Journal of Stored Products and Postharvest Research Vol. 2(6), pp. 113-119, June 2011
Available online http://www.academicjournals.org/JSPPR
ISSI 2141 - 6567 ©2011 Academic Journals

Full Length Research Paper

Toxicity and repellant potency of *Hyptis spicigera* extracts on *Sitophilus zeamais* motschulsky (Coleoptera: Curculionidae)

Wekesa I.[1], Onek L. A.[1], Deng A. L.[2], Hasanali A.[3] and Othira J. O.[1]*

[1]Department of Biochemistry and Molecular Biology, Egerton University, P. O. Box 536 - 20115, Egerton, Kenya.
[2]Department of Biological Sciences, Egerton University, P. O. Box 536 - 20115, Egerton, Kenya.
[3]Department of Chemistry, Kenyatta University, P. O. Box 43844, Nairobi, Kenya.

Accepted 2 March, 2011

Bush mint, *Hyptis spicigera* has been used traditionally as a cereal and legume protectant against storage insect pests. The objective of this study was to evaluate the toxic and repellant activity of *H. spicigera* extracts against *Sitophilus zeamais,* a major insect pest of stored cereal grains in the tropics. Essential oils and solvent extracts were tested against the maize weevil in fumigation, contact and repellence bioassays. Results obtained showed that essential oils had strong fumigant toxicity against *S. zeamais* with a LC_{50} value of 48.11µL/l air at 48 h. Contact toxicity assay gave more than 50% mortality at 48 h of exposure for methanol extracts and not hexane extracts. Hexane extracts had higher repellant activity than the methanol extracts at the same dosage. GC-MS revealed that essential oils is composed of at least fifteen mono- and sesquiterpenoids, with the major constituents being α-pinene (27.4%), β-pinene (17.6%), L-phellandrene (12.2%), α-thujene (12.2%), isocaryophyllene (9.2%), and limonene (9.4%). Bush mint may be exploited for the development of botanical insecticides to be used in weevil management.

Key words: *Hyptis spicigera*, essential oil, solvent extract, pesticidal potency, *Sitophilus zeamais*.

INTRODUCTION

Maize is an important component in the diet of inhabitants in the tropics. Between 60 and 70% of grain in Africa is stored at farm level, generally to provide a food reserve as well as seed for planting (FAO, 1998, 1999). However, maize storage conditions in developing countries are inappropriate and farmers experience postharvest grain losses due to insect attack (Niber, 1994). The maize weevil, *Sitophilus zeamais* Motschulsky, is a key pest of cereal grains whose infestations start in the field before harvest and extend throughout the storage period (Oliveira et al., 2007). Use of insecticides is considered most effective in controlling *S. zeamais* in storage. Chemical pesticides such as phosphine and methyl bromide are favoured for fumigation due to their rapid action and ease of penetration into the commodity. However, chemical insecticides have disadvantages such as development of insect resistance and environmental hazards (Leelaja et al., 2007), and may lead to mammalian toxicity (Wolansky et al., 2007). This has fueled the search for alternative methods of pest control.

Currently, botanicals constitute 1% of the world insecticide market, despite the knowledge that plants constitute a rich source of bioactive chemicals and may provide alternatives to regular insect control agents (Kim et al., 2003). Several species from the *Labiatae* family have been tested for their insecticidal potency (Belmain et al., 2001; Ogendo et al., 2004) and are widely used insect pest control (Ke'ita et al., 2000). Bush mint, *Hyptis spicigera* (family: *Labiatae*), is an important medicinal plant used in treatment of gastrointestinal disturbances, wounds, skin infections and insect bites (Rogelio et al., 2001) and also an effective insect repellant in traditional grain storage structures among the Acholi of Uganda. This study evaluated the insecticidal and repellant activity

*Corresponding author. E-mail: othirajack@gmail.com. Fax: +254-5162145.

of essential oil and solvent extracts of *H. spicigera* against *S. zeamais* in an effort to scientifically rationalize its use as grain protectant in traditional grain stores.

MATERIALS AND METHODS

Preparation of extracts

Extraction of essential oils from H. spicigera

Seeds of *H. spicigera* were collected and grown to maturity at Egerton University experimental field. Fresh aerial parts were harvested, air-dried for one week and ground to powder using a grinding mill (Retsch Muhle, Germany). The essential oil was extracted by water-distillation using a Clevenger-type apparatus for 6 h. The superior phase was collected from the condenser, dried over anhydrous sodium sulphate and stored in amber-colored vials at 4°C for further experiments.

Preparation of solvent extracts from H. spicigera

50 g of the ground leaves were extracted twice with 300 ml methanol solvents at room temperature for two days and filtered using Whatman No. 2 filter papers. The filtrate was concentrated to dryness by rotary evaporation at 40°C. The yield was calculated and recorded as methanol extracts (Kim et al., 2003). The procedure was repeated using hexane as solvent and yield calculated and recorded as hexane extract.

Insecticidal bioassays

Fumigant toxicity of H. spicigera extracts

Ten pairs of three-week old weevils were placed in 10 ml vials capped with polypropylene stoppers. Essential oils 0, 5, 10, 20, 30 and 40 µL /l air were also placed in similar vials and capped with polypropylene stoppers. Vials containing insects were turned upside down over the vials containing oils, to allow saturation of atmosphere with oil vapours. The treatments were arranged in a completely randomized design (CRD) and replicated 5 times. Mortality counts were made 24, 48 and 72 h after setup. The insects were considered dead if appendages did not move when probed with a camel brush (Këita et al., 2000). Mortality rate was calculated using the Abbott's formula for natural mortality in untreated controls (Abbott, 1925). Probit analysis was used to estimate the lethal concentration (LC_{50}) values (Finney, 1978). Fumigation bioassays for the methanolic and hexane extracts were done similarly.

Contact toxicity of H. spicigera extracts

Contact toxicity assay was done according to Kim et al. (2003) with some modifications. 20 g of maize grains in Petri dishes were coated with 50 mg plant extracts dissolved in 3 ml methanol and hexane, respectively. The grains were then placed under a fume hood for ten minutes to allow the solvent to evaporate. Ten unsexed insect pairs were then introduced into each dish and exposed to treatments. Controls contained maize grains mixed with 2 ml of methanol and untreated maize grains. The treatments were laid out in a CRD with 3 replicates per treatment. Numbers of dead insects were recorded 1, 2, 3, 4, 7, 14 and 21 days after setup. Percent mortalities were then determined and the weevils removed at the end of day 21. Corrected percent mortality was calculated using Abbot's formula (Abbot, 1925) as follows: % Mortality (adjusted) = (% AC-%AT) / %AC = (% DT- % DC) / [(100-%DC) x 100]. Where: AC = Alive in control; DT = Dead weevil in test; AT = Alive in treatment; DC = Dead weevil in control. Similar procedure was followed for hexane extracts.

Repellant activity of H. spicigera extracts

Repellant activity of *H. spicigera* extracts against the maize weevil was performed using a choice bioassay system according to Bekele (1995). The system consisted of two 1-litre glass jars connected at their rims by means of a nylon mesh tube. Fifty adult insects were introduced into the system via a circular hole at the middle of the upper side of the nylon mesh. Each jar contained maize grains; treated with leaf powders (1, 4, and 7 g, w/w) or solvent extracts (100, 50 mg) or essential oils (10, 40, 70, and 100 µL, v/w) while the control jars contained untreated maize seeds. The treatments were laid out in a CRD with 4 replicates per treatment. The number of insects present in the control jar (N_C) and the treated jars (N_T) were recorded after 24 h of exposure. Percent repellence (PR) values were computed using the method by Hassanali et al. (1990); $PR = N_C - N_T / N_C + N_T \times 100$, where Nc was the number of insects on the control half and N_T was the number of insects on the treated half. The original data from toxicity and repellency trials were transformed using $\log_{10}$ (x+1) to correct for heterogeneity of treatment variances and subjected to general linear model (GLM). The transformed means were separated by Tukeys Studentised Range Test (HSD) at 0.05% level of significance (Gomez and Gomez, 1984). Statistical Analysis System (SAS), version 8.2 was used in data analysis (SAS, 2000).

The GC-MS analysis of essential oils

Gas chromatographic (GC) analyses were performed according to Tapondjou et al. (2005) but using a Hewitt Packard ultra 1 methyl silicone capillary column of 50 m × 0.2 mm, with a phase thickness of 0.33 µm. The temperature program for the analyses were as follows: The initial temperature was 60°C for 5 min, which was increased to 280°C at 5°/min and maintained at a temperature of 280°C for 20 min. Nitrogen was the carrier gas. Sample for injection into the GC was prepared as follows: 1 µl of essential oil dissolved in 1 ml of DCM. 1 µl of this was then injected into the GC for analysis. For GC-MS analyses, Helium (100.0000%) was used as the carrier gas and maintained at a flow rate of 1 ml/min, in a split/split less flow. The electron impact ionization conditions were; ion energy 70 eV and the mass range scanned was 38 to 650 a.m.u in the full acquisition mode. The column and column temperature program were the same as those used in GC analyses. The essential oils constituents of the different oils were identified by visual comparisons of their mass spectra with those of libraries (NIST and WILEY), co-injection with known available authentic standards and use of retention times.

RESULTS

Fumigant toxicity of *H. spicigera* extracts

The *H. spicigera* essential oils showed fumigant toxicity against *S. zeamais* (Table 1). The mortality rate of *S. zeamais* increased with the concentration and duration of exposure to the essential oils. Total mortality was obtained within 48 h of exposure for three doses higher than 10 µL, of the essential oils tested. The LC_{50} of

Table 1. Percent mortality (Mean ± SE; n=5) of adult *S. zeamais* exposed to varying concentrations of *H. spicigera* essential oils applied as a fumigant.

Quantity of oil	Exposure duration			
	1 24 h	2 48 h	3 72 h	5 120 h
0 µL	0±0.00[e]	0±00[e]	0±0.00[b]	0±0.00[b]
10 µL	8.75±0.82[d]	71.25±6.25[b]	96.25±2.39[a]	100.00±0.00[a]
20 µL	23.75±1.57[c]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
30 µL	47.50±2.11[b]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
40 µL	83.75±2.80[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
LC_{50}, µg/L	48.11	46.82	46.67	44.32

Means in columns with the same letter are not significantly different (P>0.05) by Tukey's HSD test.

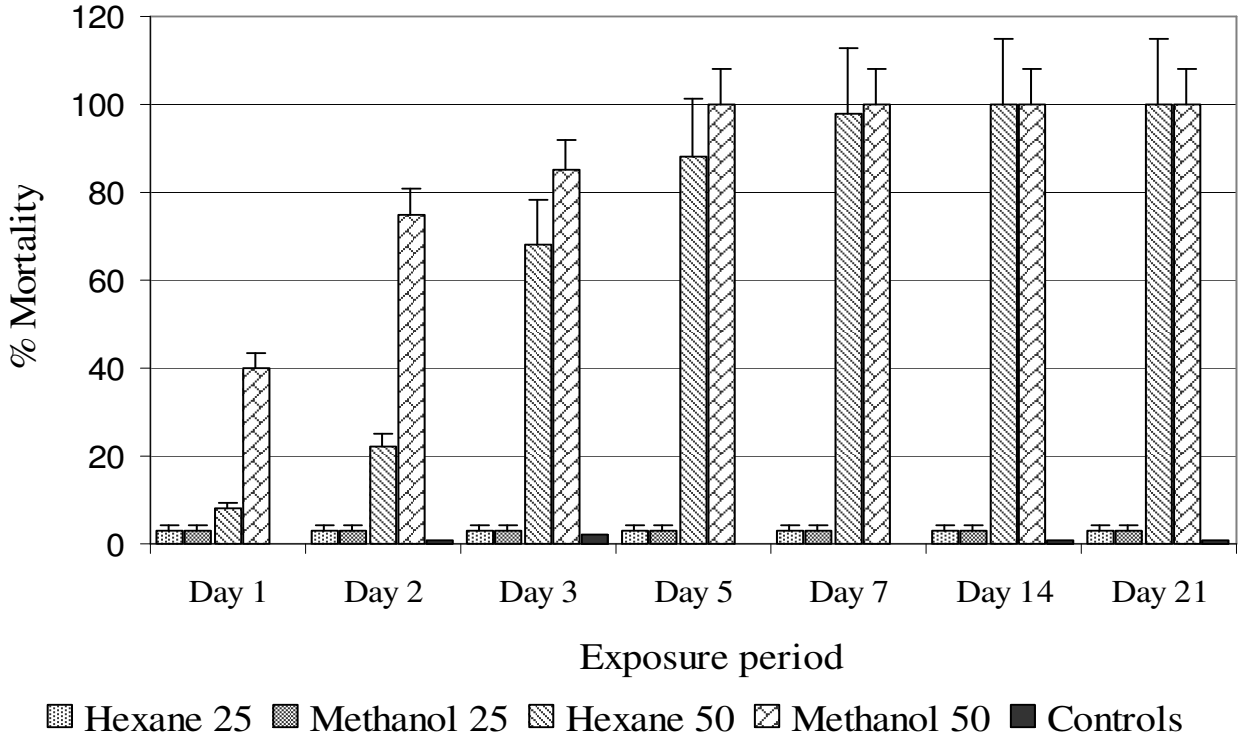

Figure 1. Mean Percent adult mortality (Mean±SE) of *S. zeamais* in grains treated with *H. spicigera* solvent extracts.

essential oils was 44.32 for *H. spicigera.*

Contact toxicity of *H. spicigera* extracts

Stronger contact toxicity of *H. spicigera* extracts was observed at higher concentrations than lower concentrations (Figure 1). There was no significant difference in mortality rates at low dose levels of both hexane and methanol extracts for the whole exposure time. In both treatments at all doses applied, the end-point mortality was reached after day 14. Nonetheless in both extracts mortality increased with exposure time. Total mortality was attained at 5 and 14 days of exposure to methanol and hexane extracts, respectively. There was significantly higher emergence of F1 progeny in the controls compared to the treatments (Figure 2). However, the progeny emergence in treatments with hexane and methanol extracts at 25 mg were not significantly (P<0.05) different from each other. At 50 mg, treatment with methanol extract had significantly (P<0.05) higher progeny than those with hexane extract.

Repellent activity of *H. spicigera* extracts

The repellency of the ground leaf powder were dose dependent with 7 g (w/w) evoking the highest repellent action, though, not significantly different from 4 g, within the same exposure duration (Figure 3). Results showed a

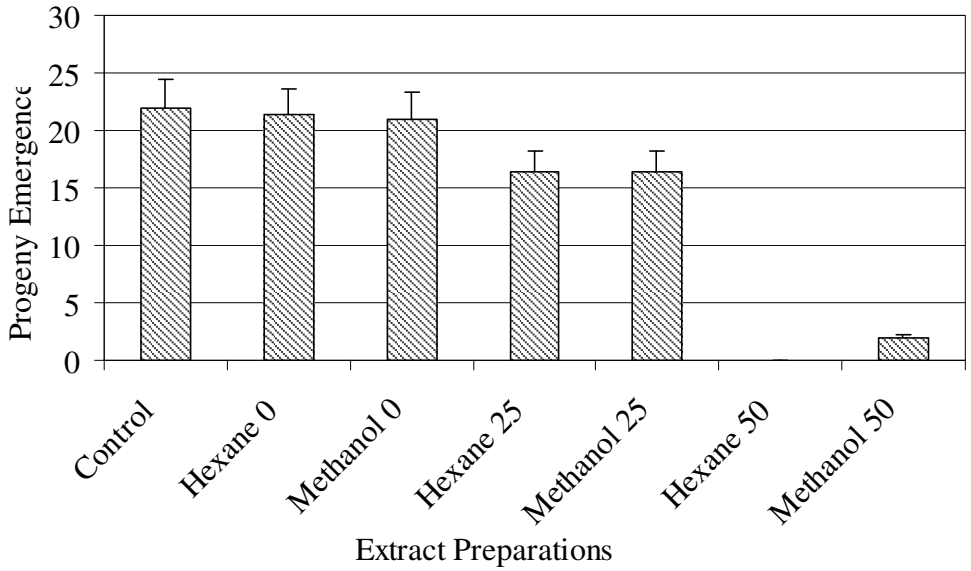

Figure 2. Number (Mean ± SE) of adult *S. zeamais* progeny (F_1) emergence 60 days after application of *H. spicigera* extracts on maize grains.

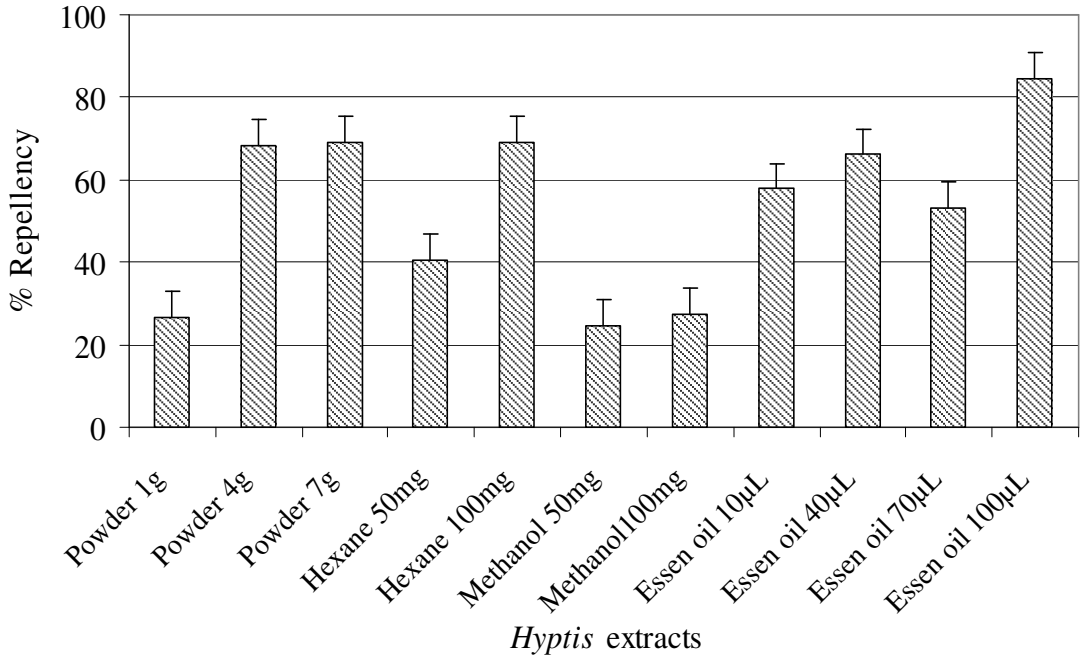

Figure 3. Percent repellence (Mean ± SE; n = 4) of adult *S. zeamais* after 24 h exposure to maize grains treated with various preparations of *Hyptis spicigera.*

dose- and extract-dependent ($P<0.05$) repellant effects of *H. spicigera* against adult *S. zeamais*. The repellency of solvent extracts were also dose-dependent pattern, however, hexane extract had higher repellency at lower concentrations than methanol extracts. No feeding was observed in grains treated with hexane extracts while low feeding was observed in those treated with methanol extracts.

Essential oils applied at different doses generally evoked higher repellency to *S. zeamais* than the powders and solvent extracts.

GC-MS analysis of essential oils

The quantity of pale yellow essential oil obtained was

Table 2. Major chemical constituents of essential oils from *H. spicigera* leaves.

Retention time	Name of constituent	Concentration (%)
17.18	α-pinene	27.4
18.53	β-phellanderene	5.3
18.80	β-pinene	17.6
19.73	L-phellandrene / α –thujene	12.2
20.65	Limonene / phellandrene	9.4
23.10	α –copaene	1.2
24.43	Terpinene-4-ol	1.0
25.80	Bourbonene	<1.0
27.60	Isocaryophyllene	9.2
32.68	α –humulene	1.1
34.05	Germacrene D	1.5
35.60	{ β –cadinen	1.1
36.35	Caryophyllene oxide	<1.0
49.03	Unknown	1.9

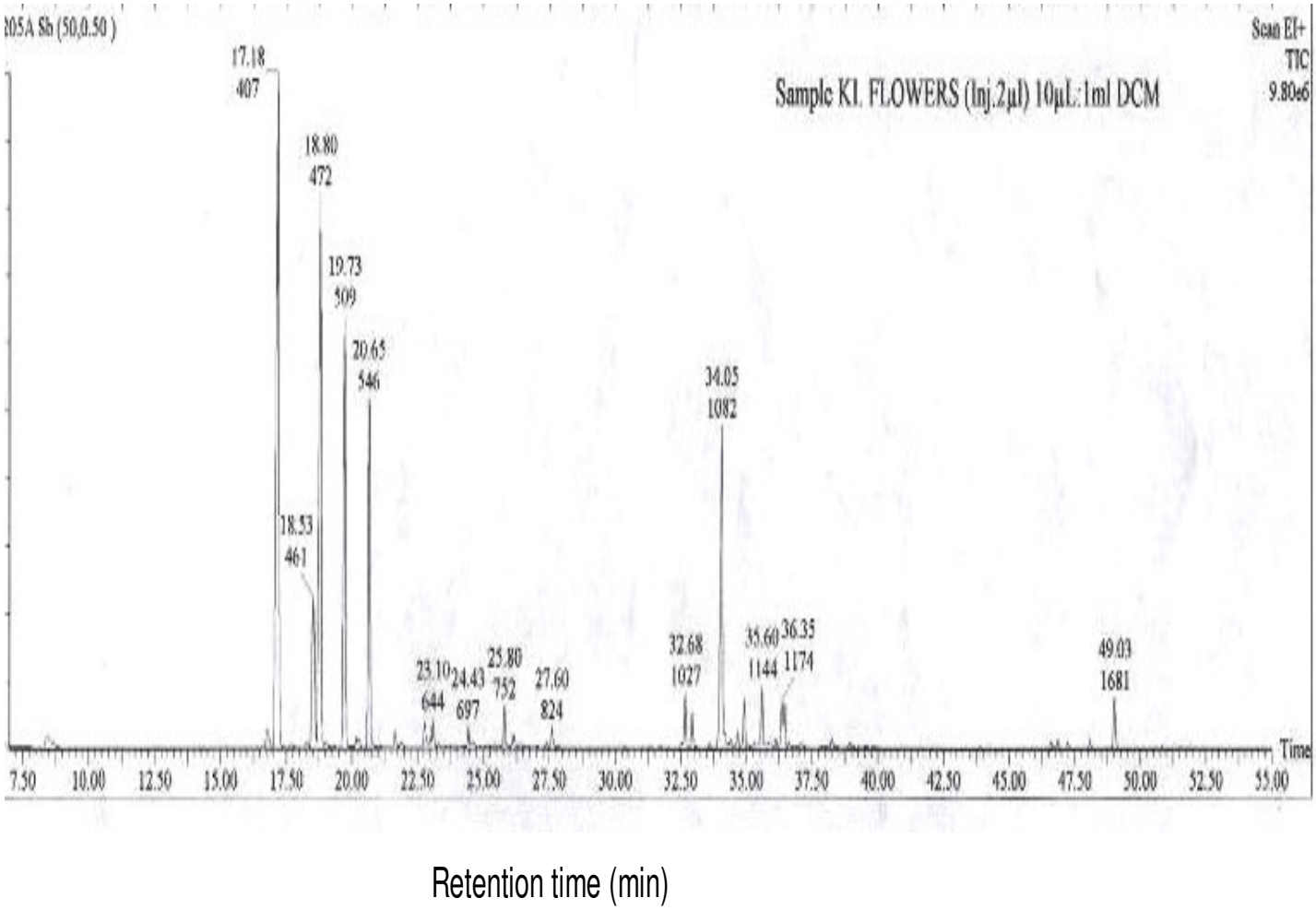

Figure 4. The GC-MS chromatogram showing composition of essential oil extracts from *H. spicigera* leaves.

2.235 g, equivalent to 0.363% of sample collected. The results of the chemical analysis of the essential oils from leaves and inflorescence of *H. spicigera* revealed that the oils mainly contain mono- and sesquiterpenoids (Table 2, Figure 4). The monoterpenes were more than 55%, with the main constituents being identified as α-pinene, β-phellandrene, α-thujene, β- pinene, limonene and terpinen-4-ol.

DISCUSSION

Fumigation studies carried out showed that the essential oils of *H. spicigera* had a 'knock down effect' on the test insect. Essential oils act by inhibiting insect acetyl cholinesterase and thus, ultimately blocking the nerve functions. This is in agreement with studies by Obeng-Ofori and Amitaye (2005) that observed signs of immobilization with flexed legs and clinging to the grain, outstretched meta thoracic wings from the elytra and paralysis of the dead or dying insects. The enzyme acetyl cholinesterase is also the target site of inhibition by organophosphates and carbamate insecticides (Matsumura, 1985). The observed rapid action of essential oils could be attributed to their property of acting in the vapour phase, hence gaining entry into the insect's internal systems with ease through the spiracles. In topical application procedures, the insect is protected by its exoskeleton against external influences. Lack of feeding in grains treated with hexane extracts suggests that hexane extracts had more bioactive constituents than methanol extracts.

These findings are consistent with the study using Chinese herbs in which hexane extracts caused high mortality of *S. zeamais* compared to methanol extracts (Liu et al*.,* 2007). High F_1 progeny numbers observed at treatments with low extract concentrations could be attributed to the ability of the weevils to bore oviposition pits while feeding. This is supported by earlier studies on terpenoids isolated from tropical rutales and *Rhaponticum pulchrum* which indicated a phago repressive effect against *S. oryzae* and *S. granaries,* close relatives of *S. zeamais* (Cis et al., 2006; Omar et al., 2007).

The repellant activity observed confirms the field studies carried out by Othira et al., (2009) on insecticidal potency of *H. spicigera* powders and fresh stems. The repellent action observed in the leaf powders is due to the action of essential oils from the leaves glandular trichomes which rapture to release the volatile oils when disturbed. Similar studies on powders and essential oils from *Ocimum suave* and *Ocimum kenyense* had also been shown to have repellent activity against the maize weevil in olfactometric and choice bioassay systems (Hassanali et al., 1990; Bekele et al., 1997). Studies by Ogendo et al. (2004) found similar repellency against maize weevil using powders from *Lantana camara* L. and *Tephrosia vogeli* (Hook). These findings indicate good potential of *H. spicigera* for the use as repellent and toxic agents in the management of the maize weevil. Since plant derived pesticides are biodegradable and safer to higher animals, they offer a viable alternative to synthetic agrochemicals (Bouda et al., 2001; Soon-II et al., 2003). Appropriate effective doses of *H. spicigera* plant extracts could save the resource poor farmer from storage pest losses. Although this study has demonstrated the scientific rationale for use of *H. spicigera* in ethno-botanical grain storage practices, further research on chemistry and mechanism of action of bioactive principles extracted from *H. spicigera* is necessary.

ACKNOWLEDGEMENTS

We are grateful to International Centre for Insect Physiology and Ecology (ICIPE), Nairobi for providing laboratory space and resources to carry out this study. Our thanks also go to Egerton University, Kenya.

REFERENCES

Abbot WS (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265- 267.

Bekele JA (1995). Effects and use of some *Ocimum* plant species and their essential oils on some storage insect pests. PhD Thesis. Kenya.

Bekele JA, Obeng-Ofori D, Hassanali A (1997). Evaluation of *Ocimum kenyense* (Ayobangira) as a source of repellents, toxicants and protectants in storage against three major stored product insect pests. J. Appl. Entomol., 121: 169-173.

Belmain RS, Neal EG, Roy DE, Golob P (2001). Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. Food Chem. Toxicol., 39(3): 287-291.

Bouda H, Tapondjou LA, Fontem AD and Gumedzoe DYM (2001). Effects of essential oils from leaves of *Ageratum conyzoides*, *Lantana camara* and *Chromolaena odorata* on the mortality of *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Stored Prod. Res., 37(2): 103-109.

Cis J, Nowak G and Kisiel W (2006). Antifeedant properties and chemotaxonomic implications of sesquiterpene lactones and syringin from *Rhaponticum pulchrum*. Biochem. Systematics Ecol., 34(12): 862-867.

FAO (1998). Information Network on Post-Harvest Operations. www.fao.org

FAO (1999). The use of spices and medicinals as bioactive protectants for grains. FAO Agricultural Services Bulletin No. 137. FAO, Rome.

Finney DJ (1978). Statistical methods in Biological assay, 3rd Ed., Charles Griffin & Company, London, pp. 508.

Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research, 2nd Edition. Wiley Interscience Publications, New York.

Hassanali A, Lwande W, Ole-Sitayo N, Moreka L, Nokoe S, Chapya A (1990). Weevil repellent constituents of *Ocimum suave* leaves and *Eugenia caryophyllata* cloves as grain protectants in parts of Eastern Africa. Discovery Innov., 2: 91-95.

Këita SM, Vincent C, Jean-Pierre S, Ramaswamy S, B'elanger A (2000). Effects of various essential oils on *Callosobrunchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Prod. Res*.,* 36: 355-364.

Kim Soon-Il, Myung-Hee O, Hyung-Chang C, Young-Joon A (2003). Contact and fumigant activities of aromatic plant extracts against *Lasioderma serricorne* (Coleoptera: Anobiidae). J. Stored Prod. Res., 39(1): 11-19.

Liu LZ, Goh HS, Ho HS (2007). Screening of Chinese medicinal herbs for bioactivity against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). J. Stored Prod. Res., 43(3): 290-296.

Matsumura F (1985). Toxicology of insecticides (2ed.), Plenum Press, New York.

Niber BT (1994). The ability of powders and slurries from ten plant species to protect stored grain from attack by *Prostephanus truncatus* Horn. (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae). J. Stored Prod. Res., 30(4): 297-301.

Obeng-Ofori D, Amitaye S (2005). Efficacy of mixing vegetable oils with pirimiphos-methyl against the maize weevil, *Sitophilus zeamias* Motchulsky, in stored maize. J. Stored Prod. Res., 41(1): 57-66.

Ogendo JO, Deng AL, Belmain SR, Walker DJ, Musandu AAO (2004). Effects of Insecticidal Plant Materials, *Lantana camara* L. and *Tephrosia vogelli* Hook, on the quality Parameters of Stored Maize Grains. J. Food Technol. Afr., 9(1): 29-35.

Oliveira EE, Guedes CRN, Tótola MR, De Marco Jr. P (2007). Competition between insecticide-susceptible and -resistant populations of the maize weevil, *Sitophilus zeamais.* Chemosphere, 69(1): 17-24.

Omar S, Marcotte M, Fields P, Sanchez EP, Poveda L, Mata R, Jimenez A, Durst T, Zhang J, MacKinnon S, Leaman D, Arnason TJ Philogène BJR (2007). Antifeedant activities of terpenoids isolated from tropical Rutales. J. Stored Prod. Res., 43(1): 92-96.

Othira JO, Onek LA, Deng' AL, Omolo EO (2009). Insecticidal potency of *Hyptis spicigera* preparations against *Sitophilus zeamais* (L.) and *Tribolium castaneum* (herbst) on stored maize grains. Afr. J. Agric. Res., 4(3): 187-192

Rogelio PM, Mabel FS, Carlos M (2001). Application of molecular mechanics in the total stereochemical elucidation of spicigerolide, a cytotoxic 6-tetraacetyloxyheptenyl-5, 6-dihydro-pyrone from *Hyptis spicigera*. Tetrahedron, 57(1): 47-53.

SAS Institute Inc (2000). SAS/STAT® User's guide. Release 8.2 Edition. SAS Institute Inc., SAS Campus drive, Cary, NC 27513.

Soon-Il K, Young-Joon A, Do-Hyoung K, Han-Seung L (2003). Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobrunchus chinensis*, J. Stored Prod. Res., 39(3): 293-303.

Tapondjou AL, Alder C, Bouda H, Fontem AD, Reichmuth C (2005). Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motchulsky and *Tribolium confusum* du val. J. Stored Prod. Res., 41: 91-102.

Wolansky MJ, McDaniel KL, Moser VC, Crofton KM (2007). Influence of dosing volume on the neurotoxicity of bifenthrin. Neurotoxicol. Teratol., 29(3): 377-384.