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Insecticidal and Repellent Activities of *Toddalia asiatica* (L.) Lam. Extracts against Three Major Stored Product Pests

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Abstract

Fumigant toxicity and repellent activity of *Toddalia asiatica* (L.) Lam. (Rutaceae) leaf and fruit extracts were screened against *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) adults. All the three solvent extracts of leaf and fruits recorded mortality and repellency against the three tested insects in a concentration dependent manner. *C. maculatus* was the most susceptible pest to the treatments. Lethal concentrations for 50 percent mortality (LC_{50}) of *C. maculatus*, *S. oryzae* and *T. castaneum* were recorded as 39.19, 44.13 and 61.10 µL/L, respectively. Diethyl ether fruit extract exhibited 100% repellent activity against *C. maculatus* and *S. oryzae* and 92% against *T. castaneum* adults at 20 µL concentration. These results suggested that Diethyl ether fruit extract of *T. asiatica* can be used as an ecofriendly fumigant and repellent against *C. maculatus*, *S. oryzae* and *T. castaneum*.

Keywords: *Callosobruchus maculatus*; *Sitophilus oryzae*; *Tribolium castaneum*; Botanical insecticide

Introduction

Agricultural products including animal and plant products are stored in different types of storage structures for future consumption or trade purposes. During storage these products are damaged by pest organisms among which insects are the most serious. More than 600 species of beetles and 70 species of moths have been reported to be associated with various stored products, including food commodities [1]. Insect infestation causes qualitative and quantitative losses of food commodities. During the year 2010-2011 the food grain production in India reached 250 million tonnes, in which nearly 20-25% of grains were damaged by insect pests [2,3]. Insect contaminants in food materials are believed to cause some health risks to humans [4].

The cowpea weevil *Callosobruchus maculatus* is an important pest of pulses. It damages cowpeas, chickpea and grams after harvest. *Sitophilus oryzae* is a major pest of stored rice and wheat. The flour beetle *Tribolium castaneum* Herbst is a serious pest of milled products in many parts of the world. The efficient control and removal of stored grain pests from food commodities is largely relying on synthetic fumigants such as methyl bromide and phosphine. The use of methyl bromide is restricted in some countries because of its potential damage to the ozone layer [5,6]. Unrestrained application of chemical fumigants caused pesticide resistance in stored product pests. Pests have developed resistance against phosphine [7] and resistance to phosphine is high in Australia and India leading to control failures [8,9].

Several plant extracts, volatile oils and compounds have been reported as effective fumigants and repellents against many stored product pests [10-12]. *Toddalia asiatica* (L.) Lam. (Rutaceae) is a medicinal plant. It is commonly called Wild orange tree or Forest pepper. It is found in South Africa, Sri Lanka and in the lower subtropical Himalayas, South India, Western Nilgiri, Palani hills and Tirunelveli District [13]. The plant has many medicinal properties. The twig is used to treat toothache and gum infection, while the fruits are used to treat irregular menstrual cycle, fever and weakness [14]. The leaves, flowers and roots are used to treat lung and skin diseases, rheumatism, malaria, arthritis, diabetes, cough and throat pain [15-19], stomach ache, to relieve pain in the bowel and used as tooth power [20]. Antioxidant effects of different solvent extracts of *T. asiatica* roots, leaves and stem bark have been reported [21].

In the present study the fumigant toxicity and repellent activity of hexane, diethyl ether and methanol extracts of *T. asiatica* leaves and fruits were studied against *C. maculatus, S. oryzae* and *T. castaneum*.

Materials and Methods

Preparation of plant extracts

Fresh leaves and fruits of *T. asiatica* were collected from natural habitats in and around Chennai, India, during December 2013. The leaves and fruits were washed in tap water and shade dried at room temperature until crisp. The dried plant materials were powdered in an electrical blender and about 3 kg powder was sequentially extracted using hexane, diethyl either and methanol. Each solvent extract was concentrated using rotary vacuum evaporator until the solvent was completely evaporated. The extracts were stored at 4°C for further study.

Insects

The test insects *C. maculatus, S. oryzae* and *T. castaneum* adults (3-5 days old) were obtained from a stock culture maintained at Entomology Research Institute laboratory at 27 ± 1°C and 65 ± 5% relative humidity. All the experiments were carried out under the same environmental conditions.

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Fumigation toxicity

Fumigant toxicity of three solvent extracts of *T. asiatica* leaves and fruits was tested separately against each test insect. An aliquot of 0, 5, 10, 20 and 40 µL of each solvent extract dissolved in acetone was evenly applied to Whatman No. 1 filter paper strips (2 cm diameter) corresponding to the dosages of 0 (as a control), 20, 40, 80 and 160 µL/L air. Each treated paper strip was fixed inside the screw cap of 50 ml glass bottle that contained 10 g of cowpea seeds (for *C. maculatus*), wheat (for *S. Oryzae*) and wheat flour (for *T. castaneum*). After release of 10 adult insects the glass bottles were closed air tight by screw caps. After 24 h of treatment the insects were observed and when there was no leg or antennal movements, insects were considered dead. Percent insect mortality was calculated and corrected by Abbott's formula [22].

Repellent activity

The repellent activity of leaf and fruit extracts was assessed using a Y-tube glass olfactometer. The base and end tubes (arms) of the Y were 20 cm long. The inner diameters of the base tube and arms were 2.5 cm. The extract was applied on a piece of filter paper (2 cm × 3 cm) and placed inside one of the end tube near the opening. In the other end of the tube a blank filter paper strip, the untreated control, was placed. An air current was created by an aerator near the arms and it passed through the base of the Y tube. The rate of air flow was adjusted as 1.25 L/min near each arm. Twenty beetles were released into the olfactometer through the opening of the base tube. The number of insects that moved into the control side and treatment side was recorded after every one hour period and the entire experiment lasted for 3 h. The experiment was replicated five times. Percent repellency was calculated by the formula of [23]:

Percent repellency = $100 \times (C-T)/(C+T)$

Where, C is the number of insects on the control side and T is the number of insects on the essential oil treatment side.

Statistical analysis

Mean values were calculated from the replication values for insecticidal and repellent effects of different concentrations of treatments. The results were statistically analyzed by one way analysis of variance (ANOVA). Significant differences between treatments were determined using Tukey's multiple range test at $P \leq 0.05$. Probit analysis was done to calculate Median Lethal Concentration of LC_{50} and LC_{90} using SPSS 11.5 version software package.

Results and Discussion

Fumigation toxicity

Hexane, diethyl ether and methanol leaf and fruit extracts of *T. asiatica* presented concentration dependant insecticidal activity against all the three test insects (Table 1). The highest toxicity was recorded in diethyl ether extract of fruit at all concentrations against the three insects tested. The highest concentration (160 µL/L) of diethyl ether fruit extract recorded 100 percent mortality in *C. maculatus* and *S. oryzae* and 95.78 percent in *T. castaneum* in 24 h. Among the leaf extracts the diethyl ether extract was found to be the most effective treatment against the three test insects. At the highest concentration (160 µL) the diethyl ether leaf extract presented 86.98, 84.78 and 75.7 percent mortality in *C. maculatus*, *S. oryzae* and *T. castaneum*, respectively. This result coincides with the findings of Lü and He [24] who reported that diethyl ether extracts of *Ailanthus altissima*, *Atractylodes lancea* and *Elsholtzia stauntonii* caused 100, 98.7 and 98% insecticidal activities respectively against *Oryzaephilus surinamensis*. Pascual-Villalobos and Robledo [25] reported that hexane extract of *A. altissima* produced 80% of mortality in *T. castaneum* larvae after topical application of 3 µg of the extract per insect. In the present study methanol extract was found to be the least effective. In contrast to our findings Kim et al. [26] reported that methanol extract of *Cinnamomum sieboldii* root and bark presented 100% mortality and methanol extracts of *Acorus calamus* var. angustatus rhizome, *Acorus gramineus* rhizome, *Illicium*

Solvent	Plant part	Exposure concentration (µL/L air)			
		20	40	80	160
C. maculatus					
Hexane	Leaf	20.22 ± 1.99^{bc}	32.66 ± 1.30^{bc}	40.89 ± 1.60^{b}	47.11 ± 2.53^{c}
	Fruit	32.89 ± 1.82^{a}	46.44 ± 1.72^{b}	55.88 ± 2.05^{a}	71.22 ± 1.88^{a}
Diethyl ether	Leaf	26.56 ± 2.24^{ab}	36.89 ± 2.46^{bc}	65.56 ± 2.41^{bc}	86.98 ± 1.49^{b}
	Fruit	41.44 ± 2.50^{a}	55.89 ± 2.66^{a}	80.33 ± 2.59^{a}	100.0 ± 0.0^{a}
Methanol	Leaf	10.22 ± 0.15^{def}	17.33 ± 1.54^{f}	26.56 ± 1.68^{b}	33.78 ± 2.74^{b}
	Fruit	20.67 ± 0.34^{bc}	29.89 ± 0.96^{cde}	41.22 ± 2.92^{a}	50.56 ± 2.33^{a}
S. oryzae					
Hexane	Leaf	12.71 ± 1.99^{cde}	21.44 ± 1.79^{def}	30.67 ± 1.56^{c}	40.89 ± 2.19^{c}
	Fruit	20.56 ± 2.07^{bc}	30.89 ± 2.04^{bcd}	42.33 ± 1.84^{b}	56.78 ± 2.75^{b}
Diethyl ether	Leaf	24.67 ± 1.93^{ab}	30.67 ± 2.15^{bcde}	59.33 ± 1.78^{c}	84.78 ± 2.19^{b}
	Fruit	30.89 ± 2.04^{a}	45.56 ± 2.10^{b}	71.11 ± 2.93^{ab}	100 ± 0.0^{a}
Methanol	Leaf	6.22 ± 1.70^{ef}	12.33 ± 2.97^{fg}	18.47 ± 1.44^{c}	25.69 ± 1.93^{bc}
	Fruit	12.56 ± 1.61^{cde}	19.67 ± 2.39^{f}	25.89 ± 2.39^{b}	31.89 ± 1.66^{bc}
T. castaneum					
Hexane	Leaf	9.22 ± 1.81^{def}	15.33 ± 2.72^{fg}	22.44 ± 1.97^{d}	31.78 ± 2.08^{d}
	Fruit	12.44 ± 2.09^{cde}	20.78 ± 2.75^{ef}	30.89 ± 2.04^{c}	44.44 ± 2.32^{c}
Diethyl ether	Leaf	15.22 ± 2.65^{cd}	22.76 ± 1.55^{def}	49.11 ± 2.70^{d}	75.70 ± 2.62^{c}
	Fruit	26.89 ± 1.78^{ab}	40.56 ± 2.33^{b}	59.89 ± 2.13^{c}	95.78 ± 1.73^{a}
Methanol	Leaf	3.11 ± 1.59^{f}	6.33 ± 2.34^{g}	10.22 ± 2.23^{d}	15.44 ± 2.47^{d}
	Fruit	5.44 ± 1.46^{ef}	7.33 ± 1.61^{g}	17.56 ± 1.60^{cd}	24.67 ± 1.48^{c}

Values represent Mean ± SD, Similar alphabets in a column do not differ significantly using Turkey's test ($P \leq 0.05$).

Table 1: Insecticidal activity of *T. asiatica* against three stored pests.

verum fruit, and *Foeniculum vulgare* fruit presented 90% mortality against *S. oryzae*. Similarly Jovanovic et al. [27] reported that ethanol extract, the high polar solvent extract of *Urtica dioica* and *Taraxacum officinale* presented 100% mortality against bean weevil *Acanthoslides obtectus* Say.

At lower concentrations the fruit extract showed 41.11, 30.89 and 26.89% mortality against *C. maculatus*, *S. oryzae* and *T. castaneum* respectively. The insecticidal activity progressively increased with the increasing concentrations. Hexane fruit extract showed insecticidal activity of 71.22, 56.78 and 44.44% and leaf extract exhibited 47.11, 40.89 and 31.78% insecticidal activity against *C. maculatus*, *S. oryzae* and *T. castaneum* respectively after 24 h at higher concentration (Table 1). The fruit methanol extract exhibited least activity than hexane and diethyl ether against tested insects (Table 1). Methanol leaf and fruit extracts of *T. asiatica* exhibited less than 35.00% insecticidal activity against *T. castaneum*. Similarly, Liu et al [28] reported that hexane extract of *Evodia nutaecarpa*, *Artemisia argyi* and *Quisqualis indica* killed *T. castaneum*. Talukder and Howse [29] reported that ethanol extracts of *Aphanamix polystachya* Wall and Parker produced insecticidal activity against *T. castaneum*. The fruit diethyl ether extract had LC_{50} values of 39.19, 44.13, 61.10 µL/L and LC_{99} 124.58, 171.72 and 183.95 µL/L against *C. maculatus*, *S. oryzae* and *T. Castaneum* respectively, followed by hexane and methanol extracts. All the treatments with fruit extract were superior than leaf extracts (Table 2). In all the treatments *C. maculatus* was more susceptible than *S. oryzae* and *T. Castaneum*. Similarly, *Cymobogan nardus*, *Mentha arvensis*, *M. piperata* and *M. spicata* recorded significant mortality against *C. maculatus* [30].

Repellent activity

Fruit extract of diethyl ether produced 100% repellent activity against *C. maculatus* and *S. oryzae*. It exhibited 92.0% repellent activity against *T. castaneum*. The present finding corroborates with the findings of Ukeh et al. [31] who found that diethyl ether extracts of *Aframomum melegueta* and *Zingiber officinale* repelled the adults of *S. zeamais*. Plenty of literature is available to support the repellent activity of plant extracts against stored product pests. Dwivedi and Shekhawat [32] reported that acetone extract of *Emblica officinalis*, *Datura alba*, *Ziziphus jujuba* and petroleum ether extract of *Ziziphus jujuba* exhibited 88.66, 77.58, 77.55 and 66.22 percent repellency against *Trogoderma granarium* (Everts) respectively. Acetone seed extract of *Aphanamixis pofystachya* showed cent percent repellent effects on red flour beetles [29]. Jovanovic et al. [27] reported that ethanol extracts of *Urtica dioica* and *Taraxacum officinale* showed 99.4 and 98.8% repellency respectively after 48 h; The ethanol extract of *Achilloa milletolium* provided 79.1% repellency. Pavela [33] reported that essential oils of *Carum carvi* L., *Cinnamomum osmophloeum* Kaneh., *Citrus aurantium* L., *Nepeta cataria* L. and *Thymus vulgaris* L. produced repellent activity against *Meligethes aeneus* adults at 10 µL/mL concentration after 1 h.

The diethyl ether leaf extract repelled 50.89, 41.8 and 36.9% against *C. maculatus, S. oryzae* and *T. castaneum* respectively at 20 µL concentration after 3 h exposure (Table 3). The fruit and leaf hexane extracts exhibited less than 50 percent repellent activity against all the tested insects. At the highest concentration (20 µL) of hexane and methanol fruit extracts the repellency was 45.78 and 41.33 percent against *C. maculatus*, but in leaf extracts it was 23.56 and 25.56 percent (Table 3). Many plant products, such as essential oils, have been screened for their repellent activity against stored grain pests [23,34,35]. Other studies have shown that *T. castaneum* can also be repelled by essential oils from *Evodia rutaecarpa* [36], *Ocimum gratissimum* L. [37] and *Artemisia vulgaris* L. [38].

Plant products have considerable potential as insecticidal compounds and are gaining tremendous importance in recent years. The presence of volatile compounds is responsible for strong odour that could block the tracheal respiration of the insects leading to their death [39].

Conclusion

In the present study the diethyl ether extract of *T. asiatica* showed higher insecticidal and repellent activities than rest of the solvent

Solvent extracts	Plant part	LC_{50} (µL/L)	95% confidence limit		LC_{99} (µL/L)	95% confidence limit		Chi-square	*P* - value
			Lower	Upper		Lower	Upper		
				C. maculatus					
Hexane	Leaf	140.24	118.20	175.50	463.20	373.18	625.88	34.78	0.923
	Fruit	79.35	67.33	92.69	312.26	266.39	382.71	45.57	0.573
Diethyl ether	Leaf	68.81	60.73	77.60	217.89	193.86	250.90	36.11	0.897
	Fruit	39.19	34.15	44.4 8	124.58	110.09	145.19	40.98	0.754
Methanol	Leaf	196.44	162.04	259.83	535.68	421.11	759.76	23.32	0.996
	Fruit	131.07	111.69	160.31	430.60	352.43	565.57	31.02	0.973
				S. oryzae					
Hexane	Leaf	168.19	142.14	211.45	475.52	384.30	639.60	24.96	0.998
	Fruit	118.35	102.32	140.64	383.92	321.28	485.58	31.44	0.969
Diethyl ether	Leaf	76.53	68.07	85.95	230.79	205.34	265.72	32.35	0.599
	Fruit	44.13	33.85	50.86	171.72	101.75	182.48	40.30	0.999
Methanol	Leaf	240.49	191.89	342.78	608.48	462.13	929.32	34.27	0.932
	Fruit	208.24	166.71	293.62	603.31	457.99	919.17	30.81	0.975
				T. castaneum					
Hexane	Leaf	209.03	171.14	281.09	553.34	431.74	797.09	29.15	0.986
	Fruit	157.13	134.11	193.61	448389	367.29	590.33	30.42	0.978
Diethyl ether	Leaf	96.50	86.71	107.99	264.93	235.08	306.45	32.66	0.956
	Fruit	61.10	54.18	68.70	183.95	163.87	211.51	35.37	0.912
Methanol	Leaf	325.50	239.86	574.27	761.32	532.98	1441.19	43.63	0.650
	Fruit	240.30	192.71	338.67	597.49	456.54	900.92	35.57	0.900

Table 2: Effective concentration for insecticidal activity of *T. asiatica* against three stored pests.

Solvent	Plant part	Exposure concentration (µL/L air)			
		5.0	10	15	20
C. maculatus					
Hexane	Leaf	2.86 ± 1.85[a]	8.89 ± 2.22[ab]	20.75 ± 2.94[abc]	23.56 ± 2.30[bc]
	Fruit	7.30 ± 2.03[a]	13.02 ± 0.78[a]	36.67 ± 2.18[a]	45.78 ± 2.99[a]
Diethyl ether	Leaf	10.07 ± 2.71[abc]	14.52 ± 2.69[cd]	31.67 ± 2.21[c]	50.89 ± 2.66[b]
	Fruit	22.38 ± 2.63[a]	50.24 ± 3.89[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]
Methanol	Leaf	2.22 ± 1.21[a]	7.30 ± 2.03[a]	17.30 ± 2.19[b]	25.56 ± 2.06[b]
	Fruit	5.08 ± 2.15[a]	10.79 ± 2.76[a]	32.86 ± 2.68[a]	41.33 ± 3.74[a]
S. oryzae					
Hexane	Leaf	0.0 ± 0.0[a]	4.44 ± 2.78[c]	12.44 ± 2.69[bc]	18.22 ± 1.77[c]
	Fruit	2.86 ± 1.85[a]	10.16 ± 2.63[ab]	26.67 ± 2.21[ab]	36.44 ± 2.77[ab]
Diethyl ether	Leaf	5.08 ± 1.15[bc]	9.52 ± 2.61[d]	26.01 ± 2.07[c]	41.78 ± 2.30[b]
	Fruit	15.79 ± 1.75[ab]	37.22 ± 1.63[ab]	83.56 ± 3.13[c]	100.0 ± 0.0a
Methanol	Leaf	0.0 ± 0.0[a]	0.0 ± 0.0[a]	8.89 ± 1.97[b]	17.44 ± 2.74[b]
	Fruit	0.0 ± 0.0[a]	5.08 ± 1.15[a]	19.71 ± 2.39[ab]	29.33 ± 1.99[ab]
T. castaneum					
Hexane	Leaf	0.0 ± 0.0 [a]	0.0 ± 0.0[c]	6.22 ± 2.05[c]	12.89 ± 1.77[c]
	Fruit	0.0 ± 0.0 [a]	0.0 ± 0.0[c]	20.22 ± 2.56[abc]	28.89 ± 2.06[abc]
Diethyl ether	Leaf	2.22 ± 1.22[c]	6.67 ± 2.72[d]	17.90 ± 2.07[d]	36.89 ± 2.16[b]
	Fruit	10.16 ± 2.63[abc]	27.56 ± 2.23[bc]	66.22 ± 3.18[b]	92.00 ± 2.89[a]
Methanol	Leaf	0.0 ± 0.0[a]	0.0 ± 0.0[a]	4.44 ± 2.72[b]	13.89 ± 2.77[b]
	Fruit	0.0 ± 0.0[a]	0.0 ± 0.0[a]	13.65 ± 0.63[b]	22.78 ± 2.89[b]

Values represent Mean ± SD; Similar alphabets in a column do not differ significantly using Tukey's test (P ≤ 0.05).

Table 3: Percent repellent activity of solvent extracts of *T. asiatica* against *C. maculatus*, *S. oryzae* and *T. castaneum* after 3 h in the filter paper test.

extracts. This extract may be further studied for the identification of active compounds.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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