Tithonia Diversifolia (Hemsl.) A. Gray Essential Oil: A Potential Biopesticide for Management of Sitophilus zeamais (Maize Weevils)

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ABSTRACT

This study evaluated the pesticidal activities of essential oils (EO) from the leaves of T. diversifolia against Sitophilus zeamais using fumigation, repellency and contact toxicity assays. Forty-five compounds were identified in the EO constituting 96.7% of the oil from GC/MS analysis. The main constituents included α-pinene (42.2%), β-pinene (16.2%) and β-caryophyllene (12.2%). The oil displayed strong activities against adult insects as expressed by fumigant activity with LC_{50} value 10.2 mg of oil/L of air ($p<0.05$), a contact toxicity mortality with LD_{50} value of 12.3 µg/adult insect and class III repellency achieved within 30 min at a very low conc (30 ul/cm^2 paper discs). The high potency of the oils could be attributed to the major components (α-pinene, β-pinene and β-caryophyllene), making it a potential biopesticide for protection of maize grains from Sitophilus zeamais.

Key words: Tithonia diversifolia, essential oils, Sitophilus zeamais, pesticidal.

INTRODUCTION

The repetitive use of synthetic pesticides for protection of cereal grains and control of pests may result in development of resistant insect populations, toxic residues adhering to food and accumulation in the environment [1]. The use of biopesticides including essential oils (EO) to control pests has gained momentum since they are effective, present smaller effects on the fauna and flora, pose greater availability, and degrade easily compared to synthetic compounds [2]. They are also cheap, non-toxic to non-target organism, and are less likely to result in resistance in the target organisms. Many plants that are environmentally-friendly and readily available in nature can be used in minimization of grain losses during storage by control of pests in stored products. The susceptibility of crop plants to insect pests and the increase in costs of synthetic pesticides have caused the need for application of alternative effective and biodegradable substances including EO, extracts and isolated compounds [3].

Plant secondary metabolites including alkaloids, monoterpenes, sesquiterpenes, phenols and coumarins, are known to exhibit toxicity, antifeedant, repellency and growth regulating effects against a range of insect pests including Sitophilus zeamais (Motsch.) (Coleoptera: Curculionidae), commonly referred to as the maize weevil [4]. This is a storage pest that has been associated with destruction of maize grains resulting in a decline in production over the years [5].

Most synthetic pesticides are environmental hazards causing serious problems including direct toxicity to predators, pollinators, fish and man. Over the years, synthetic pesticides such as organochlorines, organophosphates, carbamates, pyrethroids and neonicotinoids have been effective for control of S. zeamais [6]. However, the use of these chemicals has resulted in numerous challenges including development of resistant insect strains, toxic residues in foods and humans, workers' safety and most of them are expensive [6]. Tithonia diversifolia [Hemsley] A. Gray (Asteraceae), also referred to as wild sunflower is a perennial plant known to contain compounds with antioxidants, antibacterial, pesticidal and medicinal properties [7]. It is commonly found scattered along rivers and roadsides, wastelands, hedges, along narrow paths and around crop fields, and homesteads [8]. It grows both within and outside the tropics parts of central, south and North America, Pacific islands like Hawaii, Guam, New Caledonia, French, Polynesia, Palau and Indian island [9]

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and in tropical parts of Asia as well as Africa [10]. In Africa, it is common in many countries including Egypt, Guinea, Nigeria Cameroon, South Africa, Zimbabwe, Malawi, Congo, Ethiopia, Tanzania, Uganda and Kenya [7, 11, 12]. In Kenya it was first introduced as an ornamental plant in 1940s and now has since spread to many regions including Coastal, Mount Kenya, parts of the Rift Valley and western Kenya [7, 13, 19].

T. diversifolia has been evaluated for antibacterial, antimalarial and insecticidal activities. It has been used for treatment of menstrual pains and diabetes mellitus and for control of insects pests such as aphids, beetles, mosquitoes, maize weevil [13, 14]. Most studies have focused on the potency of extracts (aqueous, methanol, ethyl acecate, DCM and hexane extracts) against insects such as aphids, beetles, mosquitoes, maize weevil, bean weevil and whiteflies among others [7, 14, 15, 16]. However, information on activity of essential oils against insect pests including Sitophilus zeamais is limited. This study aimed at characterizing of the EO of T. diversifolia and determination of its pesticidal activities against S. zeamais.

MATERIALS AND METHODS

Rearing of Sitophilus zeamais

The insects were cultured under laboratory conditions of 25 ± 2 ºC and 65 ± 5% relative humidity in Maseno University Department of Zoology laboratory. A mass of 300 g of the maize grains were transferred into 1 L glass jars which were covered with a fine mesh cloth for ventilation and to prevent the insects from escaping. Fifty adult of S. zeamais were then introduced into the each glass jar and kept in the incubated for seven days for the insects to lay eggs and multiply.

Plant material, extraction and analysis of the essential oil

Fresh leaves of T. diversifolia were collected in April 2019 at the beginning of flowering season from Maseno forest within Maseno University. The species were identified at Department of Botany Maseno University and a voucher specimen (MU-TDV-020-2019) was deposited. 500 g of fresh leaves was subjected to hydro distillation in a Clevenger-type apparatus for 6 h. The EO obtained was extracted with n-hexane, dried using anhydrous sodium sulphate and analysed using gas chromatography mass spectrometry (GC/MS). The GC/MS system used comprised of a HP 6890 Series GC system (Agilent Technology, California, USA), coupled with a 5973 mass selective detector and an HP-5ms fused silica capillary column with a 5% phenyl-methylpolysiloxane stationary phase (30 m X 0.32 mm X 0.25 µm). The oven temperature program was initiated at 40 °C for 1 min then raised to 230 °C at a rate of 3 °C min^{-1} for 10 min. Helium was used as the carrier gas at 1.0 $mLmin^{-1}$ flow rate with a split ratio of 1/50. Detector and injector temperatures were 250 and 230 °C, respectively and spectra were obtained following electron impact ionization at 70 eV (35 to 550 m/z). The essential oil compounds were identified by comparing their retention indices, mass spectra fragmentation with those on the NIST® library version 2004.

Fumigant toxicity

Fumigation assay involved impregnating 3-cm diameter Whatman No. 41 filter paper discs with 10 µL of oil at a different concentration (0: acetone, 1.6, 3.2, 4.6 and 6.15 mg/L in acetone). This corresponded to 0, 6.4, 12.8, 18.6 and 24.6 mg of oil/L of air in the glass jars. Acetone was used as negative control while MeBr was used as the positive control. Each impregnated filter paper was allowed to dry for 15 min and thereafter, attached to the bottom of the screw caps of a gas tight 250 mL glass jars. Preliminary experiments showed that that 15 s were adequate for the evaporation of acetone. The caps were tightly screwed on glass jars containing 10 adult insects. The insects had no contact with the suspended impregnated filter paper and stayed at the bottom of the jars throughout the experiments. Control insects were kept under the same conditions although the filter paper discs were impregnated with only acetone but no oil. The treatments and controls were incubated at 28–30 °C and 70–80% relative humidity for 24 and 48 h. This was done in five replicates. The number of dead and live insects in each jar was counted at the end of a 24 and 48 h exposure periods. Insects were considered dead when no leg or antennal movements were observed. Percentage insect mortality was calculated using Abbott's [32] correction formula for natural mortality in untreated controls. Experiments were arranged in a completely

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randomized design. The LC_{50} (conc required to attain 50% mortality for a given time for each conc of extract) values with their fiducial limits were calculated by probit analysis using SPSS version 16.0 software package.

Insect repellency assay

Insect repellency tests were conducted according to method by Islam et al. [33] with slight modifications. 14 cm diameter filter papers (Whatman no. 41) were each placed in a petri dish with same size. A volume of 1 ml essential oil was then was uniformly applied to half of the papers to result in concentrations of 10, 20, 30, 40 and 50 ul/cm^2 and absolute acetone was used a negative control was applied to the rest of the filter papers. The contents of each petri dishe were left for 10 min at 25 ± 1 °C and 70 ± 10% relative humidity to evaporate the solvent and ten S. zeamais adults were placed in the centre of each dish. The numbers of weevils in the control (NC) and treatment (NT) dishes were recorded after 30, 60, and 90 min in 5 replicates. The percentage of repellency (PR) was obtained by using the equation 1.

$$PR = \frac{(NC - NT)}{(NC + NT)} \times 100 \quad \text{..... Equation 1}$$

Repellency was further classified into class 0 (PR ≤ 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%), and class V (PR = 80.1–100%) in as described by Benzi et al. [26].

Contact Toxicity

Determination of contact toxicity was conducted as described by [19] with modifications. Five serial dilutions of EO (120.0, 200.0, 350.0, 500.0 mg/L) were prepared using acetone which was also used as negative control while pyrethrine (120.0 mg/L) was used as positive control. An aliquot of 50 µL of each dilution and control was applied topically to the dorsal thorax of each insect, using a 1 mL Hamilton micropipette. This translated to conc levels of 6.0, 10.0, 17.5 and 25.0 µg of EO/adult insect respectively for the EO, and 0 and 6.0 µg/adult insect for the negative and positive controls respectively. For each concentration level, ten insects were treated and transferred to glass vials with culture media (maize flour). This was repeated for the insects treated with the control solvent. The experiment was conducted in six replicates. The vials containing the insects (10 insects/vial) were kept in incubators at 29-30 °C and 70- 80% relative humidity. Mortality of the insects was observed after 6, 12, 24, 36 and 48 h. The observed mortality data were corrected using Abbott's [32] correction formula. Results from all replicates were subjected to probit by analysis using SPSS version 16.0 software package for determination of LD_{50} (the amount of a chemical that is lethal to one-half (50%) of the experimental animals exposed to it).

RESULTS AND DISCUSSION

Characterization of essential oil from T. diversifolia leaves

The essential oil (EO) obtained from hydro-distillation of T. diversifolia leaves was light yellow in colour with a yield of 0.0025% w/w and the density of the concentrated essential oil was determined to be 0.80 g/mL. This yield was higher than that reported by Wanzala et al. [13] from hydro distillation of T. diversifolia leaves obtained from the slopes of Mt Elgon (0.000015% w/w) in western Kenya but lower than value reported by Moronkola et al. [17] (0.019% w/w) from Nigeria. The difference in the yields could be due to changes in environmental and climatic conditions in the different places [18]. The oil was observed to be insoluble in water but soluble in organic solvents including n-hexane, diethyl ether, DCM and DMSO.

Forty five compounds were identified in the EO constituting 96.7% of the oil (Table 1). The major components included α-pinene (42.2%), β-pinene (16.2%), β-caryophyllene (12.2%), followed by limonene (8.5%) and (E)-nerolidol (6.5%). Although the percentage weight of monoterpenes (68.3% w/w) in the oil was higher than those of sesquiterpenoids (29.3% w/w), a majority of the constituents comprised of sesquiterpenoids representing 46.7% (21 of the 45 components), followed by monoterpenes accounting for 42.2% (19 of the 45 compounds) of the whole oil. The main components were similar to those obtained from leaves of T. diversifolia obtained from Mt Elgon in western Kenya (α-pinene: 63.64%, β-pinene: 15.0%, iso-caryophyllene:

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7.62% and nerolidol: 3.70%) [13], and in Osun state, Nigeria (α-pinene: 32.9%), β-pinene (10.9%), and β-caryophyllene (20.8%) [17], although their respective percentages in the oils varied.

Table 1: Composition of the essential oil obtained from the leaves of T. diversifolia

Peak No.	Compounds	RI	%Composition
1	α-Pinene	942	42.2
2	Camphene	953	0.5
3	Sabinene	969	0.8
4	β-Pinene	980	16.2
5	β-Myrcene	990	0.2
6	α-Phellandrene	1002	Tr
7	∂-3-carene	1011	0.1
8	p-Cymene	1020	Tr
9	Limonene	1027	7.5
10	1, 8-Cineol	1030	Tr
11	(Z)-β-Ocimene	1032	Tr
12	(E)-β-Ocimene	1050	Tr
13	α –Terpinene	1054	0.2
14	α-Terpinolene	1088	0.3
15	Linalool	1100	0.2
16	Terpinen-4-ol	1177	0.1
17	α-Terpineol	1188	Tr
18	Decane-2,6,8-Trimethyl	1205	0.2
19	Geraniol	1249	Tr
20	α-Cubebene	1345	0.1
21	α-Copaene	1376	1.0
22	α-Gurjunene	1409	0.6
23	Bicyclo-2,2,2-octa-2,5-diene, 1,2,3,6-tetramethyl	1412	0.2
24	β-caryophyllene	1419	12.2
25	β-Gurjunene	1428	Tr
26	Aromadendrene	1440	Tr
27	α-Humulene	1452	0.2
28	β-Humulene	1454	0.3
29	(E)- β-Farnesene	1456	Tr
30	β-Ionane	1462	0.1
31	Germacrene D	1481	0.3
32	1-Tridecanone	1497	2.1
33	β-Bisabolene	1509	0.2
34	γ-Cadinene	1513	0.3
35	δ-Cadinene	1525	0.2
36	Elemicin	1555	Tr
37	(E)-Nerolidol	1564	7.5
38	Caryphyllene oxide	1581	0.4
39	Juniper camphor	1594	0.3
40	Cyclodecene	1605	0.9
41	Pentadecanone	1612	0.8
42	Cycloundecanone	1630	0.2
43	α-Bisabolol	1685	0.3
44	Farnesol	1717	Tr

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45	1-Octadecanol	2024	Tr
	Total		96.7

*Tr: trace, i.e., <0.1%; [a]Retention index on HP-5ms fused silica capillary column.

Fumigant toxicity assay

High mortality rates (>80%) were obtained with concentrations T. deversifolia EO above 18.6 mg of oil/L of air after 24 h and 12.8 mg of oil/L of air for 48 h exposure times (Table 2). Concentrations of 24.6 and 18.6 mg of oil/L of air for 24 h and 48 h exposure times respectively, were adequate to result in 100% death of the insects indicating a dose/time-dependent response in mortality rates of S. zeamais. The essential oil was less toxic than the MeBr (positive control) which attained 100% mortality within 24 h at a conc of 12.8 mg of oil/L.

Table 2. Cumulative percentage mortality of adult S. zeamais after 24 and 48 h at different concentrations of T. diversifolia essential oil by fumigation assay.

Conc of EO(mg/L of air)	%mortality 24 h	%mortality after 48 h	LC_{50} at 24 h (mg/L of air)	Slope ± SE	Chi-square	df	p
0(Acetone)	0.0±0.0a	0.0±0.0a	10.2 (8.7- 12.3)	1.21±0.18	1.98	3	0.54
6.4	32.5±3.5b	60.6±4.4b					
12.8	60.4±5.1c	84.8±6.0c					
18.6	81.1±6.4d	100.0±0.0d					
24.6	100.0±0.e	100.0±0.0d					
MeBr	100.0±0.0e	100.0±0.0d					

*EO=essential oil. **Values on the table represent mean±SD (N= 5). ***Column means followed by different letters are significantly different by Turkey test ($p<0.05$). Acetone solvent =-ve control; MeBr = +ve control.

The LC_{50} of the EO was 10.2 mg of oil/L of air at 95% upper and lower fiducial limits (FL) (8.7 to 12.3 mg oil/L of air), for 24 h exposure time. The linear regression equation of S. zeamais mortality after 24 h duration was y= 4.84x - 3.26. The activity of the essential oil was lower than that of methyl bromide (MeBr) which has been used as the positive control in the study with a reported LC_{50} value of 0.67 mg oil/L air [19].

This EO exhibited stronger fumigant activity against S. zeamais than that obtained from of aerial parts of A. subdigitata with LC_{50} of 17.01 mg oil/L [20], A. sieversiana with LC_{50} of 15.0 mg/L [21] and Illicium simonsii with LC_{50} of 14.95 mg/L [22]. The EO of T. diversifolia had weaker fumigant activity compared to oil from Artemisia giraldii with LC_{50} of 6.29 mg oil/L of air [20], Murraya exotica with LC_{50} of 8.29 mg/L of air [23] and A. mongolica with LC_{50} of 7.35 mg/L of air [21]. Therefore, the essential oil of T. diversifolia has great potential for used as a possible natural fumigant for the control of S. zeamais.

Insect repellency assay

The EO from T. diversifolia leaves repelled S. zeamais adults in a dose and time dependent manner as expressed in Table 3. The essential oil attained class III repellency within 30 min for a 30 ul/cm^2. Similar level of repellency was attained at 60 min for essential oil conc of 20 ul/cm^2 and 90 min for a conc of 10 ul/cm^2. This results are in agreement with results by Tavares et al. [24], where methanol extract of T. diversifolia flowers exhibited class III of repellency after 90 min exposure against Sitophilus zeamais adults in corn grains. Repellency against S. zeamais adults was also observed in a dose and time dependent manner for DCM and ethylacetate extracts of T. diversifolia as indicated by Gitahi et al. [25]. The major components in the DCM extract were β-Amyrin, squalene, and hexadecanoic acid, whereas those of the ethyl acetate extract were hexadecanoic acid, squalene, methyl linoleat and phytol. These components differed from the major components in the EO of T. diversifolia, indicating that repellency in the different case studies could be due to different components.

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Table 3. Repellency of Sitophilus zeamais adult insects by different concentrations of essential oil from the leaves of T. diversifolia at different exposure times.

Conc of EO (ul/cm^2)	% mean repellency of adult insects		
	30 min	60 min	90 min
Acetone (control)	0.0±0.0a (I)	0.0±0.0a (I)	0.0±0.0a (I)
10	5.0±2.3b (I)	23.0±3.2b (II)	48.0±4.1b (III)
20	36.0±3.5c (II)	59.0±6.3c (III)	69.0±5.5c (IV)
30	53.0±5.2d (III)	71.0±7.6d (IV)	100.0±0.0d (V)
40	75.0±7.5e (IV)	100.0±0.0e (V)	100.0±0.0d (V)
50	100.0±0.0f (V)	100.0±0.0e (V)	100.0±0.0d (V)

*Repellency Class: class 0 (PR≤ 0.1%), class I (PR = 0.1–20.0%), class II (PR = 20.1–40.0%), class III (PR = 40.1–60.0%), class IV (PR= 60.1-80.0%), and class V (80.1-100.0%) [26], Cumulative mean±Sd followed by the same letter within each vertical column are not significantly different ($p<0.05$).

Contact Toxicity

The lowest concentration of EO (6.0 µg/adult insect) had a mortality rate of 94.0±5.4% in 48 h while the highest concentration (500.0 µg/adult insect) exhibited a mortality rate 100±0.0% in 12 h ($p<0.05$) (Table 4). The activity of the EO was lower than that of pyrethrine (6.0 µg/adult insect) the positive control, which attained 100% mortality within 12 h exposure period. The EO of the leaves of T. diversifolia exhibited contact toxicity against S. zeamais adults insects, with LD_{50} values of 12.3 µg/adult insect at 95% upper and lower fiducial limits (FL) (10.5 to 14.7 µg of oil/adult insect), for an exposure time of 12 h. The linear regression equation of S. zeamais mortality after 12 h duration was y= 4.94x–0.355. This indicated that this EO was approximately three times less toxic against S. zeamais than pyrethrine with a reported LD_{50} value of 4.29 µg/adult insect [21]. This EO was however, more toxic to S. zeamais than those obtained from Artemisia giraldii with LD_{50} (40.51 µg/adult insect) and A. subdigitata with LD_{50} of 40.51 and 76.34 µg/adult insect respectively, at an exposure time of 24 h [20].

Table 4. Mean cumulative percentage mortality for contact toxicities of essential oil from T. diversifolia against S. zeamais (the maize weevil)

Conc (µg EO /Insect)	Percentage mortality				
	6 h	12 h	24 h	36 h	48 h
0(-ve control)	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
6.0	12.0±5.5b	38.0±5.4b	44.0±5.5b	72.0±8.4b	94.0±5.4b
10.0	25.0±6.4c	46.0±6.2c	66.0±7.1c	96.0±4.0c	100.0±0.0c
17.5	42.0±8.4d	72.0±7.1d	100.0±0.0d	100.0±0.0c	100.0±0.0c
25.0	68.0±10.0e	100±0.0e	100.0±0.0d	100.0±0.0c	100.0±0.0c
6.0(+ve control)	86.0±6.2f	100.0±0.0e	100.0±0.0d	100.0±0.0c	100.0±0.0c

*Values on the table represent cumulative percentage mean±SD (N= 6). ** Means followed by different letters in column are significantly different by Turkey's test ($p<0.05$). –ve control = acetone, +ve control = pyrethrine.

Monoterpenes including α-pinene, β-pinene and limonene, have been reported to exhibit fumigant activity, antifeedant, insect repellents and growth regulatory activities against numerous pests, including S. zeamais (maize weevil) and Callosobruchus maculatus (bean weevil) [27, 28]. Sesquiterpenoids such as α-copaene, germacrene D, γ-cadinene and δ-cadinene, have also been reported to possess insecticidal properties. According to Choi et al. [29], the toxicity of EO for stored-product insects could be influenced by their chemical composition. Therefore, insecticidal activities of T. diversifolia EO could be associated with components including α-pinene, β-pinene, and caryophylene among other constituents. These three components accounting to approximately 70.6% of the oil could be responsible for these properties since they

have been reported to possess insecticidal activities against several insect pests including aphids, termites and beetles [29, 30]. Resistance development in insects can be reduced by the use of essential oils due to synergistic action between different molecules of the oil [31].

CONCLUSIONS

The essential oil of T. diversifolia as determined by GC/MS mainly comprises of monoterpenes and sesquiterpenes, with α-pinene, constituting the greatest percentage. This oil exhibited strong fumigant, repellency and contact toxicity activities against S. zeamais adult insects and thus has great potential of for use a potent insecticide. For the practical application of these essential oil as novel insecticide, further studies on the safety of the essential oil to humans and isolation and identification of the active constituents in this essential oil are necessary. In addition, studies of individual and combined activities of the different components in the oils are necessary to ascertain whether the compounds act individually, synergistically or antagonistically.

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DECLARATION OF INTEREST STATEMENT

The author reports there are no competing interests to declare.

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