Tropical and Subtropical Agroecosystems

CONSTITUENTS OF THE ESSENTIAL OIL OF *Vernonia amygdalina* AS MAIZE WEEVIL PROTECTANTS

[ACEITES DE *Vernonia amygdalina* COMO PROTECTORES CONTRA EL GORGOJO DEL MAÍZ]

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SUMMARY

A laboratory study was conducted to determine the efficacy of *Vernonia amygdalina* essential oil for *Sitophilus zeamais* control. The experimental design was a completely randomized design with four replications. The bioactivity of the essential oil extracted by hydro distillation from leaves of *Vernonia amygdalina* was assessed under laboratory conditions at 27 ±2 ºC, 60-65% R.H and 12: 12h light: dark regimes for it's biological activity against *S. zeamais* (Maize weevil) in maize grains. The essential oil at three levels of application was mixed with 250 g of disinfested maize in one litre volume glass jar. The individual components of the essential oil were identified through GC, GC-MS and GC- Co injection with authentic standards. The identity of a total of 17 constituent compounds of the essential oil of the plant was confirmed and their relative proportion determined. Eucalyptol (1, 8 Cineole) had the highest percentage composition in *V. amygdalina* essential oil (25.1%). All the treatments with the essential oil showed significant level of toxicity to the weevil. The highest dosage (0.3%) of the essential oil of the plant material tested, induced the highest mortality in the *S. zeamais* after 7 days. Grains treated with the essential oil extract significantly reduced the number of Progeny produced by *S. zeamais,* and evoked a high repellent action against the weevil. There was no observable feeding damage on grains treated with the highest dosage of the essential oil extract. It is suggested that the plant is suitable for possible exploitation in insect pest control.

Key words: *Vernonia amygdalina*, essential oil, *S. zeamais,* maize grains, bioactivity.

RESUMEN

Se realize un estudio de laboratorio para determiner la eficacia de los aceites esenciales de *Vernonia amigdalyna* para el control de *Sitophilus zeamais*. La actividad biológica del aceite extractado contra el gorgojo del maíz fue evaluado en condiciones de laboratorio (27 ±2 ºC, 60-65% Humedad relativa y un régimen de luz de 12:12 h). Se aplicó el aceite a tres niveles mezclado con 250g de maíz no infestado. Los componentes del aceite fueron identificados por CG, CG-SM y CG inyección de estándares. Se identificaron 17 compuestos y se determinó su proporción relativa. El Eucaliptol (1,8 cineole) represento la mayor proporción (25.1%). Todos los tratamientos tuvieron un efecto tóxico significativo contra los gorgojos. La dosis mayor (0.3%) indujo la mayor mortalidad a los 7 d. Los granos tratados indujeron una menor progenie de *S. zeamais* e invocaron una mayor acción repelente contra el gorgojo. Se sugiere que Vernonia es una planta con potencial para el control de plagas.

Palabras clave: *Vernonia amygdalina*, aceites esenciales, *S. zeamais,* grano de maíz grains, bioactividad.

INTRODUCTION

Maize (*Zea mays* L.) is one of the foremost cultivated cereals cultivated in the world today (Purseglove, 1975; Rouanet, 1992) It is a major source of dietary carbohydrate in the tropics (Wudiri and Fatobi, 1992). The maize weevil, *Sitophilus zeamais (*Motschulsky) Coleoptera:Curculionidae is a serious pest of stored grains in Africa (Appert 1987). It is associated primarily with maize although it is capable of developing on all cereal grains and cereal products (Walgenbach and Burkholder, 1986; Tipping *et al*.,

1987). Initial infestations of maize grain occur in the field just before harvest and insects are carried into the store where the population builds up rapidly (Appert 1987; Adedire and Lajide 2003). The huge post harvest losses and quality deterioration caused by this pest is a major obstacle to achieving food security in developing countries (Rouanet, 1992).

The efficient and effective control of storage insects like *S. zeamais* has centered mainly on the use of synthetic insecticides (Menn, 1983; Redlinger *et al*., 1988). However, the use of these chemicals is hampered by many attendant problems such as the development of insect resistant strains, toxic residues in food and humans, workers safety and high cost of procurement (Sighamony *et al*., 1990). These problems have necessitated research on the use of alternative eco-friendly insect pest control methods amongst which are the use of botanical pesticides (Cobbinah and Appiah-Kwarteng, 1989, Hassanali *et al*., 1990; Niber, 1994; Jembere *et al*., 1995; Bekele *et al*., 1996; Lajide *et al*, 1998; Asawalam and Adesiyan, 2001; Asawalam and Adesiyan, 2002; Bekele, 2002; Asawalam and Arukwe, 2004).

Vernonia amygdalina (bitterleaf) (Compositae) is a shrub of up to 5m high with minute toothed leaves. It is a perennial crop frequently found in gardens and is native to Africa. The plant is generally multiplied by planting shoots in the soil (Schippers, 2000). The leaves are bitter and are traditionally used in curing stomach ache in the Eastern part of Nigeria.

The overall objective of this study was to evolve a sustainable strategy that will reduce damage of maize grains during storage, by the maize weevil *S. zeamais* through the use of locally available materials at low cost to the peasant farmers and also ensuring environmentally friendly approach.

The specific objectives of this study were:

i To analyze the chemical composition of *V. amygdalina* leaves and to identify the active chemical constituents of the essential oil

ii To evaluate the effect of the essential oil of *V. amygdalina* leaves on mortality, reproduction (on number of F1 progeny) and repellency of *S. zeamais;*

iii To determine the effects of the essential oil on the weight loss of stored maize grains.

MATERIALS AND METHODS

Sitophilus zeamais culture

S. zeamais was cultured in the laboratory at 27 ±2 ºC, 60-65% R.H and 12h: 12h light: dark regime. The food media used was whole maize grains. Fifty pairs of *S. zeamais* were placed in 1-litre glass jar containing 400 g of maize grains. The jars were then covered with nylon mesh held in place with rubber bands. Maize grains used for the study were purchased locally from Dikomba market in Kenya. Grains were disinfested in the oven at 40 °C for 4 hours (Jembere *et al.*, 1995) and kept in the laboratory before use.

Plant materials collection and isolation of their essential oil

V. amygdalina leaves were collected from Umudike, Nigeria. The identity of the plant materials was confirmed at the Michael Okpara University of Agriculture, Umudike, Nigeria herbarium, before using them for the bioassays. Plants were air dried in a well-ventilated area for five days before extraction procedure. Voucher specimens are kept at the University of Agriculture, Umudike herbarium, Nigeria.

The essential oil was extracted by steam distillation using Clavenger apparatus (Guenter, 1949). The condensing oils were collected in n-hexane solvent (Aldrich HPLC grade) and the solution was filtered with a filter paper containing anhydrous sodium sulphate in a funnel to remove any remaining traces of water. Hexane was then removed by distillation at 60 ºC from 'Contes' Short Path distillation apparatus. When condensation stopped, the oil was collected and weighed into small amber- coloured vials.

Analysis of essential oil

Characterisation, identification and determination of relative amounts of components of the essential oil were done through Gas Chromatography (GC), Gas Chromatography - Mass Spectrometry (GC-MS), and GC Co injection of the essential oil with authentic standards.

Gas - Liquid Chromatography (GC) of essential oil

GC analyses were performed on a capillary Gas - Chromatograph Hewlett Packard (HP) 5890 Series II equipped with a split-less capillary injector system, 50 m x 0.20 mm (i.d) cross-linked with HP - ultra 1methylsilicone 0.33 µm (film thickness) capillary column, and Flame Ionization Detector (FID) coupled to HP 3393A Series II integrator. The integrator was used to calculate the peak area.

The carrier gas was nitrogen at a flow rate of 0.84 ml/min. The flow rates of air and hydrogen were 400 and 30.5 ml/min, respectively. The temperature programme comprized of an initial temperature of 40 °C (0 min) to 90 °C at 7 °C/min for 5 mins, then to 115 at 3 °C/min for 5 mins, and finally to 280 °C at 4 °C/min where it was maintained for 20 min.

Gas Chromatography - Mass Spectrometry (GC-MS), of essential oil

GC - MS analysis was carried out on a HP 8060 Series II Gas Chromatograph coupled to VG Platform II Mass Spectrometer in order to identify the essential oil constituents. The MS was operated in the Electron impact mode (EI) at 70 eV and an emission current of 200μA. The temperature of source was held at 180 ^{o}C and the multiplier voltage at 300V.

The pressure of the ion source and MS detector were held at 9.4x 10 $^{-6}$ and 9.4x 10 $^{-6}$ mbar, respectively. The MS had a scan cycle of 1.5 sec (scan duration of 1 sec and inter-scan delay, 0.5 sec). The mass and scan range was set at m/z 1-1400 and 38-650, respectively. The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3(CF_2)_3]_3N$, (Apollo Scientific Ltd., UK). Column used for GC - MS temperature was programmed as in the case of GC analysis but film thickness was 0.5 μm. All GC - MS analysis were made in the splitless mode with helium as the carrier gas.

Preliminary identification of constituents was based on computer matching components of mass spectral data against the standard Wiley and NIST library spectra, constituted from spectra of pure substances and components of the known essential oils, and literature MS data. 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 standards. The standards used were obtained from Aldrich Chemicals UK. Relative proportion of the essential oil was computed in each case from GC- MS peak areas.

Mortality, Progeny development and damage assessment assays

Essential oil was applied to the grains at the rate of 0.012, 0.06 and 0.3% (30, 150 and 750 mg/250 g of grain dissolved in 10 ml of 95% n-hexane and shaken thoroughly to ensure uniform distribution over grain surface. Treated grains were kept for 24 h to allow the hexane to evaporate completely before bioassays were conducted.

Essential oil extracts were mixed separately with 250 g of maize grains in glass jars at the three different dosages indicated above. Two blank controls were run periodically consisting of hexane treated grain and the untreated grain. Ten pairs of 5-7 day old *S. zeamais* adults were introduced into jars containing the different treated and untreated maize grains. Jars were covered with nylon mesh held with rubber bands, and shaken gently for proper admixture. Each treatment was replicated four times. The experiment was arranged in completely randomized design in the laboratory.

Number of dead insects in each vial was counted after 1, 2, 3, 4, 5, 6, and 7 days to estimate maize weevil mortality.

100 (Number dead insects) / (Total number insect)

Data on percentage adult weevil mortality were corrected using Abbott's (1925) formula:

$P_T = (P_o - P_c) / (100 - P_c)$

Where:
P_T = Corrected mortality (%)
P_O = Observed mortality (%)
P_C = Control mortality (%)

In a similar experiment, 10 pairs of *S. zeamais* were introduced into treated and untreated grains. After 30 days oviposition period, parent adults were removed. Insects subsequently emerging were counted to estimate F_I progeny production. Counting was stopped after 33 days to avoid overlapping of generation.

Damage assessment was carried out on treated and untreated grains. Samples of 100 grains were taken from each jar and the number of undamaged and damaged (grains with characteristic holes) grains were counted and weighed. Percentage weight loss was calculated, using FAO (1985) method as follows:

% Weight loss =[UaN-(U+D)] / UaN X 100

Where:
U = weight of undamaged fraction in the sample
N = total number of grains in the sample
Ua = average weight of one undamaged grain
D =weight of damaged fraction in the sample

Repellency

Repellent action of the essential oil extracts against *S. zeamais* was assessed in a choice bioassay system, consisting of two 1-L glass jars connected together at their rims by means of a 30x10 cm nylon mesh tube. A 5.0 cm diameter circular hole was cut at the middle of the mesh for the introduction of test insects. In this device, it was possible to test large quantities of materials. 250 g of maize were put into the glass jars. Grains in one jar were treated with essential oil extracts while untreated grains in the other jar acted as control. Twenty-five adults of *S. zeamais* were introduced into the nylon mesh tube through the circular hole by means of a 5 cm-diameter funnel. Number of insects present at the control (N_C) and treated (N_T) jars were recorded after 1 hour exposure. All repellency assays were replicated four times and

were carried out in the laboratory at 27 ± 2 ºC and 60-65% R.H. Percent repellency (PR) values were computed as:

$PR= [N_C - N_T / N_C + N_T] X 100$

PR data were analyzed using ANOVA after arcsine transforming them.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using a general linear model procedure (SAS, 2000) and significant difference (P>0.05), means were separated by using Student Newman-Keuls (SNK) test.

RESULTS AND DISCUSSION

Essential oil composition

GC profile of V. *amygdalina* essential oil is shown in Figure 1. The analysis of the oil revealed a complex mixture of constituents. A total of 17 compounds were identified in the essential oil of *V. amygdalina* by GC - MS and GC Co - injection with authentic standards (Table 1). Constituents present in >0.94% were the ones identified. The oils are a mixture of monoterpenes and sesquiterpenes. In figure 2 chemical structures of 1- 1, 8 Cineole, 2- Beta pinene 3-Myrtenal are shown. 1, 8 Cineole occurred in the largest quantity (25.11%). This is followed by Beta pinene (14.54%), Myrtenal (6.52%), Transpinocarveol (6.24%), Alpha pinene (4.93%) and Linalool (4.28%).

Table 1. Major identified constituents of *V. amygdalina* essential oil and their relative proportion in the oil.

GC peak number	Component	Peak area (%)	Retention Time
1	Alpha pinene	4.93	16.6
2	Sabinene	2.79	18.43
3	Beta pinene	14.54	18.8
4	1,8 Cineole	25.11	21.53
5	Gamma terpinene	1.03	23.00
6	Ascaridol	1.75	23.38
7	Linalool	4.28	25.40
8	Nopinone	1.49	27.28
9	Transpinocarveol	6.24	28.48
10	Thujen alcohol	2.31	28.75
11	Pinocarvone	3.37	29.43
12	Cryptone	3.87	30.68
13	Unknown	4.90	30.98
14	Myrtenal	6.52	31.48
15	Myrtenol	3.50	32.00
16	Cuminal	0.97	33.85
17	Phellandral	1.47	35.88

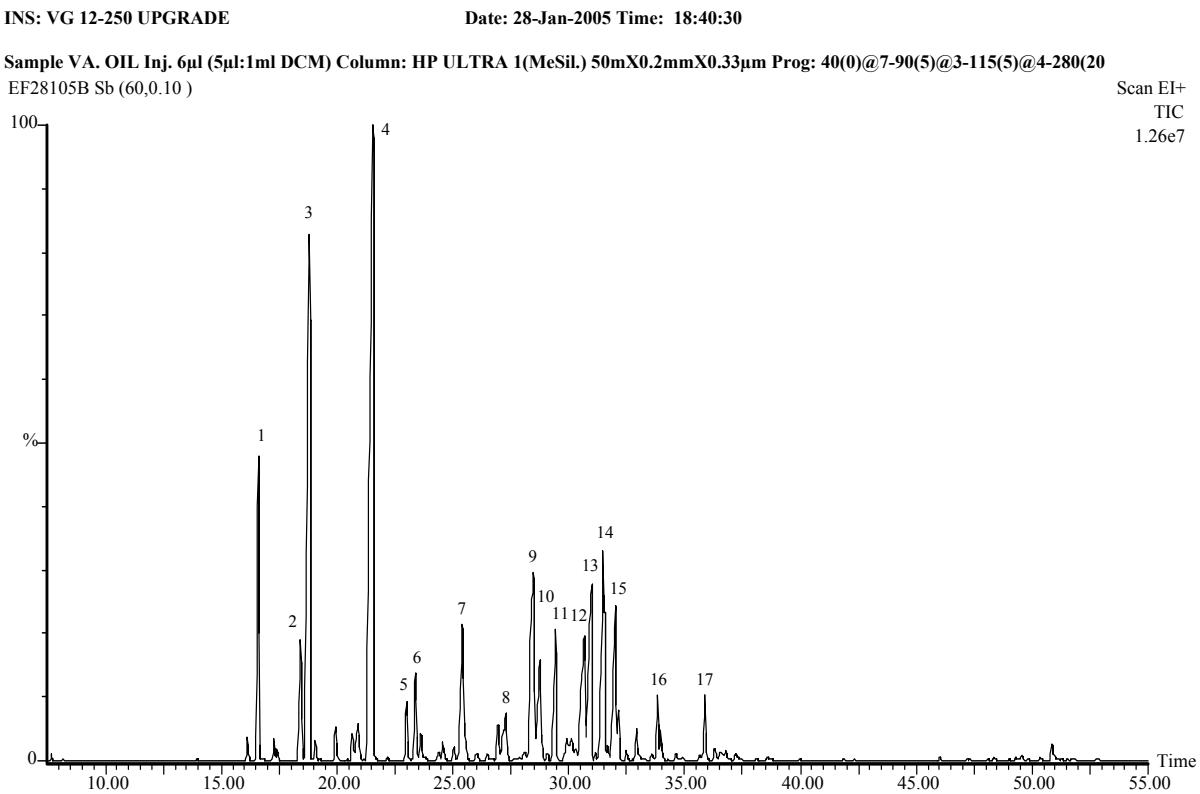
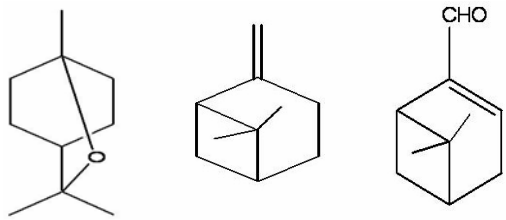


Figure 1. GC-MS Chromatogram for *Vernonia amygdalina* essential oil.



1, 8 Cineole Beta pinene Myrtenal

Figure 2. Chemical structures of major identified constituents of *V. amygdalina* essential oil.

Adult mortality in grains

Figure 3 shows the cumulative mean percentage mortality of *S. zeamais* in maize grains treated with different concentrations of *V. amygdalina* essential oil.

All treatments with essential oils showed a significant level of mortality. The highest dosage (750 mg corresponding to 0.3%) of essential oils of the plant material tested induced the highest mortality in *S. zeamais* at 7 days after treatment. There was no mortality of *S. zeamais* in the untreated grains. Essential oil of *V. amygdalina* evoked high mortality in the weevil (82%) at 0.3%.

Toxicity is one of the various effects of plant terpenoids to insects. Thus, the toxic effects of plant materials might be attributed to their essential oil composition. The toxic effect of plant essential oils has been reported by various authors (Hassanali *et al.,* 1990; Weaver *et al.,* 1991; Jembere *et al.,* 1995; Bekele *et al*., 1996; Bekele *et al.,* 1997; Bekele and Hassanali 2001; Renault - Roger *et al*., 1993; Bouda *et al*., 2001), who attributed their effect to different terpene constituents of the essential oils. Similarly, toxicity of *V. amygdalina* essential oil against *S. zeamais* in the present study might be attributed to their essential oil constituents. The essential oil of *V. amygdalina* contained various terpenoids and induced 82% mortality with essential oil at 750 mg/250 g application rate after 7 days of treatment.

Lethal toxicity of the major components of essential oil of the plant materials, and those of selected blends of essential oil require further investigation. This will help to elucidate the role and relative importance of the major constituents of the essential oil in conferring some of the observed biological effects.

Effect of essential oil on progeny development

The number of progeny produced by *S. zeamais* in untreated grains and grains treated with different concentrations of *V. amygdalina* essential oil is shown in Figure 4. Significantly higher number of F1 progenies was produced by *S. zeamais* in the untreated grains compared, with grains treated with the essential oil extract. The essential oil extract, significantly reduced the number of F1 progeny produced by the weevil. No progeny was produced in grains treated with the highest dose of 750 mg (0.3%), of the essential oil extract tested.

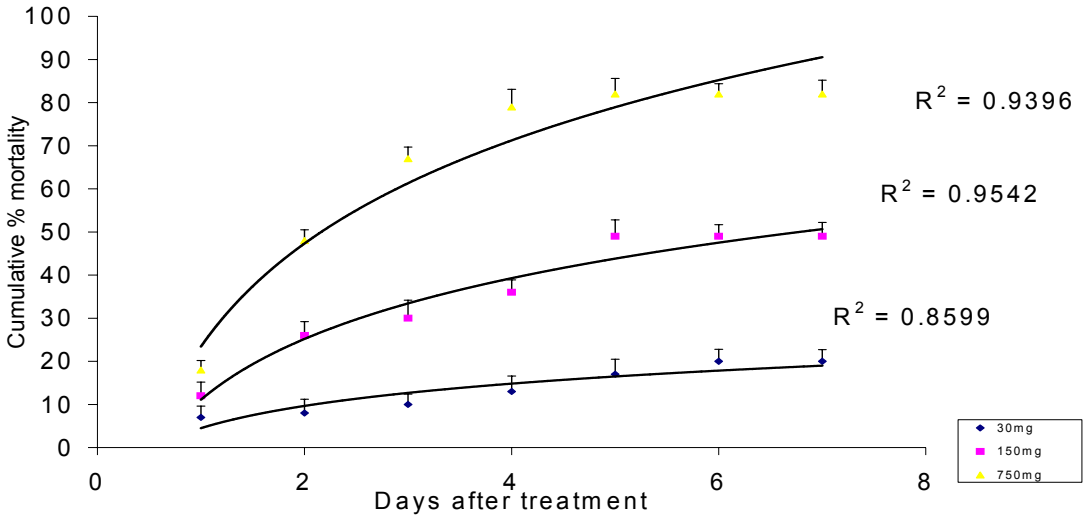


Figure 3. Cumulative mean % mortality of *S. zeamais* in maize grains treated with different concentrations of *V. amygdalina* essential oil (n = 4).

This study has shown that the essential oil gave good protection to the stored maize grains by suppressing reproduction (F1 progeny emergence). Bekele et al., (1997) observed similar result, where grains treated with essential oil extract of *Ocimum kenyense* significantly reduced the number of progeny produced by *S. zeamais*.

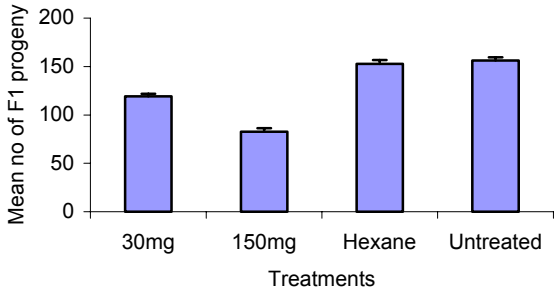


Figure 4. Effect of *V. amygdalina* essential oil on the number of F1 progeny produced by *S. zeamais* in stored maize grains.

Effect of the essential oil on weight loss of grains

Weight loss caused by *S. zeamais* to treated and untreated grains are shown in Figure 5. Weight loss caused by *S. zeamais* was significantly ($P < 0.05$) higher in the control compared with grains treated with essential oil extract of *V. amygdalina*. Grains with higher dosages of essential oil extract 150 and 750 mg/250g grain gave better protection against infestation by *S. zeamais* compared to the untreated grains. There was no weight loss in the 750 mg of the essential oil extract.

The result obtained from this study indicated that weight loss in stored maize grains is related to the number of insects present. This finding is also in agreement with Jembere *et al*., (1995). Since there was no weight loss recorded in the 750 mg of the essential oil, this result suggests that the chemical constituents of the essential oil may have inhibited feeding by the weevils.

Repellency effect of the essential oil on *S. zeamais*

Figure 6 represents the mean repellency values of the essential oil extract at different dose levels against *S. zeamais* in stored maize grains. All the dosages were repellent to *S. zeamais*, with the 750 mg evoking the highest repellent action. Analysis of variance indicated significant differences ($P < 0.05$) between the responses of the three dosages tested. The untreated grains induced no repellency against *S. zeamais*.

Essential oil was significantly ($P < 0.05$) repellent to the weevil relative to the control. This is similar to the findings of Hassanali *et al*., (1990) who demonstrated the repellent effect of essential oils of *Ocimum suave* leaves and the dried unopened flower buds of *Eugenia caryophyllata* cloves against *S. zeamais* in olfactometric assays. Eugenol was found to be highly repellent to the four beetle species tested, with overall repellency in the range of 80 - 100% (Obeng - Ofori and Reichmuth, 1997).

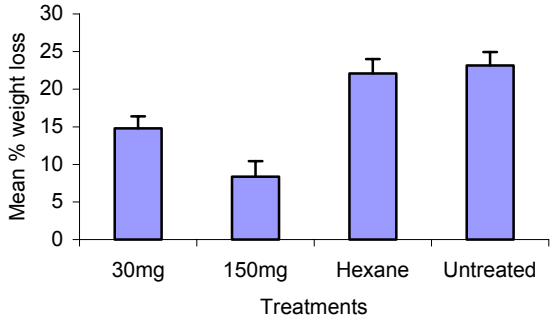


Figure 5. Mean percentage weight loss for different dose levels of *V. amygdalina* essential oil against *S. zeamais* in stored maize grains.

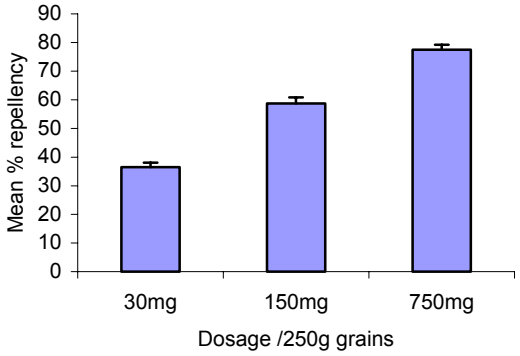


Figure 6. Mean percentage repellency for different dose levels of *V. amygdalina* essential oil against *S. zeamais* in stored maize grains.

All the aliphatic ketones and aldehydes of *Commiphora rostrata* constituent have been demonstrated to possess greater repellency against *S. zeamais* than the synthetic commercial insect repellent N, N - diethyl toluamide (DEET) (Lwande *et al*., 1992).

Ndungu *et al* (1995) reported that 1 - alpha terpenol and 2 - dodecanone were the most repellent

components of *Cleome monophylla* essential oil against *Rhipicephalus appendiculatus* and *S. zeamais*. Thus, the repellent effect of the essential oil in this study could be attributed to its major components.

CONCLUSION

This study demonstrates the potential of plant volatile essential oil for use against *S. zeamais*. The essential oil deserve further investigation to help scientists in identifying cheap and readily available environmentally friendly pest control agents.

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