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## Potentials of Three Plants Leave oils mixture in Protecting Maize Grains against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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### Abstract

Over the last decade, *Sitophilus zeamais* are being control using synthetic insecticides. The over-use of these chemicals has led to the development of insecticides resistance, environmental pollution, and killing of non-target organisms. The efficacy of *Hyptis suaveolens*, *Ocimum gratissimum*, and *Psidium guajava* leaf oils combination against adult *S. zeamais* was investigated on maize grain during the study. The leaves of the plants were grounded into powders and oils were extracted using Soxhlet apparatus separately. Maize variety 99% EDVT used was obtained from the International Institute for Tropical Agriculture (IITA) Kano. The insects were cultured in a containers and identified using a standard taxonomic key for *Sitophilus spp.* Different oils combination of two plants leaves oils were made in the ratio of 50%:50% of the test dose 0.1, 0.2, 0.3, and 0.4ml/20g. Ten newly emerged *S. zeamais* were introduced separately for examination of adult mortality test and lethal concentration determination. Results showed that mortality increases with exposure period. The leaves oils combination show a significant difference in adult mortality of *S. zeamais* ( $P < 0.05$ ). Isobutylcyclohexane, Oleic Acid and 11-octadecenoic acid were the most occurring compound present in the individual plant extract. *H. suaveolens* and *O. gratissimum* combination protecting maize grains against *S. zeamais* infestation and therefore, the active ingredient of these plant combinations should be evaluated.

**Keywords:** Gas Chromatography-Mass Spectroscopy (GS-MS), Maize, Mortality, Plant leaves,

### INTRODUCTION

The maize weevil *Sitophilus zeamais* (Motsch.) is one of the most important post-harvest insect pest causing severe damage to stored maize grain in the tropics and it also results in total damage of the grain kernels (FAO, 2005). The insect has a wide host range with a high capacity to penetrate grain mass and accounts for about 50% of loss in stored maize (<https://eduproject.com.ng> retrieved 7<sup>th</sup> June, 2021). Abraham (1991) reported that the extent of damage during storage depends upon the number of emerging adult during each generation and the duration of each life cycle and seeds permitting more rapid and higher levels of adult maize weevil emergence will be more seriously damaged. Maize weevils can consume as much as 15 % of the harvest in some months and have the ability to reduce maize quality (Bergvinson, 2004). Maize damage by *S. zeamais* causes food loss, increased poverty, and lower nutritional values of grain, increased malnutrition, reduced weight, and market values (Keba and Sori, 2013). *Sitophilus zeamais* destroys seeds kept for planting in subsequent season(s). Similarly, the pest reduces germination percentage and maize

production as most farmers in developing countries store grain and seed together (Pingali and Pandey, 2001). The weevils were responsible for causing more than 20% weight loss of hybrid maize stored in traditional structures, 40% loss due to poor post-harvest storage, and 80% loss on farm stores in tropics (Gerald, 2008). Small holders could loss 80% of their stock because of insects after 6 to 8 months of storage (Nukenine, 2002) and grain weight loss of 20-90% due to maize weevil for untreated maize in tropical countries (Muzemu *et al.*, 2013).

Chemical control includes the use of insecticides to prevent or manage insect infestations. They have proven to be the simplest and most cost-effective means of dealing with the pest. Even though synthetic chemicals continue to play important role in reducing storage losses due to insect pest activities, they are un-friendly as a result of their toxic residues in food and environmental pollution, adverse effects on beneficial and non-target insects, increased risk to workers safety and the high cost of the chemicals (Aswalam, 2006; Niber, 1994). Thus, there is an urgent need to develop new alternatives which

will be safe, low cost and eco-friendly insect pest control methods to avoid the hazards of chemical insecticides. However, botanical insecticides can be an alternative. These compounds of plant materials affect insect populations by reducing their developmental, survival, and reproductive rates (Carlini and Grossi-de-sa, 2002). The present study was therefore chosen to investigate the combined insecticidal potential of oils from the leaves of *H. suaveolens*, *O. gratissimum* and *P. guajava* against adult *S. zeamais*.

## MATERIALS AND METHODS

### Study Area

The experiment was carried out at Entomology Laboratory, Department of Biological Sciences, Gombe State University, Gombe for the period of six months (6) between February - July 2016 at latitude 12° 8' and 10° 24' N longitude 11° 22' and 11° 24' under ambient condition of temperature (28±2°C) and relative humidity (70-75%).

### Plants and Extraction of Leave Oils

Leaves of *H. suaveolens*, *O. gratissimum* and *P. guajava* were collected in along Dadin Kowa Dam, Gombe. Leaves of the plants were washed and air dried separately at room temperature for 14 days. The dried leaves were grounded with a pestle in a mortar and sieved using 80µm laboratory sieve to get fine powder particles (Efidi *et al.*, 2009). One hundred and sixty grams (160 g) of powdered plant materials was wrapped in a filter paper and then put in the thimble-holder of the Soxhlet apparatus compartment. Chiller was connected to a hose respectively for the recycling of the cold water and steam during the process. Two hundred and fifty ml (250 ml) of the solvent (n-Hexane) was added. The targeted oil was extracted for five hours (5hrs) at 60-80 °C and subsequently stored in a refrigerator (4 °C) until used (Ahmed *et al.*, 2004).

### Insect Culture

One hundred (100) of both male and female of undetermined age of adult *S. zeamais* from the stock were introduced into three (3) litre plastic container, containing five hundred grams (500 g) of the disinfested maize grain and then sealed with a clean fine muslin cloth and tight with rubber bands. The insects were allowed to oviposit for ten (10) days before they were sieved out and the container was sealed again with the cloth to prevent possible escape new emergence and/or re-infestation.

The F<sub>1</sub> adults that emerged were used for the experimental test (Aswalam, 2006).

### Adult Weevil Bioassay

Four different concentrations of the combined plant leaf oils was made by mixing the individual plant leaf in the following ratio of 0.05: 0.05; 0.1:0.1, 0.15:0.15 and 0.2:0.2 ml. Ten (10) ml of n-Hexane was separately mixed with 20g of maize grain in plastic container (5cm x 5cm x 3cm). Positive control (Dichlovos) and negative control were also set along the treatments. The oil was thoroughly agitated to ensure uniform coating. Ten (10) newly emerged unsexed adults of *S. zeamais* were introduced separately into the containers (5cm x 5cm x 3cm), and covered with the lid perforated by a needle to allow proper circulation of air (Zapata and Guy, 2010). All treatments were replicated three (3) times and arranged in a completely randomized design (CRD). The mortality of the insect was observed and recorded. The weevils were confirmed dead when there is no response after probing the abdomen with a sharp object (Adedire *et al.*, 2011).

### GC-MS Analysis of the individual Plant Leaf Oils

Gas chromatographic (GC) analyses were performed on a capillary gas-chromatograph (GCMS-QP2010 plus Shimadzu, Japan) equipped with a split-less capillary injector system. The integrator was used to calculate the peak areas. The carrier gas was n-Hexane at a flow rate of 6.2 ml/min. The temperature program comprised of initial temperature of 80°C (0 min) to 200°C a hold at this temperature for 1 min, then to 4 min followed by another hold for 5 min, and finally to 280°C at 4°C/min where it was maintained for 3.0 min. The sample (8µl) was injected with a split ratio of 1:0. The MS had a scan cycle of 1.5 s (scan speed 1250). The mass and scan range were set at m/z 40.00 and 600.00, respectively. Preliminary identification of constituents was based on computer matching components of mass spectral data against the standard NIST library spectra.

### Statistical Analysis

Data were subjected to two way Analysis of Variance (ANOVA) to find out the differences among the activity of the plant extracts using LSD Test at P <0.05 level of significance. The Lethal Concentration (LC<sub>50</sub>) was calculated using Probit analysis. All the statistical analyses were carried out using Open-Stat statistical software (version 08.12.14) and the results were represented as mean ± standard error.

**RESULTS**

**Adult Mortality of *S. zeamais***

The treatment combination of 0.15:0.15ml of *H. suaveolens* and *O. gratissimum* and *P. guajava* and *H. suaveolens* recorded complete adult mortality (100%) at 72hrs post-exposure period. The result indicated that none of the oil

was comparable to the chemical control (Dichlorvos) treatment but all the leaves oils were observed with varying activities resulting in adult mortality. All the plant powder showed significant difference  $P < 0.05$  adult mortality against *S. zeamais* (Table 1).

**Table 1: Adult Mortality of *S. zeamais* Treated with Combined Plant Leaves Oils.**

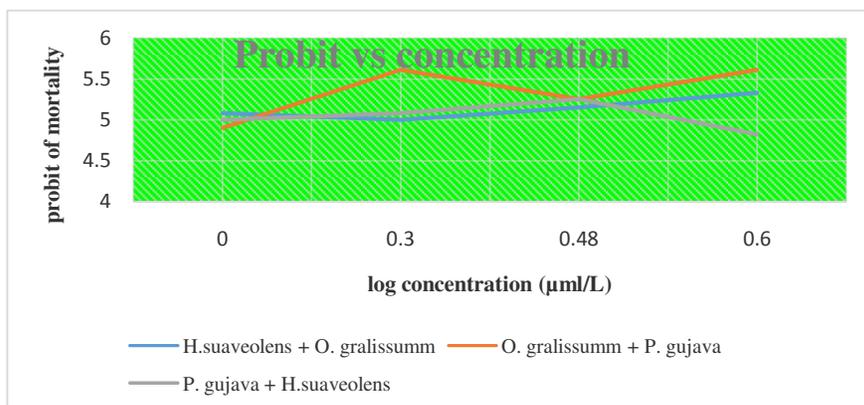
Treatment	Conc. Of Mixture (ML: ML)	Weight of maize (g)	No of insect used	% mortality (Mean ±S.E) In hours		
				24hr	48hr	72hr
<i>H. suaveolens</i> + <i>O. gratissimum</i>	0.05:0.05	20	10	6.70±0.66 <sup>a</sup>	60.00±0.57 <sup>ab</sup>	93.30±0.00 <sup>b</sup>
	0.1:0.1	20	10	10.00±0.00 <sup>a</sup>	50.00±1.15 <sup>ab</sup>	96.70±0.33 <sup>b</sup>
	0.15:0.15	20	10	26.70±0.33 <sup>a</sup>	56.70±0.33 <sup>abb</sup>	100.00±0.00 <sup>b</sup>
	0.2:0.2	20	10	23.30±1.85 <sup>a</sup>	63.30±0.33 <sup>ab</sup>	100.00±0.00 <sup>b</sup>
<i>O. gratissimum</i> + <i>P. guajava</i>	0.05:0.05	20	10	13.30±0.33 <sup>a</sup>	33.30±0.33 <sup>ab</sup>	93.30±0.66 <sup>b</sup>
	0.1:0.1	20	10	16.70±0.66 <sup>a</sup>	56.70±1.33 <sup>ab</sup>	96.70±0.33 <sup>b</sup>
	0.15:0.15	20	10	0.00±0.00 <sup>a</sup>	66.70±1.52 <sup>ab</sup>	90.00±0.57 <sup>b</sup>
<i>P. guajava</i> + <i>H. suaveolens</i>	0.2:0.2	20	10	6.70±0.33 <sup>a</sup>	73.30±1.20 <sup>ab</sup>	100.00±0.00 <sup>b</sup>
	0.05:0.05	20	10	6.70±0.66 <sup>a</sup>	50.00±0.57 <sup>ab</sup>	96.70±0.33 <sup>b</sup>
	0.1:0.1	20	10	13.30±0.33 <sup>a</sup>	53.30±1.20 <sup>ab</sup>	86.70±0.88 <sup>b</sup>
	0.15:0.15	20	10	20.00±0.57 <sup>a</sup>	60.00±1.15 <sup>ab</sup>	100.00±0.00 <sup>b</sup>
	0.2:0.2	20	10	13.30±0.66 <sup>a</sup>	43.30±0.33 <sup>ab</sup>	86.70±0.88 <sup>b</sup>
Control (-Ve)	0:0	20	10	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Control (Dichlovos)	0.1	20	10	100±0.00 <sup>b</sup>	-	-
LSD (0.05)				<b>36.50</b>	<b>61.50</b>	<b>30.90</b>

Values with the same letter in the same column are not significant difference by LSD  $P < 0.05$

**Least Effective Concentrations**

Based on the  $LC_{50}$  value recorded, the treatment combination of *H. suaveolens* and *O. gratissimum* at 48 hours has the lowest

(4.44 $\mu$ ml/L) value compared with the other treatment combination (Figure 1).



**Figure 1:  $LC_{50}$  values of *H. suaveolens* + *O. gratissimum*, *O. gratissimum* + *P. guajava* and *P. guajava* + *H. suaveolens* against *S. zeamais* at 48 hours of Post-exposure GC-MS Analysis of the experimental leaf oils**

The analysis of the leaf oils of the individual plants revealed a complex mixture of constituent's compound of *H. suaveolens*, *O. gratissimum* and *P. guajava*. A total of 32 compounds were identified in Leave oils composition of *H. suaveolens* plant, with Isobutylcyclohexane recorded the highest percentage composition of 13.09% followed by n-Decane (10.37) and the lowest (0.54%) was Naphthalene (Table 2). The oil composition of *O. gratissimum* from comparison of mass spectra of individual constituents and with NIST

data through GC-MS led to the identification of 12 compounds. The highest percentage composition was recorded (18.38 %) in Oleic acid (9) while the least compound was cis-2- $\alpha$ -bisabolene (4) with 0.77% (Table 3). From GC profile of *P. guajava* leaves oil constituents present were identified by GC-MS and co-injected with the standards. The highest percentage was recorded at 18.74% in 11-octadecenoic acid and the lowest (0.71%) was Caryophyllene oxide (Table 4).

**Table 2: Chemical Constituents of *H. suaveolens* Leaf Oil and their Relative Proportion**

Peak	Compound	Retention index	Retention time	%Chemical Composition	Molecular Weight
1	4-methyltridecane	1349	3.061	9.32	198
2	Cyclohexane	941	3.234	5.21	126
3	Isobutylcyclohexane	1015	3.473	13.09	140
4	4-Methylnonane	951	3.749	7.43	142
5	n-Decane	1015	4.203	10.37	142
6	4- methyldecane	1051	4.489	4.57	156
7	2,6,10,14-tetramethylheptane	1852	4.639	3.51	296
8	Dodecanoic acid 2- penten-1-ylester	1886	4.704	2.54	268
9	Dodecylester	1375	4.937	2.34	332
10	Methylundecane	1150	5.035	2.42	170
11	3,7-dimethylnonane	986	5.116	2.13	156
12	Undecane	1115	5.524	6.84	156
13	Trans-pinane	937	6.092	1.60	138
14	Propanoic acid	1501	6.398	1.87	204
15	Dodecane	1214	6.906	2.86	170
16	2,6-Dimethylundecane	1185	7.094	1.21	180
17	2,7,10-Trimethyldodecane	1320	7.910	1.48	212
18	n-Tridecane	1313	8.288	2.05	184
19	4,6-dimethyldodecane	1285	9.317	1.00	198
20	n-hexadecane	1612	9.623	2.09	226
21	Cis- $\alpha$ -bisabolene	1518	10.142	1.04	204
22	3,7-dimethyldecane	1086	10.420	0.77	170
23	1-Decen-3-yne	1222	12.230	1.28	164
24	Palmitic acid	1968	17.979	1.87	256
25	Naphthalene	1909	19.008	0.54	272
26	Isopropyl-1,1,4a-tri	2004	19.669	0.85	270
27	9-Octadecanoic acid/Oleic acid	2175	20.811	2.78	282
28	Aqua Cera	2694	21.078	1.18	372
29	10- bromo-11-phenylundecanoic acid	2376	22.996	0.75	340
30	11-beta-hydroxyandrosterone	2316	23.104	1.60	306
31	1-phenanthrenemethanol,1,2,3,4	2247	23.579	0.65	286
32	Squelene	2914	27.828	2.75	410

**Table 3: Chemical Constituents of *O. gratissimum* Leave Oils and their Relative Proportion**

Peaks	Compound	Retention index	Retention time	%chemical Composition	Molecular Weight
1	2-tert-butylphenol	1223	8.525	9.29	150
2	α-farnesene	1458	10.143	1.49	204
3	α-seline	1474	11.021	1.18	204
4	Cis-2-α-bisaboleneepioxide	1934	12.235	0.77	262
5	Tridecanoic acid methylester	1580	16.884	3.06	228
6	n-Hexadecanoic acid	1968	17.989	7.30	256
7	Linolelaidic acid methylester	2093	19.912	6.56	294
8	11-octadecanoic acid methylester	2085	19.997	5.27	296
9	Oleic acid	2175	20.832	18.38	282
10	Aquacera	2694	21.124	15.42	372
11	Pentafluoropionic acid heptadecylester	1872	25.693	14.39	402
12	Squalene	2914	27.853	16.90	410

**Table 4: Chemical Constituents of *P. guajava* Leave Oils and their Relative Proportion**

Peaks	Compounds	Retention index	Retention time	%Chemical Composition	Molecular Weight
1	Cis-α-bisabolene	1518	10.141	2.03	204
2	α-seline	1474	11.019	2.55	204
3	Caryophyllene oxide	1507	11.533	0.71	220
4	α-farnesene	1458	12.895	1.48	204
5	Limonene	1031	13.118	2.97	152
6	Palmitic acid methylester	1878	16.168	13.92	270
7	Octadecanoic acid	2167	17.973	5.07	284
8	9,12-octadecadienoic acid	2093	19.698	16.37	294
9	11-octadecenoic acid	2085	19.806	18.74	296
10	Hexadecanoic acid	1914	20.179	5.26	284
11	Oleic acid	2175	20.825	14.62	282
12	Aquacera	2694	21.130	10.65	372
13	Phenanthrenemethanol	2247	23.563	1.56	286
14	Farnesyl cyanide	1812	27.828	4.08	231

**DISCUSSION**

The study reveals that leave oils combination of *H. suaveolens* and *O. gratissimum*, completely controlled *S. zeamais* on maize grains at 72hrs post-exposure. These findings confirmed earlier work for combining two or more plant materials in botanical formulations is more potent than when only one plant material used (Oparaeke *et al.*, 2002). Similarly, oil palm products mixed with *Paperoma pellucid* proved effective in protecting maize grain against infestation by *S. zeamais* (Ibe and Nwufa, 2001). Also, the powdered leaves of *H. suaveolens* and *O. gratissimum* were very effective in enhancing adult mortality of *S. zeamais* and *C. maculates* where they performed well in reducing adult emergence (Iloba and Ekrakene, 2006). Various

combinations of Niger seed oil and 5% malahtion dust provided complete protection to maize seed from the maize weevil up to 90 days after infection (Yuya *et al.*, 2009). Research findings revealed that a combination of aqueous extract of the bush mint *H. suaveolens* with a lower dose of insecticides such as Thionex 350 EC (Endosulfan (350) or Laser 480 EC (Spinosad (48)) helped to successfully control cotton bollworms (Sinzogan *et al.*,2006). The efficacy might be due to the major active compounds contained in the respective materials when in combination with each other may act synergistically to enhance the toxic substances in the extract mixtures by increasing their effects.

The GC- MS revealed the presence of bioactive compounds which were mostly alkanolic and aldehydes from the leaf oils of *H. suaveolens*, *O. gratissimum* and *P. guajava*. Several reports concerning the insecticidal activity of 1, 8-cineol against stored product insects exists (Kordali *et al.*, 2006). Existing variations in oil content and composition may be attributed to factors related to ecotype, phenophases and the environment including temperature, relative humidity, irradiance, and photoperiod (Fahlen *et al.*, 1997). Some of these plant materials used in this study have been found individually effective in storage pest's control (Okonkwo and Okoye, 1996). Information on the combined effect of these plant oil extracts in pests control is limited.

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## CONCLUSION

The findings of this study revealed that the combination of *H. suaveolens* and *O. gratissimum* was found to be better than all the treatment leave oil combinations which could be considered as an alternative agent for the control of *S. zeamais* and may become important supplement to toxic and non-sustainable synthetic insecticides. Also, more studies are needed to extract the bioactive compounds in the leave oils of the combined plant species.

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