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Full Length Research Paper

Contribution of different constituents to the toxicity of the essential oil constituents of *Vernonia amygdalina* (Compositae) and *Xylopia aetiopica* (Annonaceae) on maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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The lethal toxicity of the major constituent of the essential oils of *Vernonia amygdalina* and *Xylopia aetiopica*, and of selected blends of these against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) was compared with those of the full blends of the essential oils. The compounds were assayed in amounts and proportions present in the minimum 100% lethal dose of the essential oils. The major component of *X. aetiopica* was found to be largely responsible for the toxic action of its essential oil against *S. zeamais*, On the other hand the toxic action of *V. amygdalina* was due to the combined effects of its constituents, either with or without significant individual toxic action of their own against the insects. The implication of these results and their implication in screening and using plants and their phytochemicals for *S. zeamais* control is highlighted.

Key words: *Vernonia amygdalina, Xylopia aetiopica*, essential oils, *Sitophilus zeamais,* synergism.

INTRODUCTION

Ethnobotany has played a very important role in traditional methods of protection against storage pests in Africa and Asia (Hassanali et al., 1990; Niber, 1994; Okonkwo and Okoye, 1996). In view of the potential of plant products in small-scale farm holdings typical of the bulk of grain production in Africa, there has been growing interest in evaluating their efficacies and in elucidating the basis of their protective action (Weaver et al., 1991; Niber, 1994; Jembere et al., 1995; Bekele et al., 1996, 1997; Obeng-Ofori et al., 1997a; 1997b; Bekele and Hassanali 2001).

Xylopia aethiopica (Dunal) A. Rich (African Pepper) is a peppery spice of Nigeria and is often used as food condiments and for medicinal purposes. Among the Ibos in Eastern part of Nigeria, it is used to aid uterine contraction after baby delivery (Ijeh et al., 2004). *Vernonia amygdalina* Del (bitterleaf) (Compositae) is a shrub of up to 5 m high with minute toothed leaves. It is a perennial crop frequently found in gardens and is native to Africa. The plant is readily available and generally multiplied by planting shoots in the soil (Schippers, 2000).

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The leaves are bitter and, in the eastern part of Nigeria, are traditionally used in post-harvest protection. *V. amygdalina* is also widely eaten as a vegetable, ethnopharmacologically used against a number of ailments including relieving stomach aches, and has been confirmed to have anti-bacterial, anti-fungal, nematicidal, antiplasmodial and insecticidal properties (Iwalokun et al., 2004).

Previously we evaluated the essential oils obtained from *X. aetiopica* and *V. amygdalina* against the maize weevil, *Sitophilus zeamais* Motschulsky a serious post-harvest pest in Africa and found varying degrees of repellent, toxic and protective effects against the weevil (Asawalam et al., 2006a, 2006b). The aim of the present study was to compare the toxicity of the essential oils of *X. aetiopica* and *V. amygdalina* with those of their major constituents, individually and in selected blends, with the purpose of identifying the constituents important in conferring the biological activities of the two plants against the maize weevil. It was envisaged that this information would help guide the selection of such plants in traditional post-harvest practices.

MATERIALS AND METHODS

Sitophilus zeamais culture

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

Plant collection and isolation of their essential oil

X. aetiopica and *V. amygdalina* leaves were collected from Umudike, Nigeria in Jauary 2006. The identity of the plant was confirmed at the herbarium of Michael Okpara University of Agriculture, Umudike, Nigeria, before using them for the present study. Plants were air-dried in a well-ventilated area for five days before extraction. Voucher specimens are kept at the University herbarium. The essential oil was extracted by steam distillation using Clevenger apparatus. The condensing oils were collected in n-hexane (Aldrich HPLC grade) and the solution filtered and exposed to anhydrous sodium sulphate to remove any traces of water. Hexane was then removed by distillation at 60°C from 'Contes' Short Path distillation apparatus and the oil collected and weighed, and stored in small amber-coloured vials.

Analysis of essential oil

Gas chromatographic (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 areas. The carrier gas was nitrogen at a flow rate of 0.84 ml/min. The temperature programme comprised of an initial temperature of 40°C (0 min) to 90°C at 7°C/min, a hold at this temperature for 5 min, then to 115°C at 3°C/min followed by another hold for 5 min, and finally to 280°C at 4°C/min where it was maintained for 20 min.

Gas chromatography–linked Mass Spectrometry (GC-MS) analysis was carried out on a HP 8060 Series II gas chromatograph coupled to VG Platform II Mass Spectrometer (manufactured by Micromass, UK, formerly known as VG Biotech). The MS was operated in the Electron impact mode (EI) at 70 eV and an emission current of 200 µA. The temperature of source was held at 180°C and the multiplier voltage at 300 V. The pressure of the ion source and MS detector were held at 9.4×10^{-6} and 9.4×10^{-6} mbar, respectively. The MS had a scan cycle of 1.5 s (scan duration of 1 s and inter-scan delay, 0.5 s). 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 (film thickness 0.5 µm) temperature was programmed as in the case of GC analysis. All GC-MS analyses 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 samples. The samples used were obtained from Aldrich Chemicals UK. Relative proportion of the essential oil was computed in each case from GC-MS peak areas.

Comparative toxicity tests of essential oils and their constituents

These were conducted in pyrex glass petri dishes, 9 cm diameter (Weaver et al., 1991; Bekele and Hassanali, 2001). A sample of each essential oil was weighed, dissolved in 1 ml of acetone Merck (95 - 97%) and delivered to a Whatman No 1 filter paper (9 cm dia.) in the petri dishes. Various doses of the essential oils (2.5 - 0.5 mg cm^{-2}) were used to identify the minimum doses, which gave 100% mortality of the weevil. Acetone was allowed to evaporate off (20 min) prior to the introduction of the weevil. Acetone treated filter papers were used as controls. 10 pairs of *S. zeamais* were introduced and the petri dishes were kept for 24 h in the laboratory maintained at 26 ± 1°C and 60 ± 5% relative humidity.

The number of dead insects was counted after 24 h. Insects were considered dead if they were immobile and did not react to three probings with a blunt dissecting probe. The experiment was repeated with commercially available constituents (Aldrich) of the essential oils and blends of these at their natural proportions (GC) in the oils and in amounts present in the minimum 100% lethal dose of the oils, with each component subtracted from the blend in turn to determine its relative contribution to the overall toxicity of the natural oil (Bekele and Hassanali, 2001).

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) procedure (SAS, 2002) and means were separated by Student Newman-Keuls (SNK) test at $P < 0.05$. Data were arcsine-transformed prior to analysis. Probit analysis for the determination

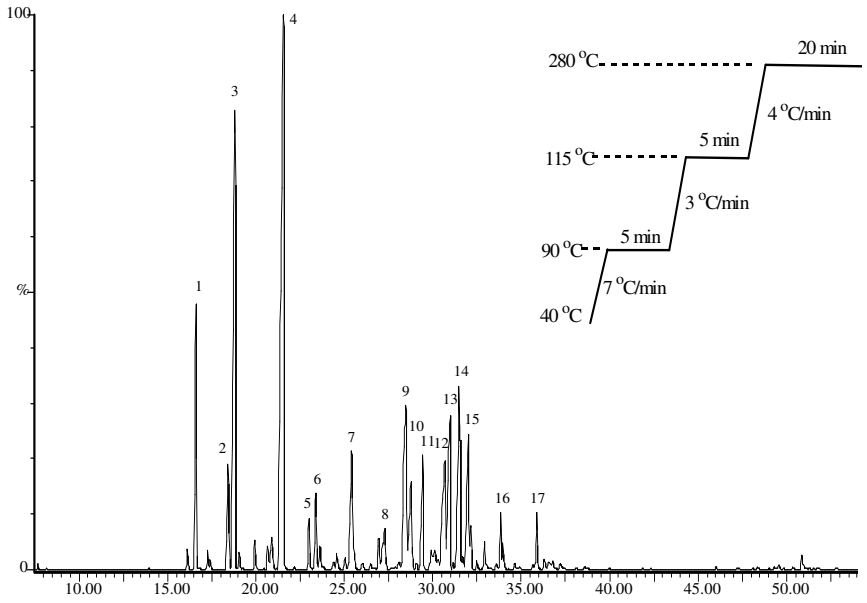

Figure 1. GC-MS chromatogram for *V. amygdalina* essential oil.

Table 1. Major identified constituents of *X. aetiopica* essential oil and their relative proportion in the oils.

GC peak number	Component	Peak area (% composition)	Retention time
1	α-Pinene	5.14	16.55
2	β-Phellandrene	2.66	18.35
3	β-Pinene	15.34	18.73
4	1,8-Cineole	26.63	21.45
5	γ-Terpinene	1.53	23.30
6	Linalyl acetate	3.88	25.33
7	Pinanol	6.02	28.38
8	Verbenene	2.06	28.60
9	Pinocarvone	3.19	29.33
10	L-Carveol	4.44	30.53
11	Terpinene-4-ol	5.24	30.85
12	Myrtenal	6.64	31.35
13	Myrtenol	2.81	31.88
14	Cuminal	1.79	33.78
15	Phellandral	1.13	35.83

of LC_{50} was based on the methods of Busvine (1971) and Finney (1978).

RESULTS

Essential oil composition

The analysis of *V. amygdalina* and *X. aetiopica* essential oils revealed a complex mixture of constituents (Figures 1 and 2). Components were identified by comparison of the mass spectral fragmentation pattern with those in Wiley and NIST library and by GC-co injection with authentic samples (Tables 1 and 2).

Toxicity of individual and blends of constituent compounds of the essential oils

In a minimum dose of 1.74 $mgcm^{-2}$ of filter paper, *X. aetiopica* essential oil caused 100% mortality of *S. zeamais*. When all the six components of the essential oil

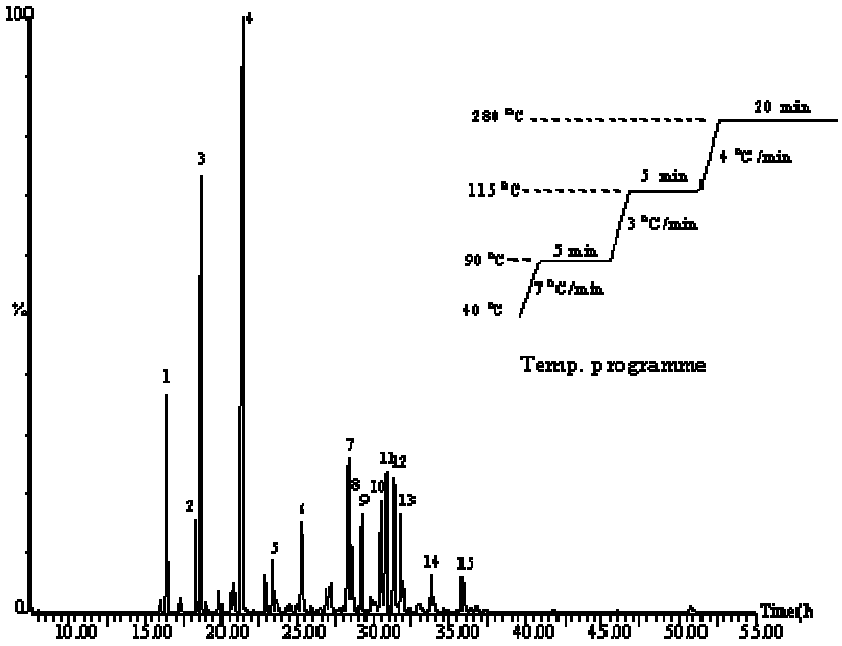

Figure 2. GC-MS chromatogram for *X. aetiopica* essential oil.

Table 2. Major identified constituents of *V. amygdalina* essential oil and their relative proportion in the oil.

GC peak number	Component	Formula	Peak area (% Composition)	Retention time
1	*α*-Pinene	$C_{10}H_{16}$	4.9	16.6
2	Sabinene	$C_{10}H_{16}$	2.8	18.4
3	*β*-Pinene	$C_{10}H_{16}$	14.5	18.8
4	1,8-Cineole	$C_{10}H_{18}O$	25.1	21.5
5	γ-Terpinene	$C_{10}H_{16}$	1.0	23.0
6	Ascaridol	$C_{10}H_{16}O_2$	1.8	23.4
7	Linalool	$C_{10}H_{18}O$	4.3	25.4
8	Nopinone	$C_9H_{14}O$	1.5	27.3
9	Trans-Pinocarveol	$C_{10}H_{16}O$	6.2	28.5
10	Thujen alcohol	$C_{10}H_{18}O$	2.3	28.8
11	Pinocarvone	$C_{10}H_{14}O$	3.4	29.4
12	Cryptone	$C_9H_{14}O$	3.9	30.7
13	Unknown		4.9	30.9
14	Myrtenal	$C_{10}H_{14}O$	6.5	31.5
15	Myrtenol	$C_{10}H_{16}O$	3.5	32.0
16	Cuminal	$C_{10}H_{12}O$	1.0	33.9
17	Phellandral	$C_{10}H_{16}O$	1.5	35.9

were present, 100% mortality of the insect was obtained (Table 3). Absence of 1,8-Cineole caused a large drop in the activity of the resulting 5-component blend showing that this component contributes most to the activity of the essential oil. Similar results were obtained with *V. amygdalina* (Table 4) essential oil.

DISCUSSION

Our previous studies showed that the essential oil derived from these plants provided varying degrees of protection against *S. zeamais* (Asawalam et al., 2006a, 2006b). This present study compared the toxic effects of

Table 3. Percent mortality of *S. zeamais* adults exposed to *X. aetiopica* constituents individually and in blends at doses equivalent to the relative proportion of each compound in the 100% lethal dose of the essential oil.

No.	Treament[a] (compounds)	Mean % mortality of *S. zeamais* ±SE
1	Essential oil [b]	100±0.0a
2	1,8-Cineole	54±2.6b
3	*B*-Pinene	46±2.1c
4	Myrtenal	0±0d
5	Pinanol	0±0d
6	***α*** -Pinene	0±0d
7	L- Carveol	0±0d
8	2-7 combined	100±0.0a
9	3-7 combined without 2	42±2.4c
10	2-7 combined without 3	53±1.5b
11	2-7 combined without 4	100±0.0a
12	2-7 combined without 5	100±0.0a
13	2-7 combined without 6	100±0.0a
14	Acetone	0±0.0d

S.E ± = Standard error of the mean.
[a]Treatment means (average of six replicates) within each column followed by the same letter are not significantly different at 5% level of probability according to Student – Newman – Keuls (SNK) test.[b]Minimum lethal dose of 1.74 mg cm^{-2}.

Table 4. Percent mortality of *S. zeamais* adults exposed to *V. amygdalina* constituents individually and in blends at doses equivalent to the relative proportion of each compound in the 100% lethal dose of the essential oil.

No.	Treament[a] (compounds)	Mean % mortality of *S. zeamais* ±SE
1	Essential oil [b]	100±0.0a
2	1,8-Cineole	63±2.1b
3	*β*-Pinene	37±2.4d
4	Myrtenal	0±0f
5	Transpinocarveol	0±0f
6	*α*-Pinene	0±0f
7	Linalool	0±0f
8	2-7 combined	100±0.0a
9	3-7 combined without 2	27±2.4e
10	2-7 combined without 3	52±2.0c
11	2-7 combined without 4	100±0.0a
12	2-7 combined without 5	100±0.0a
13	2-7 combined without 6	100±0.0a
14	Acetone	0±0.0d

S.E ± = Standard error of the mean.
[a]Treatment means (average of six replicates) within each column followed by the same letter are not significantly different at 5% level of probability according to Student – Newman – Keuls (SNK) test.[b]Minimum lethal dose of 2.44 mg cm^{-2}.

major constituents of the essential oils of *V. amygdalina* and *X. aetiopica* and various blends of these with those of the full blends of the essential oil against *S. zeamais*. The aim was to elucidate the roles and relative importance of the major constituents of the oils in conferring some of the observed biological effects of the plants, and to use this information to guide effective deployment of such plants by small-scale farmers in post harvest grain protection.

This study shows that the major components of the oil

contributed to its toxicity to *S. zeamais.* None of the individual components (*β*-pinene, myrtenal, pinanol, α-pinene, L-carveol, transpinocarveol and linalool) that were tested, had significant toxic effect against *S. zeamais*. However, the mixture of the major components were as effective as the essential oil showing clearly that the toxic action of the oil results from the combined action of some of its major constituents. Absence of 1,8-cineole in both blends resulted in the largest drop in the toxic action of the resulting blend.

The results stress the importance of evaluating plant components in blends to elucidate their full potency in a given bioactivity. Most previous studies on effects of essential oils on post-harvest and other pests have focused on the identification of active components rather than mixtures. In the present study, the result obtained with 1,8-cineole (the major component of the oil of *V. amygdalina* and *X. aetiopica*) against *S. zeamais*, where it appears to be responsible for the toxicity of the oil, may be less common. Blend effects in bioactivity of plant products may well be the norm. Bekele and Hassanali (2001) obtained similar results in their study on blend effects in the toxicity of the essential oil constituents of *Ocimum kilmandscharicum* and *Ocimum kenyense* (Labiateae) on two post-harvest insect pests. Indeed, there is accumulating evidence of adaptive value of phytochemical diversity in ecological interactions among plants and their associated herbivores and pathogens (Bekele and Hassanali, 2001). This has an important practical implication in the strategy adopted in the search for and use of plants and their phytochemicals for pest control.

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