with the same letter in the same column are not significantly different by LSD P<0.05

Table 5: Assessment of Seed Germinability of Treated Maize Seed

Treatment	No of seed planted	Mean no of seed germinated	% germinability
<i>O. gratissimum</i>	10	3.5	35
<i>P. guajava</i>	10	5.0	50
Control -Ve	10	4.3	43
Control +Ve	10	8.0	80

DISCUSSION

Effect of Plant Leave Oil on Adult Mortality of *S. zeamais*

The result of the present study revealed that the leaf oil of *O. gratissimum* and *P. guajava* could serve as poison against adult *S. zeamais*. The mortality of *S. zeama is* in the maize grains treated with various concentrations of *O. gratissimum* and *P. guajava* leaf oil was found to be dose dependent. Similarly, Akunne *et al.*, (2014) reported that the efficacy of root powder of *L. cyanescens* on adult *S. zeamais* in maize grains was dose dependent. In all the treated maize grains, the activities of weevils were greatly reduced. This is an indication that the plant oil extracts have actually controlled the weevils. Iram *et al.* (2013) reported that *P. guajava* leaves and fruit peels showed promising effects of seed protection and insecticidal properties against *Tribolium castaneum* Herbst and also Guava leaf oil has been reported as a wheat grain protectant against *S. oryzae* (Akunne *et al.*, 2014e). Ileke and Bulus, (2012); Kerdchoechuen *et al.*, (2010) tested the toxicity effect of plants extract using, *Ocimum lenuiflorum*, *Citrus aurantifolia*, *Ocimum basilicum*, *Citrus hystrix*, and *Eugenia caryophyllus* against *Sitophilus zea mais* and the finding from their studies shows that 96% percent mortality was achieved by *Ocimum basilicum* at 72 hours after treatment which is similar with the present study. At 6 days a total control was achieved with the highest concentration of *P. guajava* oil extract this agree with investigation conducted to compare the products of *A. sativum* and *C. citratus* in the control of *C. maculatus* on stored cowpea grains by Oparaeke and Dike (1996). They found that both of the plant powders

showed effectiveness by exhibiting 100% mortality 7 days after treatment.

Effect of Leave Oils from Various Plants on Adult Emergence

Highly significant difference on the emergence of adults *S. zeamais* on treated and untreated maize indicates that insecticidal materials tested had significant effects on the developmental stages which in turn affected emergence. In the present study, the toxic action of the *O. gratissimum* and *P. guajava* oil is also reflected in the growth and development of eggs, which corresponds to decreased numbers of F₁ and F₂ progeny that emerged in treated grains. Similar results were obtained in experiments that involved treating maize with the essential oil of *Ocimum kenyense* (Bekele *et al.*, 1997). Asawalam and Emosairue (2006) and Asawalam *et al.* (2007) recorded 10.0% and 5.0% adult emergence of *S. zeamais* on maize treated with the same plant powder respectively. *C. frutescens* and *N. tabacum* were also reported to affect adult emergence of *S. zeamais* on maize grains revealing 10.0% and 12.0% emergence respectively (Asawalam *et al.*, 2007) which is higher than the present study.

Effect of Leaves Oil on Weight Loss and Seed Damage by *S. zeamais* on Maize

Treatment of maize grain with *O. gratissimum* and *P. guajava* extracts on *S. zea mais* resulted in significant reduction of percentage weight loss. The result of an earlier study by Okonkwo and Okoye, (1996) showed that percentage weight loss was related to the population of adult *S. zeamais*.

In terms of grain damage, minimal damage from weevils was recorded in the grains treated with the three plant oil extracts. Grain damage was very low in grains treated with *P. guajava* oils. The low values recorded can be attributed to the high mortality of the weevils to the plant extract application since low numbers of weevils are ineffective in causing substantial damage in storage. This agrees with the report of Abraham (1991) which indicated that the extent of damage during storage depends upon the number of emerging adult weevils during each generation and the duration of each life cycle. Grains that had more adult maize weevil emergence were more seriously damaged.

Effect of Leaves Oil on Germinability of Maize Grain

Seed viability post-treated with the extracts showed that the treatments did not negatively affect seed germination. This agrees with the report of Adedire *et al.*, (2005) which gave no

significant differences in viability of seeds post-treated with 1% and 4.0% of four plant extract concentrations and the control.

Conclusion

The results from the experiments conducted showed that at 7 days after infestation with *S. zeamais*, *P. guajava* was the most effective with the lower LC₅₀ (1.94µml/L) amongst the treatments. At 28days and 56days after infestation with *S. zeamais*, there was a significant differences in F1 and F2 Adult emergence at p<0.05 with the untreated control. The highest percentage damage and percentage weight loss amongst the treatments dependant on the concentration and the number of adult emergence. The result also, revealed highest percentage germination in *P. guajava* among the plant oil tested. Materials from these plants could be used to control pests and thus substitute other expensive synthetic pesticides.

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