



EFFICACY OF HYPTIS SUAVEOLENS LEAF OILS IN PROTECTING STORED MAIZE AGAINST ADULT MAIZE WEEVIL (Sitophilus zeamais) M

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Abstract

Laboratory experiment was conducted in the postgraduate laboratory of the Department of Biological sciences, Gombe State University, to test the insecticidal effects of leaf oils of Hyptis Suaveolens (L.). Concentration of 0.1, 0.2, 0.3 and 0.4ml of the leaf oils and 0.1ml of Dichlorvos (treated control) were applied to 20g each of maize grains at three replicate in which five (5) adult Sitophilus zeamais Motschulsky were introduced for adult mortality, emergence, grains weight loss and seed damage. The results showed that the highest (100%) mortality of S. zeamais obtained when 0.4ml was applied after 168hrs of treatment while the least (6.66%) was recorded after 24hrs of treatment at the same concentration. The results also revealed that the highest (1.67±0.00) and (1.33±0.00) for F1 and F2 adult emergence of the weevils were obtained when 0.1ml and 0.2ml was applied, while no adult emergence (0.00±0.00) was recorded at the highest concentration (0.4ml) for both F1 and F2 respectively. The findings of this study showed that the adult mortality and adult emergence of S. zeamais was directly proportional to the concentration of the leaf oils applied. Lowest grain percentage weight loss and seed damage was recorded in 0.4ml grain treatment (0.50% and 8%) respectively. Generally, the efficacy of the leaf oils on the mortality, adult emergence and weight loss and damage of S. zeamais was found to be significantly (p<0.05) different between the test oils and the untreated control. Findings of this study showed that the leaf oil of H. suaveolens was effective in protecting maize grains against S. zeamais infestation in stores. Therefore, more research is recommended on toxicity effect of these plant leaves oil on other insect pests.

Keyword: Emergence, GC-MS Leaf oil, Mortality, S. zeamais, Weight loss

Introduction

Maize (*Zea mays* L.) is the third most important food crop in the world surpassed only by two other grains, wheat and rice (Kyenpia *et al.*, 2009; IITA, 2005). 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). In Nigeria, maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L) and rice (*Oryza sativa* L.) are the major cereals grown in the sub-savanna region of Nigeria (Fatima and Abdul, 2005). It forms a cheap source of dietary carbohydrate (Rouanet, 1992),

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for humans and livestock. 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 grains 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 activity, insecticidal easy to use. biodegradable alternative that will be 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 Hyptis suaveolens, in protecting stored maize (Zea mays) from insect pest infestation.

Methodology

Collection, Identification and Processing of *H. suaveolens*.

H. suaveolens leaves were collected in Kano metropolis along Zaria road, Kano, Nigeria and were identified and authenticated using the appropriate plant taxonomy guide Plant Biology at Department Bayero University Kano. The voucher specimen was numbered and kept in Department of Plant Biology herbarium for further reference. Healthy leaves of the H. suaveolens that were collected and identified were washed properly under running tap water followed by distilled water. The Leaves were air dried in the laboratory for 7-14 days under a room temperature. The completely dried leaves were ground with pestle in a mortar and sieved to get a fine powder of the leaves (Epidi et al., 2009). The sieved powder was kept in a glass bottle until required for extraction.

Extraction of Oil from the Leaf Power of *H. suaveolens*

Oil was extracted from the plants powder using soxhlet apparatus described by (Ahmed *et al.*, 2004) with slight modification. One hundred and sixty (160) gram of pulverized sample was wrapped in a filter paper and then put in the thimbleof the holder soxhlet apparatus compartment. Chiller was connected to a hose respectively, for the recycling of the cold water and steam during the process. Three hundred (300) ml of the solvent (nhexane) was added with the aid of a funnel passing it through the by thimble containing the sample to the round bottom flask system of the soxhlet. The target oil was extracted for five (5) hrs at 60-80°C and subsequently stored in a refrigerator at 4°C for further use.

Collection and Processing of Maize Grain



The maize grains were obtained from International Institute for Tropical Agriculture (IITA), Kano State and damage maize grains were excluded. The undamaged grains were placed in a plastic vial and stored temporarily in a deep freezer at 20 °C for 72 h to eliminate insipient infestation of insects (Adedire and Ajayi, 2003). The grains were removed from freezer and kept at room temperature for two (2) hours to equilibrate. The moisture content of the maize samples was determined before each laboratory experiment.

Insect Culture

The method described by Asawalam, (2006) was adopted. 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 five hundred (500) g of the disinfested maize grain. The insects were allowed to oviposite for ten (10) 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 used for the experimental test.

Bioassay

Effect of Leaves Oil of *H. suaveolens* Plants 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. Four different concentrations (0.1, 0.2, 0.3 and 0.4 ml) was added to 10ml of n-hexane to give 1.0%, 2.0%, 3.0%, and 4.0% of oil extract of

H.suaveolens leaves and was separately mixed with 20g of maize grain in 5cm diameter plastic container. The oil concentration was thoroughly agitated to ensure uniform coating. Ten newly emerged unsexed adults S. zeamais were introduced into the containers and covered with the lid perforated by a needle to allow proper circulation of air (Zapata and Guy, 2010). Treated and untreated control experiments were set up along the treatment. All treatments were replicated three (3) times and arranged in a completely randomized design. Mortality of the insect was observed and recorded at 24 h interval for 168 h. 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;

$$P_{\rm T} = \frac{P_{\rm O} - P_{\rm C}}{100 - P_{\rm O}} \times \frac{100}{1}$$

Where P_T = corrected mortality (%) P_O = observed mortality (%) P_C = control mortality (%).

Effect of Leaves Oil from *H. suaveolens* Plants on Progeny Emergence

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

Assessment of Damage and Weight Loss of the Treated Maize Grains



Damage assessment was carried out on treated and untreated maize grains after adult emergence using weighing balance (FAO, 1985). Samples of hundred (100) grains were randomly taken from each container and the number of damaged grains (grains with characteristic holes) and undamaged grains was counted and weighed after F1 and F2 adult emergence. Percentage weight loss and percentage damage respectively were calculated using the formulae described by Baba-Tierto (1994).

% Grain weight loss

Initial weight of grain – Final of grain	10
Initial weight of grain	10
% Crain damage – Number of damaged grain	ns
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= <u>% treated maize grains perforated</u> x<u>100/</u>% control maize grains perforated + % treated grains perforated

WPI value exceeding 50 were regarded as enhancement of infestation by the weevil or negative protectability of the essential leaf oil tested.

GC-MS Analysis of the Plant Leaf Oils

The evaluation of chemical composition of leaves oil was carried out following the procedure described by (Adams, 2001) with little modification. Gas chromatographic (GC) analyses were performed on a capillary gaschromatograph 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 programme comprised of an 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 (8ul) was injected with a split ratio 1:0. The MS had a scan cycle of 1.5 s (scan speed 1250). The mass and scan range was set at m/z 40.00 and 600.00, respectively. Preliminary identification of constituents was based computer matching on components of mass spectral data against standard NIST library the spectra, constituted from spectra of pure substances and components of the known leaf oils, and literature MS data (Adams, 2001). They were confirmed by their GC retention time comparison with those of reference compounds, peak enhancement as well as co-injection /co-elution with authentic samples. Relative proportion of the leaf oil were computed in each case from GC-MS peak areas (Stenhangen et al., 1972).

Statistical Analysis

Data collected was subjected to one-way Analysis of Variance (ANOVA). All ANOVA analyses were conducted with Open-Stat statistical software (version 08.12.14). Where the ANOVA indicated significant difference, least significant difference (LSD) was used to separate the means.

Discussions

The result shows varying mortality of *S. zeamais* after treatment on maize grain.







However, highest S. zeamais mortality (100%) was observed after 168hours of treatment with H. suaveolens leave oils at treatment level of 0.4 ml. The result also indicated that H. suaveolens oil at 0.4ml comparable the chemical was to insecticide used (Dichlorvos), even though the chemical insecticide was better than H. suaveolens leaves oil and untreated control Similarly Abdullahi et al. (Table 1). (2011) reported that at highest value of

100% mortality was observed in the treatment with lime peel oil by third day using 3.0 v/w, this is followed by 2.5 v/w at 4 days (100%), 0.5 and 1.5 v/w at 6 days (100%). The higher mortality caused by *H. suaveolens* oils might not be unconnected with the reason stated by Don-Pedro (1990) that the oils of plant origin are highly lipophilic; and therefore have the ability to penetrate the cuticle of insects.

Table 1: Effect of H. suaveolens Leaf Oil Extract on Adult Mortality of S. zean	nais
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Treatment	ConcWeightV/Wof maize(%)(g)	No of	%mortality in hours								
		of maize (g)	used	24hrs	48hr s	72hr s	96hrs	120hrs	144hrs	168hrs	LC ₅₀ (µml/l)
H. suaveolens	0.1(1)	20	5	0.00	13.33	20.00	33.33	40.00	60.00	80.00	4.44
	0.2(2)	20	5	0.00	20.00	26.66	53.33	80.00	80.00	86.66	
	0.3(3)	20	5	0.00	33.33	33.33	60.00	86.66	86.66	93.33	
	0.4(4)	20	5	6.66	40.00	53.33	80.00	93.33	93.33	100	
Control (- Ve)	0.0(0)	20	5	0.00	0.00	0.00	0.00	0.00	1.66	1.66	
Control (+Ve)	0.1(1)	20	5	100	0.00	0.00	0.00	0.00	0.00	0.00	

Table 2: Effect of H. suaveolens Leaf Oil Extract on F1 and F2 Adult Emergence of S. zeama
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Treatment	Concentration V/W (%)	Weight of maize (g)	No of insect used	Means Adult Emergence± S.E		
				F1	F2	
H. suaveolens	0.1(1)	20	5	1.67±0.00 ^e	1.00±0.00 ^{ab}	
	0.2(2)	20	5	$1.33{\pm}0.00^{d}$	1.33 ± 0.00^{b}	
	0.3(3)	20	5	1.33 ± 0.00^{d}	0.67 ± 0.00^{a}	
	0.4(4)	20	5	0.00 ± 0.00^{a}	$0.00{\pm}0.00^{a}$	
Control (-Ve)	0.0(0)	20	5	1.67 ± 0.00^{e}	5.00±0.00 ^c	
Control (+Ve)	0.1(1)	20	5	$0.00{\pm}0.00^{a}$	$0.00{\pm}0.00^{a}$	
LSD(0.05)				0.31	1.05	

Mean Adult emergence \pm 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 Emerge	ence at 28 days Post
treatment	

Treatments	Conce ntratio n V/W (%)	No of insect used	Mean Initial weight of maize grain(g)	Mean Final weight of maize (g)	Mean weight Loss ± S.E	% weight loss
. suaveolens	0.1(1) 0.2(2)	5 5	20 20	19.50 19.83	0.50 ± 0.15^{abc} 0.17 ± 0.13^{a}	2.50 0.85
	0.3(3)	5	20	19.80	0.20±0.05 ^a	1.00
Control(-Ve)	0.4(4) 0.0(0)	5 5	20 20	19.90 19.26	0.10±0.05 ^a 0.74±0.08 ^c	0.50 3.70
Control (+Ve) LSD(0.05)	0.1(1)	5	20	20.00	0.00±0.00 ^a 0.52	0.00

Table 4: Assessment of Weight Loss (%) of the	Treated Maize after F2	Adult Emergence at 56days Post
treatment		

Different leaves oils	Concentration V/W (%)	No of insect used	Initial weight of maize grain	Final weight(g)	Mean weight Loss± S.E	% weight loss	WPI
H. suaveolens	0.1(1)	5	20	19.25	$0.75{\pm}0.34^{bd}$	3.75	48.80
	0.2(2)	5	20	19.54	0.46±0.14 ^{abc}	2.30	48.19
	0.3(3)	5	20	19.62	0.38±0.05 ^{abc}	1.90	40.27
	0.4(4)	5	20	19.73	0.27±0.05 ^{abc}	1.35	15.68
Control(-Ve)	0.0(0)	5	20	18.64	1.36±0.39°	6.80	50
Control (+Ve)	0.1(1)	5	20	19.84	0.16±0.03 ^a	0.80	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





Peak	Compound	Retention	Retention time	% chemical
	-	index		composition
1	4-methyltridecane	1349	3.061	9.32
2	α-pinene	941	3.234	5.21
3	Isobutylclohexane	1055	3.473	13.09
4	Methylnonane	951	3.749	7.43
5	n-Decane	1015	4.203	10.37
6	4- methyldacane	1051	4.489	4.57
7	2,6,10,14-tetramethylheptane	1852	4.639	3.51
8	Dodecanoic acid 2- penten-1-ylester	1886	4.704	2.54
9	α-copaene	1375	4.937	2.34
10	Methylundecane	1150	5.035	2.42
11	3,7-dimethylnonane	986	5.116	2.13
12	2,6,6-trimethylbicyclo-3-1,1-heptane	1115	5.524	6.84
13	α-muurolene	937	6.092	1.60
14	Dodecane	1501	6.398	1.87
15	2,6-dimethylundecane	1214	6.906	2.86
16	ρ-cymen-8-ol	1185	7.094	1.21
17	n-tridecane	1320	7.910	1.48
18	4,6-dimethyldodecane	1313	8.288	2.05
19	n-hexadecane	1285	9.317	1.00
20	Cis-α-bisabolene	1612	9.623	2.09
21	3,7-dimethyldecane	1518	10.142	1.04
22	7-dodec-3-yne	1086	10.420	0.77
23	Palmitic acid	1222	12.230	1.28
24	Naphthalene	1968	17.979	1.87
25	Isopropyl-1,1,4-tri	1909	19.008	0.54
26	Cis-oleic acid	2004	19.669	0.85
27	Octadecanoic acid	2172	20.811	2.78
28	10- bromo-11-pheny	2694	21.078	1.18
29	Undecanoic acid	2376	22.996	0.75
30	11-beta-thadroxyandrosterone	2316	23.104	1.60
31	11-phenonthrenemethanol	2247	23.579	0.65
32	Squalene	2914	27.828	2.75

Table 5: List of compounds identified from H. suaveolens leaf oil



1996).

Figure 1: GC-MS Analysis of *H. suaveolens* leaf oil

The mortality of S. zeamais in the maize grains treated with various concentrations of H. suaveolens leaf oil was found to be dose dependent. Similarly, Akunne et al. (2014d) reported that the efficacy of root powder of L. cyanescens on adult S. *zeamais* in maize grains was dose dependent. This is also in conformity with the work of Iram et al. (2013) who reported that P. guajava leaves and fruit peels showed promising effects of seed protection and insecticidal properties against Tribolium castaneum Herbst. H. suaveolens may have been potent because of the strong odours emitted, thereby disrupting normal respiratory activities of the weevils; resulting in asphyxiation and

plant leave oils extracts provide total protection on the maize grains against *S*.

subsequent death (Adedire and Ajayi,

Result from this study confirm that all the

zeamais at higher concentration (0.4ml) which correspond to the concentration of the treated control (Dichlorvos). However, minimal emergences were observed in F2 of *H. suaveolens* at 0.3ml (0.67 \pm 0.00) (Table 2) which is in agreement with Iloba and Ekrakene (2006) who reported that powdered leaves of H. suaveolens and O. gratissimum were very effective in enhancing adult mortality in Sitophilus zeamais and C. maculatus and performed well in reducing adult emergence. Significantly lower number of emerged F₁ progeny relative to treated control suggests



the presence of some active compounds in the plants that are toxic the weevils. Control untreated recorded the highest means F1 and F2 emergence of 1.67±0.00 and 5.00 ± 0.00 (Table 2), while the higher doses of the treatments and the treated control dust offered the least mean adult emergence. The emergence of new adults in the untreated control test show that the weevils were not disturbed in their environment. However, in the treated maize with higher concentration (0.4ml), no newly emerged adults were seen (Table 2). The plant leaves oils may have prevented mating and oviposition of eggs by the females. This could also been as a result of the blockage of the hatchability of the eggs.

In term of weight loss, the highest percentage weight loss (PGWL) was observed from H. suaveolens 0.1ml (2.50%) and the lowest at H. suaveolens 0.4ml (0.50%). For the treated control (Dichlorvos) **PGWL** was observed (0.00%) whereas untreated control gave rise to PGWL of (3.70%) after F1 adult emergence (Table 3). Also highest was observed in H. suaveolens PGWL 0.1ml (3.75%) and lowest in *H. suaveolens* 0.4ml (1.35%); the untreated control gave rise to (6.80%) after F2 adult emergence (Table 4). This is in conformity with the result of an earlier study by Okonkwo and Okoye, (1996) who reported that percentage weight loss was related to the population of adult S. zeamais. Similarly Casey (1994) reported that where there was a heavy pest infestation in stored maize, weight loss as much as 30-40% could be recorded. Moreover, the high loss in weight with the lower concentration can be

attributed to the floury extraction resulting the weevils feeding activity. from Percentage grain damage (PGD) was observed in the treated grain with H. suaveolens 0.1ml (41%) and the lowest recorded in *H.suaveolens* 0.4ml (8%) when compared with the untreated control (46%) (Table 3), since grain damage and weight loss are related, the higher the grain damage, the higher the weight loss and vice versa. It can be inferred from the results that the low weight loss observed can be attributed to the low grain damage recorded. This is because, as the number of live weevils reduces in the grains in storage, the amount of damage caused to the grains also reduces as there is less feeding. Hence the lower weight loss observed. 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. The plant oils did not cause any grain damage at higher concentration used and had similar effects to that of the treated control (Dichlorvos). *H. suaveolens* at 0.4ml was observed to be more effective (Table 4). This agrees with the findings of G/Selase and Getu (2009) which reported significant reduction in cowpea grain weight loss by J. curcas and some other plants when applied against Z. subfasciatus. This action might be as a result of its effectiveness in killing all the adult weevils introduced. Leaf oil of *H.suaveolens* was also promising in reducing grain damage and weight loss. When conferred to untreated control, its



effects had been proportional to the amount used which is in agreement with the findings of Al-Moajel (2004) which reported an increase in efficacy of leaves powder of *L. inermis* with increase in concentration. The WPI of *H. suaveolens* was also less than 50 at all the varying concentrations used showing potentials for protection of grains against *S. zeamais* (Table 4).

GC profile of H. suaveolens leaf oil is shown in Figure 1. The analysis of the oil revealed complex mixture a of constituents. A total of 32 compounds were identified in the leaf oil of H. suaveolens by GC -MS and GC Co injection with authentic standards (Table 5). The oils are a mixture of monoterpenes Isobutylclohexane and sesquiterpenes. occurred in the largest quantity (13.09%). This is followed by n-Decane (10.37%), 4methyltridecane (9.32%), mythylnonane (7.43%), 2, 6, 6-trimethylbicyclo-3,-1, 1-(6.84%).and Alpha heptanes pinene (5.21%). Similarly several reports concerning the insecticidal activity of 1, 8cineol (a type of monoterpenoids, found in aromatic plants of *Lamiaceae* family) against stored product insects exists (Aggarwal et al., 2001; Lee et al., 2004; Kordali et al., 2006). Similarly the repellent properties exhibited by P. guineense against S. zeamais explains the fact that P. guineense has broad spectrum of activity as it is reputed to contain alkaloids like *piperine*, *chavicine* and piperidine (Lale and Alaga, 2000). 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).

Conclusion and Recommendations

The results obtained from this study have revealed that the leaf oil of *H. suaveolens* had significant effects on mortality, adult emergence of S. zeamais on maize grains. The observed differences on adult mortality, adult emergence, and weight loss and seed damage of this pest on treated and untreated maize grains indicated that the test plant leaf oil had effects on maize weevil. The plant oils also showed positive protectant ability against S. zeamais attacking maize grains in the store. The following recommendations are preferred.

- I. Planting of *H. suaveolens* should be encouraged in different part of the country where maize is used.
- II. Further research should be carried out on the insecticidal bioactive compound in the leaves oil of *H. suaveolens* on humans.
- III. Farmers should be trained on how to extract oils from the leaves of *H. suaveolens*.

Further research is recommended on toxicity effect of these plant leaves oil on other insect pests.

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