Journal of Plant Diseases and Protection (2021) 128:607–615
https://doi.org/10.1007/s41348-020-00424-9

SHORT COMMUNICATION

Chemical composition and fumigant toxicity of the essential oil from *Tithonia diversifolia* (Hemsl.) A. Grey against two major stored grain insect pests

Thiyam B. Devi[1,2] · Vishakha Raina[2] · Dinabandhu Sahoo[1,3] · Yallappa Rajashekar[1]

Received: 23 July 2020 / Accepted: 23 December 2020 / Published online: 22 January 2021

Abstract

The essential oil (EO) from the leaves of *Tithonia diversifolia* (Hemsl.) A. Grey was investigated to determine chemical composition and fumigant toxicity against two major stored grain pests rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). A total of 78 compounds were identified by GC-MS of which α-Pinene (63.62%), Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methyl ethyl)- (14.68%) and 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- (2.34%) were most abundant. EO from leaves of *T. diversifolia* showed strong fumigant activity against *S. oryzae* adults with LC_{50} values of 95.12, 77.00 and 74.68 µL/L air after 24, 48 and 72 h of exposure whereas for *T. castaneum* the LC_{50} values were 102.67, 98.12 and 81.54 µL/L air, respectively. Mortality rates increased with increasing exposure periods and increasing concentration. These naturally occurring materials derived from EO of *T. diversifolia* could be used to manage the populations of *S. oryzae* and *T. castaneum*. It was found that the EO's of *T. diversifolia* is very effective as a fumigant against stored grain insect pests and can also be used as an alternative to the synthetic fumigants.

Keywords Fumigant toxicity · Chemical composition · *Tithonia diversifolia* · Essential oils · *Sitophilus oryzae* · *Tribolium castaneum*

Introduction

Globally, the quantitative and qualitative loss of stored grains products after postharvest storage in the temperate zone may amount up to 5–10% and 20–30% in the tropical zone (Rajendran and Sriranjini 2008). Most of this damage is caused by the stored grain insect pests belonging to the Coleoptera which are distributed worldwide (Howe 1965; Sinha and Watters 1985). The primary beetle pests in stored grains are *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae); *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the main secondary pest is the grain beetle *Tribolium castaneum* (Herbst). (Coleoptera: Tenebrionidae) (Shaaya et al. 2006). Their effective control has been commonly based on synthetic or conventional insecticides (Rajashekar et al. 2016). However, continuous use of synthetic insecticides for decades has resulted in the widespread development of resistance and detrimental effects on non-target organisms (Subramanyam and Hagstrum 1995). These insecticides also generate environmental pollution, and their residues often persist in food products (Sharma and Meshram 2006). Therefore, due to the problem of resistance to synthetic insecticides, the situation demands a serious effort to find out some safe, viable, biodegradable, newer eco-friendly and effective substitute for these conventional insecticides from natural origin (Rajendran and Sriranjini 2008; Rajashekar et al. 2013). One alternative is the use of essential oil (EO) from aromatic plants. Several essential oils are currently being used in the manufacturing process of many industrial products in the fields of cosmetics, perfumery, cleaning,

✉ Yallappa Rajashekar
rajacftri@yahoo.co.in

1 Insect Bioresource Laboratory, Animal Bioresources Programme, Institute of Bioresources and Sustainable Development, Department of Biotechnology, Govt. of India, Takyelpat, Imphal 795001, Manipur, India

2 School of Biotechnology, Kalinga Institute of Industrial Technology, Deemed to be University Bhubaneswar, Bhubaneswar, Odisha, India

3 Marine Biotechnology Laboratory, Department of Botany, University of Delhi, Delhi 110007, India

pharmacology, chemistry and also as insecticides (Regnault-Roger et al. 2012; Devi et al. 2019). Insects either do not develop resistance or have reduced resistance against essential oils due to synergistic action between the different molecules (Varma and Dubey 1999) and with the possible advantage over the synthetic fumigants like less toxicity to mammals, a fast degradation period plus easy local availability (Isman 2000; Devi et al. 2020). The tree marigold or Mexican sunflower, *Tithonia diversifolia* (Hemsl.) A. Grey is a medicinal plant belonging to the family Asteraceae (Compositae) distributed widely across the humid and sub-humid tropics and native to Mexico, Asia, Central America and Africa (Ayeni et al. 2008; Carter 1978; Zhai et al. 2010). *T. diversifolia* essential oil has found to have insecticidal activity against the aphids (*Aphis fabae*), bean foliage beetles (*Ootheca mutabilis* and *O. bennigseni*) and flower beetles (*Epicauta albovittata* and *E. limbatipennis*) feeding on *Phaseolus vulgaris* L. common bean, plants (Mkenda et al. 2015). This plant has been used traditionally as herbs with agricultural benefits in some developing countries because of its insecticidal properties (Castano-Quintana et al. 2013). However, our knowledge concerning the chemical composition of the *T. diversifolia* essential oil is very mited, and insecticidal activity against stored grain insects has also not been investigated. Therefore, the objective of this study was mainly to identify the chemical composition and evaluate the fumigant toxicity of the *T. diversifolia* essential oils against two insect pests of stored grains namely the rice weevil, *S. oryzae* and red flour beetle, *T. castaneum* adults.

Materials and methods

Collection of plant material

The fresh leaves of *Tithonia diversifolia* (Hemsl.) A. Grey was collected from Tupul, Tamenlong district, (N-24°47’51.74”, E-093°45’56.85”) Imphal, Manipur, India and were stored in a refrigerator at 4 °C. The plant samples were identified by Dr. Biseshwori Thongam (Plant taxonomist, Institute of Bioresources and Sustainable Development, Imphal, Manipur, India).

Extraction of the essential oil

The collected leaves of *T. diversifolia* were washed carefully with running tap water to remove dust and other foreign particles. The plant materials were partially dried at the temperature of 25 °C–27 °C for 2–3 days. The partially dried plant sample was cut into small pieces making into a length of 2–3 cm by a Secateur. 1 kg of the plant material was precisely weighed, and 4 L of distilled water was added and were placed in a distillation apparatus. The essential oils of the plant sample were obtained at 60 °C after 3–4 h of steam distillation in a Clevenger-type apparatus (Borosil, India) (Demirci et al. 2008). The essential oil was dried using anhydrous sodium sulphate (HiMedia, India), filtered and stored in an amber glass vial in a refrigerator at 4 °C until it was analysed and tested for further bioassays.

Test insects

The rearing and maintenance of *S. oryzae* cultures as on whole wheat (moisture content 12.5–14%), *Triticum aestivum* (Poales: Poaceae) whereas the cultures of *T. castaneum* were in whole wheat flour containing 2% brewers yeast powder. The two test insect cultures were collected from the warehouse, Food Corporation of India (FCI), Sangaiprou, Imphal West, India. Mix-sexed adults of *S. oryzae* (3–5 d old) and *T. castaneum* (2–3 d old) were used in the experiments. The insect cultures were held and maintained at 30 ± 1 °C and 70% relative humidity in the insect incubation chamber. Experiments were conducted in the laboratory at 27 ± 2 °C and 70 ± 5% r.h. (Leelaja et al. 2007; Rajashekar et al. 2016).

Chemical analysis of the essential oil

Analysis of the chemical composition of the essential oil was done by using a gas chromatograph (Trace 1300) coupled with mass spectrometry (TSQ Duo) (Thermo Fisher Scientific Pte Ltd, Blk 33 Marsiling Industrial Estate Road 3#07-06, Singapore- 739256). The column used was a TG-5MS silica capillary column of 30 m × 0.25 mm i.d. and 0.25 µm film thickness. The ionization energy was adjusted to 70 eV for GC–MS detection, and the temperature of the injector and mass transfer line was set at 250 °C and 250 °C, respectively. Helium was used as a carrier gas at a flow rate of 1 ml/min with an injection volume of 0.5 µl for each sample (1:100 sample oil in hexane). Column temperature programmed from 40 °C for 1 min to 250 °C at a rate of 5 °C/min heating ramp and then held at 250 °C for 20 min. Identification of the chemical compounds was based on the highest reverse search index value of the mass spectra present in the National Institute of Standards and Technology (NIST) GC–MS Libraries 2017.

Fumigant toxicity assays

Fumigant activity of essential oil was determined against the adults of the two insect pests of stored grains as described by Devi et al. (2019). Filter paper discs were impregnated with different doses of the essential oils starting from 50 to 100 µL/L air. Preliminary experiments were carried out to determine the dose to be used in the fumigant toxicity tests. The fumigant bioassay was performed with 10 adults of each tested insect species for each dose in glass jars with a 1 L volume. The impregnated filter papers were placed in the middle of the fumigation chamber supported by a porcelain plate to stop direct contact to the insect pests and to provide surface area for evaporation of essential oil. Different doses of EO's were injected by using a gas-tight microsyringe in each glass jar, and injection wasw done through a rubber septum fitted in the desiccator lid. There were 4 replicates per dose with the same number of untreated control replicates for both species at exposure times of 24, 48 and 72 h at 28 ± 2 °C and 70% RH. After the fumigation exposure, the test insects were removed from the desiccators and were transferred into the clean vials with food media to determine insect mortality. Thus mortality rates were recorded for each dose rate and exposure time. Insects were regarded as dead when no movements were found when they were lightly probed or shaken in the light under mild heat.

Statistical analysis

By using the Abbott formula equation (1925), percentage mortality was calculated while the LC_{50} values with their respective confidence limits were calculated by Probit analysis (Finney 1971) using Statplus 2007 software statistical programme.

Results

Chemical composition of the essential oil

The leaves of *T. diversifolia* yielded 0.19% (v/w) of a pale-yellow oil with an unpleasant odour. The GC-MS analysis identified 78 compounds accounting for 99.99% of the total oil composition (Table 1; Fig. 1). The major EO components were *α*-pinene (63.62%), bicyclo [3.1.0] hexane, 4- methylene-1-(1-methylethyl)- (14.68%), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- (2.34%),Cyclohexene,1-methyl-5-(1-methylethenyl)-,(R)- (1.71%), Caryophyllene (1.26%), and Camphene (1.16%) that all likely contributed to the insecticidal activity through different odour types (α-Pinene-Woody odour, Bicyclo [3.1.0] hexane, 4- methylene-1-(1-methylethyl)- Spicy odour, 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- Fruity odour, Cyclohexene,1-methyl-5-(1-methylethenyl)-,(R)- Fir wood, Caryophyllene- clove or pepper odour and Camphene-Pungent odour. The high content of *α* pinene in *T. diversifolia* makes the oil similar to those previously reported by (Menut et al. (1992) Moronkola et al. (2007) and Chukwuka et al. (2014). Three monoterpenes (exclusively hydrocarbons), namely *cis*-*β*-ocimene (43.7%), *α*-pinene (28.6%) and limonene (12.0%) were reported from the essential oils of *T. diversifolia* collected from Nigeria (Adebayo et al. 2008). Moronkola et al. (2007) reported 72 essential oil components from the leaf oils of *T. diversifolia* and the chemical composition comprises mainly α-pinene (32.9%), β-caryophyllene (20.8%), germacrene D (12.6%), *β*-pinene (10.9%) and 1,8-cineole (9.1%), while germacrene D (20.3%), *β*-caryophyllene (20.1%) and bicyclogermacrene (8.0%) were characterized from the flower oils. Green et al. (2017) also reported that the sesquiterpene, namely Tagitinin A isolated from the methanolic extract from the leaves of *T. diversifolia* has contact toxicity against recently emerged adults of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) reared on cowpea seeds (*Vigna unguiculata*). The difference observed in the chemical composition of the essential oils of *T. diversifolia* may be attributed to the environment for plant growth, genetic differences, geographical and ecological variations such as soil, climatic condition, place of cultivation and experimental conditions such as stabilization and storage of EO's can also affect its oil composition of the same species. (Gobbo-Neto and Lopes 2007). Hence, Perry et al. (1999) also reported that environmental differences can affect the biochemical yield of essential oils in plants.

Fumigant activity of the essential oil

T. diversifolia oil was tested for fumigant toxicity against *S. oryzae* and *T. castaneum* adults and their LC_{50} values are shown in Table 2. The oil showed significant fumigant activity and it was clear that with an increase in the dosage and exposure time, the fumigant activity also increases. It was observed that *T. diversifolia* essential oils were more toxic to *S. oryzae* adults than to *T. castaneum* adults. The highest concentration (100 µL/L) of the oil resulted in 100% mortality of *S. oryzae* adults after 24, 48 and 72 h of exposure time, but the mortality rate of *T. castaneum* at the highest concentrations were 85%, 95% and 100% after 24 h, 48 h and 72 h exposure time, respectively. Essential oils of *T. diversifolia* also showed strong fumigant activity against *S. oryzae* adults with LC_{50} value of 95.12 µL/L air, 77.00 µL/L air and 74.68 µL/L air whereas in the case of *T. castaneum* the LC_{50} value was 102.67, 98.12 and 81.54 µL/L air after 24, 48 and 72 h of exposure time, respectively. These investigations also revealed that an increase in mortality with increasing exposure times at the dosage of 100 µL/L was observed in both tested insects.

Discussion

Many studies on the chemical profiles of EO obtained from leaves and flowers of *T. diversifolia* have been reported. In the present study, 78 compounds were identified by GC-MS from which *α*-pinene (63.62%), bicyclo [3.1.0] hexane, 4-methylene-1-(1- methylethyl)-(14.68%) and

Table 1 Chemical composition of essential oils from *Tithonia diversifoilia* (Hems.) A Grey

Peak no. RA[C]	RT[a]	Component RSI[b]	RSI[b]	%
1	8.40	Tricyclo [3.2.1.0(2,4)]octane, 8-methylene-, (1à,2à,4à,5à)-	963	0.06
2	8.55	Bicyclo [3.1.0] hex-2-ene, 4-methyl-1-(1-methylethyl)-	934	0.13
3	8.81	α-Pinene	928	63.62
4	9. 18	Camphene	945	1.16
5	9.49	Benzaldehyde	91	0.04
6	10.02	Bicyclo [3.1.0] hexane,4-methylene-1-(1-methylethyl)-	931	14.68
7	10.52	3-Penten-1-ol, 2-methylene-,(E)-	878	0.07
8	10.80	Bicyclo [2.2.1]heptane,2,2-dimethyl-3-methylene-,(1R)-	896	0.04
9	11.18	Cyclohexene,1-methyl-4-(1-methylethylidene)-	925	0.09
10	11.4	o-Cymene	954	0.14
11	11.55	Cyclohexene,1-methyl-5-(1-methylethenyl)-,(R)-	907	1.71
12	11.64	Silabenzene,1-methyl-	959	0.04
13	12.45	ç-Terpinene	919	0.33
14	12.70	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol #	939	0.36
15	13.35	Cyclohexene,1-methyl-4-(1-methylethylidene)-	912	0.10
16	13.63	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol #	921	0.66
17	14.14	(E)-4,8-Dimethylnona-1,3,7-triene	894	0.05
18	14.32	1-Methylene-2-vinylcyclopentane	898	0.08
19	14.86	Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene-,[1S-(1à,3à,5à)]-	923	0.54
20	15.01	trans-Verbenol	936	0.33
21	15.21	6-Octenal, 3,7-dimethyl-, (S)-	935	0.08
22	15.55	Bicyclo[4.1.0]heptan-3-one,7,7-dimethyl-4-methylene-,(1R)-	848	0.08
23	15.65	Bicyclo[2.2.1]heptane-7-methanol, 2-hydroxy-1,7-dimethyl-	884	0.11
24	15.97	Terpinen-4-ol	908	0.77
25	16.16	Benzenemethanol,à,à,4-trimethyl-	850	0.05
26	16.34	à-Terpineol	928	0.15
27	16.53	(-)-Myrtenol	912	0.09
28	16.68	Cyclopenta[c]pyran-1,3-dione, ,4a,5,6-tetrahydro-4,7-dimethyl-	875	0.11
29	16.90	4,7,7-Trimethylbicyclo[4.1.0]hept-3-en-2-one	872	0.05
30	16.99	2-Pentene, 2,4-dimethyl-	871	0.14
31	17.13	Cyclobutaneethanol,á-methylene-	920	0.05
32	17.34	6-Octen-1-ol, 3,7-dimethyl-,(R)-	928	0.21
33	17.74	1H-Benzotriazole,7-amino-1-methyl-	887	0.04
34	18.08	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	887	0.23
35	18.63	1H-Indene-4-carboxaldehyde,2,3-dihydro-	939	0.04
36	20.68	Cyclohexene,1,5,5-trimethyl-6-(2-propenylidene)-	919	0.11
37	20.90	Phenol,2-methoxy-3-(2-propenyl)-	934	0.17
38	21.49	à-Cubebene	916	0.69
39	21.64	S-(2-phenylethyl) ester Modephene	958	0.57
40	21.82	(1R,3aS,5aS,8aR)-1,3a,4,5a-Tetramethyl-1,2,3,3a,5a,6,7,8-octahydrocyclopenta[c]pentalene	917	0.33
41	22.35	(1R,3aS,5aS,8aR)-1,3a,5a-Trimethyl-4-methylenedecahydrocyclopenta[c]pentalene903	918	0.21
42	22.65	Caryophyllene	951	1.26
43	23.43	Tricyclo[3.2.1.0(2,4)]octane,8-methylene-, (1à,2à,4à,5à)-	869	0.05
44	23.50	Cyclohexene,4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-	887	0.11
45	23.63	1,5-Diphenylhex-3-ene	880	0.07
46	23.69	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0]unde c-4-ene	922	0.06
47	24.01	5,10-Pentadecadiyne, 1-chloro-	887	0.06
48	24.18	Germacrene D	924	0.08
49	24.24	1-Hexadecanol	917	0.08
50	24.29	Tricyclo[3.2.1.0(2,4)]oct-6-ene, 8-methylene-, (1à,2à,4à,5à)-	853	0.13

Table 1 (continued)

Peak no. RA[C]	RT[a]	Component RSI[b]	RSI[b]	%
51	24.56	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo [8.1.0]undeca-2,6-diene	925	0.29
52	24.96	Germacrene D	908	0.11
53	25.15	5,6-Decadien-3-yne,5,7-diethyl-	922	0.10
54	25.75	Cyclohexanemethanol,4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-,[1R-(1à,3à,4á)]-(1-methylethenyl)-,[1R-(1à,3à,4á)]-	910	0.10
55	26.00	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-, (E)-	948	2.34
56	26.33	Dihydro-cis-à-copaene-8-ol	919	0.05
57	26.49	Cyclohexene,1,5,5-trimethyl-6-(2-propenylidene)-	937	0.91
58	26.64	1-Formyl-2,2,6-trimethyl-3-cis-(3-methyl-but-2-enyl)-5-cyclohexene	892	1.87
59	26.73	5,10-Pentadecadiyn-1-ol	939	0.15
60	26.89	5,10-Pentadecadiyne, 1-chloro-	897	0.05
61	26.96	5,10-Pentadecadiyn-1-ol	937	0.05
62	27.12	Dihydro-cis-à-copaene-8-ol	954	0.21
63	27.24	10-Pentadecen-5-yn-1-ol, (E)-	909	0.08
64	27.62	5,10-Pentadecadiyne, 1-chloro-	906	0.05
65	27.83	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	938	0.43
66	27.90	Dihydro-cis-à-copaene-8-ol	906	0.50
67	28.25	Dihydro-cis-à-copaene-8-ol	946	1.49
68	28.58	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-	866	0.10
69	28.86	1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene	907	0.05
70	29.35	1,15-Pentadecanediol	915	0.09
71	29.61	5,10-Pentadecadiyne, 1-chloro-	906	0.15
72	30.31	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	858	0.04
73	30.87	Phenanthrene	942	0.07
74	31.30	Ethanone,1,1'-(6-hydroxy-2,5-benzofurandiyl)bis-	893	0.04
75	32.08	2-Pentadecanone,6,10,14-trimethyl-	904	0.06
76	32.39	1-Hexyl-2-nitrocyclohexane	967	0.04
77	35.68	cis-2-Methyl-á-methyl-á-nitrostyrene	855	0.04
78	37.21	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	930	0.12
Total compounds indentified (%)			99.99	

[a]Retention time

[b]Reverse similarity index on TG-5MS capillary column

[C]Relative area (peak area relative to the total peak area)

1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- (2.34%), were most abundant accounting for 99.99% of the total oil composition where the major compound was a monoterpene. The chemical composition of the EO mentioned in the present study is much the same as the previous studies, with slight dissimilarities. For example, Moronkola and Ogunwande (Moronkola et al. 2007) reported 30 compounds from the leaf oils of *T. diversifolia* in which the major compounds were found to be α-pinene (32.9%), β-c caryophyllene (20.8%) and β-pinene (10.9%) which represented 99.9% of the total oil. The differences in the chemical constituents of EO's depends on different geographical and climatic conditions (Lahlou and Berrada 2003; Raina et al. 2003).

Among the plant-derived botanical extracts, the use of essential oils is regarded as one of the promising alternatives to conventional chemical insecticides for control of stored product insects (Rajendran and Sriranjini 2008). Many plant essential oils and their constituents have been found fumigant activity against the stored product pests (Kim and Ahn 2001; Singh et al. 2005; Opolot et al. 2006; Tripathi et al. 2009; Lopez and Pascual-Villalbos 2010; Isman 2000; Caballero-Gallardo et al. 2011). The fumigant activity of the EO's obtained from the leaves of *T. diversifolia* against the two major stored grain insect pests namely, *S. oryzae* and *T. castaneum* is reported for the first time in the present study. There are no previous reports on insecticidal activity of the essential oils of *T. diversifolia* against *S. oryzae* and

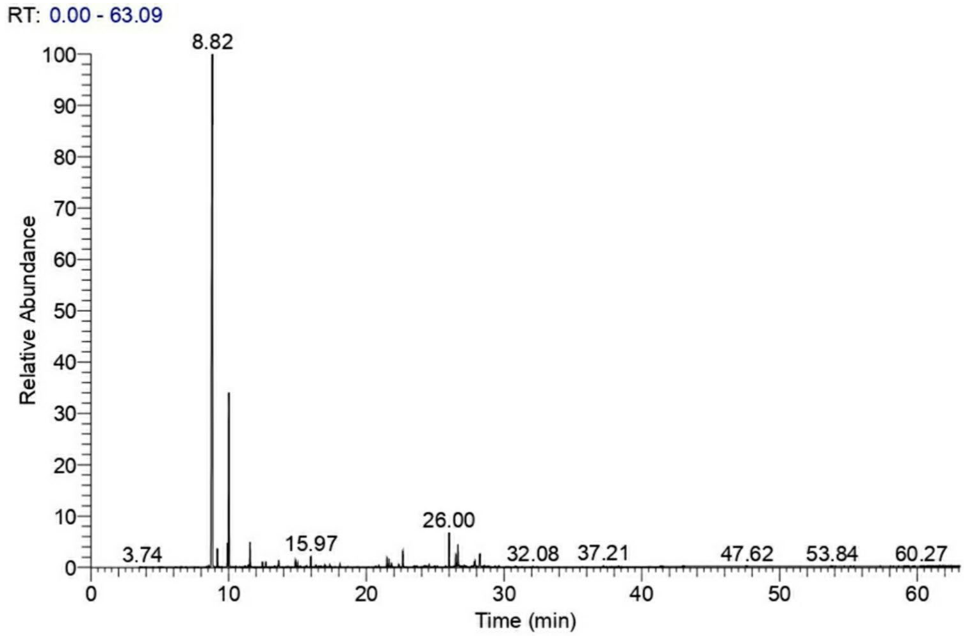

Fig. 1 Representative GC-MS total ion chromatogram of essential oils from *Tithonia diversifolia*

Table 2 Fumigant effect of the essential oil from *Tithonia diversifolia* against adults of *Sitophilus oryzae* and *Tribolium castaneum*.

		Period (h)	LC_{50} (µL/L)	Standard error	Chi-square (χ^2)	Degrees of freedom	*P* value
Sitophilus oryzae	Essential oil	24	95.12 (89.34–100.9)	2.94	0.951	4	0.091
		48	7.00 (71.8–86.29)	4.73	0.173	4	0.096
		72	74.68 (69.66–77.69)	4.59	0.0281	4	0.099
Tribolium castaneum	Essential oil	24	102.67 (96.58–108.77)	3.105	0.313	4	0.098
		48	98.12 (91.92–104.3)	3.16	0.212	4	0.094
		7 72	81.54 (74.43–88.65)	3.62	0.638	4	0.098

[a]Upper and lower limits of the confident interval of the 95% are indicated between parenthesis

T. castaneum adults. However, it has been reported that the extracts or plant samples had insecticidal activity against other stored product insect species. For instance, the leaf powder of *T. diversifolia* showed contact toxicity against the mix-sexed adults of *Sitophilus zeamais* Motschulsky (Coleoptera-Curculionidae) (Babarinde et al. 2008) and the compound Tagitinin A, a sesquiterpene isolated from the methanolic extract of *T. diversifolia* leaves has shown toxicity against *C. maculatus* (Green et al. 2017). Mkenda et al. (2015) have also reported that *T. diversifolia* was also effective on aphids, weevils and whiteflies when leaves or seeds are used in a cold infusion. Adedire and Akinneye (2004) had also previously investigated the insecticidal activities such as mortality, adult emergence and oviposition of the ethanolic extracts and powder of *T. diversifolia* against cowpea seed bruchid, *C. maculatus* (Fabricius.) (Coleoptera: Bruchidae). In this study reported by Adedire and Akinneye (2004), the ethanolic extract of *T. diversifolia* displayed activity within 24 h with mortality percentage of 73.3%, 93.3% and 100% at the concentration of 1.0% and 2.0% and 5% whereas the powder of the plant was showing moderate mortality percentage of 50–63% and 75–83% at the concentration of 1–3% and 4.0–5% at 24 h of the exposure period. These studies also reported that at 1.5% and 2% concentration of ethanolic extract, the treated cowpea seed showed a reduced effect on adult emergence when compared to the same concentration when the cowpea seed was treated with the powder of *T. diversifolia*. The mean number of eggs laid at 1.5–2% concentration of ethanolic extracts as well as powder of the plant was 9.7, 4.7 and 27, 17.3, respectively. The report of this study agrees with the findings that ethanolic extract of *T. diversifolia* possessed higher bioactivity on oviposition, adult emergence and mortality of *C. maculatus* when compared to the powder of *T. diversifolia*. Tavares et al. (2014) had also previously reported that the flower extract of *T. diversifolia, when applied to corn grains,* showed repellency and mortality against adults of *Sitophilus zeamais* (Motsch.). It has been also reported that the aqueous extract of *T. diversifolia* shows effect against the tea red spider mite *Oligonychus coffeae* (Nietner.), one of the major pest of tea (*Camellia sinensis*) (Radhakrishnan and Prabhakaran 2014). Extracts from *T. diversifolia* have also been reported in suppressing the populations of some insects and fungal pathogens under the field conditions (Owolade et al. 2004), deterrent activity against the larvae, *Chlosyne lacinia* (Geyer.) (Lepidoptera: Nymphalidae) (Ambrósio et al. 2008) and biological control of leaf-cutting ants, Atta cephalotes (L.) (Hymenoptera: Formicidae) (Castaño-Quintana et al. 2013).

The present study reported that the essential oil of *T. diversifolia* caused 100% mortality in *S. oryzae* with an exposure period of 24, 48, 72 h at the concentration of 100 uL/L whereas for *T. castaneum* mortality was 85%, 95% and 100% at the same concentration and exposure periods. These findings suggest that the oil of *T. diversifolia* may have the potential to use as a biofumigant against *S. oryzae* and *T. castaneum* in stored grains even though, the highest (100%) percentage mortality in *S. oryzae* started at the early exposure of 24 h and as late as 72 h in the case of *T. castaneum*. The LC_{50} values indicate this oil might be feasible to use as a commercial fumigant in real grain storage. Fang et al. 2002 also reported that *Tribolium* species are least susceptible and often more resistant to pesticide. They are more difficult to kill in comparison with other Coleopteran pest of stored grain products, but the toxic effect against the pests may also vary depending upon the nature of insecticide. The resistance of insects towards such insecticides could depend upon the three mechanisms. Firstly, behavioural mechanisms such as the activity of insect pest at minimum exposure periods and doses (Hemingway and Ranson 2000, Lee and Lees 2001). Secondly, physiological changes when pesticides enter or penetrate inside the body of the insect (Scott 1999). Thirdly, biochemical mechanisms like metabolic resistance and the target site insensitivity (Gao et al. 2006), (Konus 2015). Metabolic resistance includes the overproduction or modification in the function of enzymes like oxidise P450, esterase and glutathione S-transferase that detoxify the compound whereas modification in the target site fails the binding of the pesticidal compound with its target (Panini et al. 2016). Papachristos et al. (2004) have reported that monoterpenoid compounds act as active fumigants, repellents and contact insecticides against the stored product insect pests. Similarly, the insecticidal activity of the essential oils of *T. diversifolia* tested in our study may be attributed due to the major monoterpenoids, such as α-pinene, bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)- (Traboulsi et al. 2002; Papachristos et al. 2004; Miresmailli et al. 2006; Cetin et al. 2007; Kordali et al. 2008; Magdy and Samir 2008). Despite the fact there are previous studies on the insecticidal activities of the plant against various stored grain pests, there is no proper study which mentions LC50 values of the extracts and essential oil of *T. diversifolia*. The monoterpenoids are likely most effective as an insecticide by inhibiting the AchE enzymes that showed biofumigant activity against the stored grain pests. EO can act as a neurotoxicant by inhibiting the AChE activity, changing the gamma-aminobutyric acid receptors and ligands of octopamine receptors (Jankowska et al. 2017). The EO's from *T. diversifolia* can use as a possible alternative to the chemical insecticides for controlling of stored grain insect pests. Yang et al. (2012) have also reported *T. diversifolia* as a globally invasive alien species where its collection and its uses as an insecticide could minimise the environmental impacts. However, furthermore, research may be required for testing its efficacy against other life stages (pupae, eggs and larvae) and evaluating the biosafety before its practical use to control the stored grain insects. Our findings indicated that the oil of *T. diversifolia* has the potential to be used in the control of *S. oryzae* and *T. castaneum* in stored grains.

Acknowledgements The authors thank the Director, Institute of Bioresources and Sustainable Development, Imphal, Manipur, India for the research work. The author would also like to acknowledge the Department of Biotechnology, Govt. of India, Grant No. BT/PR28069/TRM/120/192/2018 for supporting financial assistance and IBSD manuscript number IBSD/2020/01/030.

Compliance with ethical standards

Conflict of interest The authors, Thiyam Bidyababy Devi, Vishakha Raina, Dinabandhu Sahoo and Yallappa Rajashekar declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

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

Adebayo A, Gbolade Biondi DM, Ruberto G (2008) Comparative analysis of the essential oils from two asteraceous plants found in Nigeria, *Acanthospermum hispidum* and *Tithonia diversifolia*. Nat Prod Commun 3:1735–1738

Adedire CO, Akinneye JO (2004) Biological activity of tree marigold, *Tithonia diversifolia,* on cowpea seed bruchids, *Callosobruchus maculatus* (Coleoptra: Bruchidae). Ann Appl Biol 144:185–189

Ambrósio SR, Oki Y, Heleno VCG, Siqueira Chaves J, Dantas Nascimento PGB, Espada Lichston J, Gomes Constantino M, Varanda EM, Batista Da Costa F (2008) Constituents of glandular trichomes of Tithonia diversifolia: relationships to herbivory and antifeedant activity. Phytochem 69:2052–2060

Ayeni AO, Lordbanjou DT, Majek BA (2008) *Tithonia diversifolia* (Mexican sunflower) in south-western Nigeria: occurrence and growth habit. Weed Res 37:443–449

Babarinde SA, Olabode OS, Akanbi MO, Adeniran OA (2008) Potential of *Tithonia diversifolia* with pirimiphos-methyl in control of *Sitophilus zeamais* (Coleoptera: Curculionidae). Afr J Plant Sci Biot 2:77–80

Caballero-Gallardo K, Olivero-Verbel J, Stashenko EE (2011) Repellent activity of essential oils and some of their individual constituents against *Tribolium castaneum* Herbst. J Agric Food Chem 59:1690–1696

Carter CD (1978) Cultivated sunflower. Oxford University Press, London, p 420

Casta-O-Quintana K, Montoya-Lermaa J, Giraldo-Echeverrib C (2013) Toxicity of foliage extracts of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) on Atta Cephalotes (Hymenoptera: Myrmicinae) workers. Ind Crops Prod 44:391–395. https://doi.org/10.1016/j.indcrop.2012.11.039

Cetin H, Erler F, Yanikoglu A (2007) Comparative evaluation of *Origanum onites* essential oil and its four major components as larvicides against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams. Pest Manage Sci 63:830–833

Chukwuka KS, Ojo OM (2014) Extraction and characterization of essential oils from *Tithonia diverssifolia* (Hemsl.) A. gray. Am J Essent Oils Nat Prod 1:1–5

Demirci F, Guven K, Demirci B, Dadandi MY, Baser KHC (2008) Antibacterial activity of two phlomis essential oils against food pathogens. Food Control 19:1159–1164

Devi MA, Sahoo D, Singh TB, Rajashekar Y (2019) Toxicity, repellency, and chemical composition of essential oils from *Cymbopogon* species against red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). J Consum Prot Food S. https://doi.org/10.1007/s00003-019-01264-y

Devi MA, Nameirakpam B, Devi TB, Mayanglambam S, Singh KD, Sougrakpam S, Shadia S, Tongbram M, Singh SD, Sahoo D, Rajashekar Y (2020) Chemical compositions and insecticidal efficacies of four aromatic essential oils on rice weevil *Sitophilus oryzae* L. Int J Trop Insect Sci. https://doi.org/10.1007/s42690-020-00102-1

Fang L, Subramanyam B, Arthur FH (2002) Effectiveness of spinosad on four classes of wheat against five stored-product insects. J Econ Entomol 95:640–650

Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, Cambridge, p 333

Gao JR, Yoon KS, Frisbie RK, Coles GC, Clark JM (2006) Esterase-mediated malathion resistance in the human head louse, Pediculus capitis (Anoplura: Pediculidae). Pestic Biochem Physiol 85:28–37

Gobbo-Neto L, Lopes NP (2007) Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim Nova. https://doi.org/10.1590/S0100-40422007000200026

Green PW, Belmain SR, Ndakidemi PA, Farrell IW, Stevenson PC (2017) Insecticidal activity of *Tithonia diversifolia* and *Vernonia amygdalina*. Ind Crops Prod 110:15–21

Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Annu Rev Entomol 45:371–91

Howe RW (1965) Losses caused by insects and mites in stored foods and foodstuffs. Nutr Abs Rev 35:285–302

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608

Jankowska M, Rogalska J, Wyszkowska J, Stankiewicz M (2017) Molecular targets for components of essential oils in the insect nervous system-a review. Molecules 23:23–34. https://doi.org/10.3390/molecules23010034

Kim DH, Ahn YJ (2001) Contact and fumigant activities of constituents of *Foeniculum vulgare* fruit against three coleopteran stored-product insects. Pest Manage Sci 57:301–306

Konus M (2015) Malathion resistance mediated by detoxification enzymes in *Sitophilus zeamais* (motschulsky) (Coleoptera: Curculionidae). Fresenius Environ Bull 24:2146–2151

Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E (2008) Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresour Technol 99:8788–8795

Lahlou M, Berrada R (2003) Composition and niticidal activity of essential oils of three chemotypes of Rosmarinus officinalis L. acclimatized in Morroco. Flavour Fragr J. https://doi.org/10.1002/ffj.1160

Lee SE, Lees EM (2001) Biochemical mechanisms of resistance in strains of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) resistant to malathion and chlorpyrifos-methyl. J Econ Entomol 94:706–13

Leelaja BC, Rajashekar Y, Reddy PV, Begum K, Rajendran S (2007) Enhanced fumigant toxicity of allyl acetate to stored-product beetles in the presence of carbon dioxide. J Stored Prod Res 43:45–48

Lopez MD, Pascual-Villalobos MJ (2010) Mode of inhibition of acetylcholinesterase by onoterpenoids and implications for pest control. Ind Crops Prod 3:284–288

Magdy IEM, Samir AMA (2008) Chemical composition and insecticidal potential of essential oils from Egyptian plants against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Appl Entomol Zool 43:599–607

Menut C, Lamaty G, Zollo PH, Kuiate RJR, Bessiere JM (1992) Aromatic plants of tropical central Africa. IX. Chemical composition of flower essential oils of *Tithonia diversifolia* (Hemsl) A. Gray from cameroon. J Essent Oil Res 4:651–653

Miresmailli S, Bradbury R, Isman MB (2006) Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Manage Sci 6:366–371

Mkenda P, Mwanauta R, Stevenson PC, Ndakidemi P, Mtei K, Belmain SR (2015) Extracts from field margin weeds provide economically viable and environmentally benign pest control compared to synthetic pesticides. PLoS One 10:30. https://doi.org/10.1371/journal.pone.0143530

Moronkola DO, Ogunwande IA, Walker TM, Setzer WN, Oyewole IO (2007) Identification of the main volatile compounds in the leaf and flowers of *Tithonia diversifolia* (Hems) Gray. J Nat Med 61:63–66

Opolot HN, Agona A, Kyamanyawa S, Mbata GN, Adipala E (2006) Integrated field management of cowpea pests using selected synthetic and botanical pesticides. Crop Prot 25:1145–1152

Owolade OF, Alabi BS, Osikanlu YOK, Odeyemi OO (2004) On-farm evaluation of some plant extracts as biofungicide and bioinsecticide on cowpea in Southwest Nigeria. Agric Environ 22:237–240

Panini M, Manicardi G, Moores G, Mazzoni E (2016) An overview of the main pathways of metabolic resistance in insects. Invertebr Surviv J 13:326–335

Papachristos DP, Karamanoli KI, Stamopoulos DC, Menkissoglu-Spiroudi U (2004) The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). Pest Manage Sci 60:514–520

Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heaney AJ, McGimsey JA (1999) Essential oils from dalmation sage (*Salvia officinalis* l.): variations among individuals, plant parts, seasons and sites. J Agric Food Chem 47:2048–2054

Radhakrishnan B, Prabhakaran P (2014) Biocidal activity of certain indigenous plant extracts against red spider mite, *Oligonychus coffeae* Nietner infesting tea. J Biopestic 7:29–34

Raina VK, Srivastava SK, Syamasunder KV (2003) Essential oil composition of Acorus calamus L. from the lower region of the Himalayas. Flavour Fragr J. https://doi.org/10.1002/ffj.1136

Rajashekar Y, Kumar HV, Ravindra KV, Bakthavatsalam N (2013) Isolation and characterization of biofumigant from leaves of *Lantana camara* for control of stored grain insect pests. Ind Crops Prod 51:224–228

Rajashekar Y, Tonsing N, Shantibala T, Manjunath JR (2016) 2, 3-Dimethylmaleic anhydride (3, 4-Dimethyl-2, 5-furandione): a plant-derived insecticidal molecule from Colocasia esculenta var. esculenta (L.) Schott. Sci Rep 6:20546

Rajendran S, Sriranjini V (2008) Plant products as fumigants for stored- product insect control. J Stored Prod Res 44:126–135

Regnault-Roger C, Vincent C, Arnason JT (2012) Essential oils in insect control: low- risk products in a high-stakes world. Annu Rev Entomol 57:405–424. https://doi.org/10.1146/annurev-ento-120710-100554

Scott JG (1999) Cytochromes P450 and insecticide resistance. Insect Biochem Mol Biol 29:757–77

Shaaya E, Kostyukovsky M (2006) Essential oils: potency against stored product insects and mode of action. Stewart Posth Rev 2:1–6

Sharma K, Meshram NM (2006) Bioactivity of essential oils from *Acorus calamus* and *Syzygium aromaticum*, against *Sitophilus oryzae* (L.) in stored wheat. Biopestic Int 2:144–152

Singh G, Maurya S, Catalan C, Delampasona MP (2005) Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract. Indian Perfum 49:441–451

Sinha RN, Watters FL (1985) Insect pests of flour mills, grain elevators, and feed mills and their control. Research Branch, Agriculture Canada Government Publishing Centre, Ottawa

Subramanyam B, Hagstrum DW (1995) Resistance measurement and management. In: Subramanyam B, Hagstrum DW (eds) Integrated Management of Insects in Stored Products. Marcel Dekker, New York, pp 331–397

Tavares WS, Faroni LRD, Freitas SS, Ribeiro PEA, Fouad HA, Zanuncio JC (2014) Effects of Astilbin from *Dimorphandra mollis* (Fabaceae) flowers and Brazilian plant extracts on *Sitophilus zeamais* (Coleoptera: Curculionidae). Fla Entomol 97:892–901

Traboulsi AF, Taoubi K, El-Haj S, Bessiere JM, Rammal S (2002) Insecticidal properties of essential plant oils against the mosquito *Culex pipiens* molestus (Diptera: Culicidae). Pest Manag Sci 58:491–495

Tripathi A, Upadhyay S, Bhuiyan M, Bhattacharya P (2009) A review on prospects of essential oils as biopesticide in insect-pest management. J Pharmacogn Phytother 1:52–63

Varma J, Dubey NK (1999) Prospectives of botanical and microbial products as pesticides of tomorrow. Curr Sci 76:172–178

Yang J, Tang L, Guan YL, Sun WB (2012) Genetic diversity of an alien invasive plant Mexican sunflower (*Tithonia diversifolia*) in China. Weed Sci 60:552–557. https://doi.org/10.1614/WS-D-11-00175.1

Zhai HL, Zhao GJ, Yang GJ, Sun H, Yi B, Sun L, Chen WS, Zheng SQ (2010) A new chromene glycoside from *Tithonia diversifolia* (Hemsl.). Chem Nat Compd 46:198–200