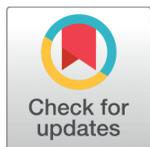


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Fumigant effect of essential oils against pulse beetle (*Callosobruchus chinensis* L.) and rice weevil (*Sitophilus oryzae* L.) in stored products

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Abstract

Objectives: To develop a method to replace the usage of synthetic fumigants in stored product pest management. **Methodology:** For this purpose, we employed four plant based essential oils namely, *Lantana camara* (*Lantana*) oil, *Citronella nardus* (*Citronella*) oil, *Cinnamomum zeylanicum* (*Cinnamon*) oil and *Trachyspermum copicum* (*Ajwain*) oil and evaluated against pulse beetle, *Callosobruchus chinensis* L., and rice weevil, *Sitophilus oryzae* L., for their fumigant toxicity and the weight loss caused by them. The oils of *Lantana* and *Citronella* were extracted from leaves, *Cinnamon* and *Ajwain* from barks and seeds respectively. **Findings:** Among the essential oils tested for fumigant toxicity, *C. zeylanicum* performed better with Lethal Concentration (LC₅₀) of 23.16 and 21.91 µl in minimal period of exposure i.e. at 12 h of treatment and found effective against adults of test insects viz., *C. chinensis*, and *S. oryzae* respectively. During the maximum period of exposure i.e. at 72 h. the fumigation efficacy of *T. copicum* was noticed to be the highest (3.43 µl) against adults of *C. chinensis* whereas *C. zeylanicum* was found better against *S. oryzae* (6.19 µl). The minimum loss of pulse grains was observed in *C. zeylanicum* treated at 60 % of 24 h LC₅₀, with 7.41 % weight loss. The minimum weight loss was noticed in *C. zeylanicum* about 60 % of 24 h LC₅₀ with 9.68 per cent against *S. oryzae*. The fumigant toxicity bioassay revealed that as the exposure period increases, mortality of test insects also found increased i.e. exposure period directly proportional to mortality of insects.

Keywords: Coleoptera; essential oils; fumigant toxicity; stored product pests; volatiles

1 Introduction

The food requirement of an increasing human population remains a major global issue. More than one third of food is lost or wasted during post harvest operations. Reducing the post harvest losses, a concrete solution will be to increase the availability of

food, reduce damage on natural resources, eliminate hunger and improve farmers' livelihoods. Cereal grains are the staple food in most of the developing nations and it account for maximum post harvest losses. Owing to lack of technical knowledge, 50 – 60 per cent of cereal grains are lost during storage [1]. As per the report of Food Corporation of India (FCI), about 62,000 tonnes of food grains have been damaged in the storage during the last six years, in 2016-17 (up to 1st March), a damage of 8,679 tonnes of food grains was noticed. Pest attacks, leakages, poor quality stocks, exposure to rains, floods and negligence are the cause for grain damage in godowns [2]. Storage losses have been estimated as 14 million tonnes of food grain and worth of ₹7,000 crore every year in India, out of which insects alone account for nearly ₹1,300 crores. The loss of food grains in the farmer holdings in Tamil Nadu was estimated as 12.9 % in paddy, 16.0 % in sorghum, 14.0 % in bajra and 12.7 % in maize. Out of all the post harvest losses, storage alone 6.58 %, in this insect alone account for 2.0 to 4.2 % followed by rodents (2.50 %), Birds (0.85 %) and moisture (0.68 %) [3].

Among the storage pests, the cowpea beetle, *C. chinensis* L. and the four spotted bean weevil, *Callosobruchus maculatus* F. (Bruchidae: Coleoptera) are the notorious pests of common legumes and pulses grown in Asia, Africa, Central and South America. It lays eggs on the seed coat, the grub hollows out the grain and cause huge yield loss [4]. The rice weevil, *S. oryzae* L. (Coleoptera: Curculionidae) is one of the big menaces and causes huge loss of stored grain both quantitatively and qualitatively worldwide [5, 6]. The most effective methods for the protection of stored products from insect pests are fumigation. Usage of phosphine leads to development of resistance in insects and may cause control failures. Although chemical insecticides are effective, their continuous use has led to several problems viz., residue, environmental pollution, and effect on humans. Many insecticides have either been banned or restricted in their use due to the problems mentioned above. This led to the need for biodegradable pesticides with greater selectivity. Plants may act as potential alternatives to the currently used insect control agents as they constitute a rich source of bioactive molecules [7].

Hence, an attention has been given to utilize the plant products against stored grain pests [8]. Further, botanical insecticides are best for organic food production during post harvest protection of food grains [9]. Botanical insecticides composed of essential oils may be an alternative to the synthetic pesticides [10]. Essential oils are volatile oil(s) comprising strong aromatic components and give distinct odour, flavour or scent to a plant and the by-products of plant metabolism. They are obtained from various plant parts namely, leaves, stems, bark, flowers, roots and/or fruits. The aromatic characteristics of essential oils may provide various functions include attracting or repelling insects, utilizing chemical constituents in the oil as defence materials [11]. Plant essential oils and their constituents have been studied against stored product pests as alternatives to classical fumigants [12]. In developing countries, aromatic plants are widely used for stored-product insects in traditional agricultural systems. Essential Oils are produced by steam distillation of plant material and contain many volatile, low-molecular-weight terpenes and phenolics [13]. Essential oils show a broad spectrum of activity against insect pests as insecticidal, antifeedant, repellent, oviposition deterrent and growth regulatory activities. The use of these oils in the control of stored product pests is safer for human and animal health [14]. Hence, essential oils are promising alternatives to chemical insecticides [15]. Many formulations are being used against stored product insects and their selection or synthesis of formulation is important to control target insects [16]. Considering the need for environment friendly, safe, economically feasible and also effective insecticide to manage the insect pests of stored products, the present study was under taken to find out the fumigant toxicity of selected essential oils against the adult stages of two coleopteran pests namely, *C. chinensis* and *S. oryzae* inflicting damage to stored produce.

2 Materials and methods

2.1 Test Insect culture

The test insects such as pulse beetle, *C. chinensis*, and rice weevil, *S. oryzae* were reared at the Department of Entomology, Annamalai University. They were mass cultured in 1kg capacity glass jar of size 15x10 cm containing respective food materials such as green gram for *C. chinensis* and rice grains for *S. oryzae* each 500 g as a nutritional source at 60-70 per cent relative humidity and temperature ranged from 30-35° C. Then glass jars were covered with a fine muslin cloth and secured with rubber band to facilitate aeration. Maximum of seven days were allowed for mating and oviposition in separate rearing containers every day. Then the parent stocks were removed and food media containing eggs were incubated in the temperature/humidity as mentioned above in darkness to obtain same aged insects. In every generation, half of the completely infested grains were replaced with the same quantity of uninfested materials [17]. Thus, a continuous culture was maintained throughout the study period and subsequent progenies of the insects were used for the experiments.

2.2 Procurement of essential oils

Based on the fumigant action of essential oils of plant species from literatures surveyed, the essential oils namely, *L. camara* (*Lantana*) oil, *C. nardus* (Citronella) oil, *C. zeylanicum* (Cinnamon) oil and *T.opicum* (Ajwain) oil were selected. The oils of *Lantana* and Citronella were extracted from leaves, Cinnamon and Ajwain from barks and seeds respectively. The essential oils used for this study were purchased from the Allins Exports Private Limited, Noida, Uttar Pradesh and Surajbala Exports (P) Ltd., New Delhi.

2.3 Fumigant toxicity of essential oils against test insects

Glass vials of 10 cm long and 3 cm diameter with polystyrene cap were used for testing the fumigant toxicity of test insects like *C. chinensis* and *S. oryzae*. For fumigation, filter paper strip (1 cm²) treated with solutions of five different concentrations (5, 10, 15, 20 and 25 µl for *C. chinensis* and 10, 20, 30, 40 and 50 µl for *S. oryzae*) of essential oils prepared in acetone was placed on the inner surface of each screw caps of the glass vials. The treated filter paper strips were air dried for 15 minutes for evaporating the solvents at room temperature. Then ten grams of respective food materials were taken, and ten adults were released in each vial. The open end of the vials was closed by the cap so that the oil treated filter paper remains inside the vial. For each treatment, five different concentrations and for each concentration three replicates were used. The vials were kept at 30 + 2°C, 75 + 5% RH and a photoperiod of 10:14 (L: D) hours. Mortality of adults was recorded after every 12 h of treatment up to 72 h. Two controls were set one is standard check (i.e.) filter paper strip treated only with acetone and another one was untreated [18].

2.4 Estimation of weight loss

Estimation of loss in weight of food sources which was fumigated with two different concentrations of essential oils at 30 % and 60 % of the 24 h LC₅₀ was done. Ten pairs of freshly emerged adults of the test insects were selected and allowed into the jars containing 100 gm of respective food material. The experiment was replicated thrice. A control was set up without any treatment. The grains were maintained for completion of one generation of test insects and weighed using weighing balance and the per cent loss in weight were determined [19].

$$\text{Per cent weight loss} = \frac{W1 - W2}{W1} \times 100$$

Where,

W1 = weight of baseline sample,

W2 = subsequent sample weight at different storage intervals.

2.5 Statistical analysis

The LC₅₀ values for selected essential oils were calculated by using POLO software program. The data obtained from the assessment of weight loss experiment was analysed statistically by using Completely Randomised Block Design (CRBD). Based on the Analysis of Variance (ANOVA) and Least Significant Difference test (LSD), treatment effects were compared and ranked [20, 21].

3 Results and discussion

3.1 Toxicity of selected essential oils against test insects due to fumigation

The fumigant effect of essential oils at different exposure periods were tested against the adult beetles of *C. chinensis* and Lethal Concentration (LC₅₀) along with Upper and Lower Confidence Limit (UCL and LCL 95 %) were obtained and the results presented in Table 1 as µl/ lit. The minimum exposure period taken for the study is 12 h and the maximum exposure period is 72 h. Among the essential oils tested, *C. zeylanicum* performed better at 12 h of exposure period followed by *L. camara*, *T.opicum* and least performance with *C. nardus*, their 12 h LC₅₀ values were 23.16 µl, 25.23 µl, 30.80 µl and 33.89 µl respectively. The results of these essential oils varied greatly at different exposure periods from 24 h to 60 h. The LC₅₀ values of *T.opicum* and *C. zeylanicum* were found almost similar during 36 h of exposure period. During 72 h of exposure period, the minimum LC₅₀ value was obtained in *T.opicum* (3.43 µl) followed by *C. zeylanicum* (6.49 µl), *C. nardus* (7.15 µl) and *L. camara* (8.60 µl).

The results revealed that, as the exposure period increased the fumigation effect of essential oils also increased. A minimum exposure period of 12 h of treatment with *C. zeylanicum* and a maximum exposure period of 72 h of treatment with *T.opicum*

Table 1. LC₅₀ of selected essential oils at different exposure periods against adults of *C. chinensis* in laboratory condition

Essential oils	Exposure periods (hours)	LC ₅₀ ^a (µl)	LCL ^b	UCL ^b
<i>T. copicum</i> (Ajwain)	12 h	30.80	17.83	53.19
	24 h	20.56	12.93	32.69
	36 h	12.97	08.46	19.35
	48 h	08.75	05.54	13.83
	60 h	06.08	03.27	11.30
	72 h	03.43	01.11	10.60
<i>L. camara</i> (Lantana)	12 h	25.23	16.21	39.29
	24 h	19.97	14.65	27.22
	36 h	16.44	10.77	25.10
	48 h	13.44	09.54	18.94
	60 h	11.20	07.30	16.62
	72 h	08.60	05.58	13.25
<i>C. nardus</i> (Citronella)	12 h	33.89	10.88	105.54
	24 h	19.24	12.65	29.26
	36 h	18.02	10.51	30.91
	48 h	12.31	08.63	17.58
	60 h	10.53	07.46	14.86
	72 h	07.15	04.93	10.38
<i>C. zeylanicum</i> (Cinnamon)	12 h	23.16	10.63	50.46
	24 h	14.01	10.08	19.48
	36 h	12.98	9.72	17.35
	48 h	10.52	7.44	14.88
	60 h	07.72	5.01	11.91
	72 h	06.49	3.63	11.58

^a LC₅₀ represents lethal concentrations that cause 50% mortality
^b LCL and UCL represents lower and upper confidence levels

found with better results against adult beetles of *C. chinensis*. The LC₅₀ values of essential oils at different exposure periods against adults of *S. oryzae* are given in Table 2. During the minimal exposure period and maximum exposure period, i.e. 12 h and 72 h of the study, *C. zeylanicum* excelled other treatments with LC₅₀ of 21.91 µl at 12 h and 6.19 µl at 72 h. This was closely followed by *L. camara*, observed with LC₅₀ of 22.27 µl. The essential oils of *T. copicum* and *C. nardus* LC₅₀ values were followed suit with 27.17 µl and 32.63 µl respectively at 12 h. The results of the essential oils of *T. copicum* and *L. camara* were found closer with each other during 48 h of exposure period recorded as 12.36 and 12.28 µl respectively while *T. copicum* and *C. zeylanicum* were also caused nearly similar effects during 60 h of exposure period and found with 9.38 and 9.11 µl respectively. At 72 h, it was observed that *T. copicum* manifests its effectiveness with a LC₅₀ of 7.24 µl, followed by *L. camara* and *C. nardus* with 8.72 µl and 10.63 µl respectively. This might be due to the presence of phytochemicals i.e. secondary plant metabolites in these essential oils had the capability to emit the strong pungent fumigant odour that acts against the target insects. Due to this insecticidal action, the insect became susceptible and showed promising lethal effects.

Table 2. LC₅₀ of selected essential oils at different exposure periods against adults of *S. oryzae* in laboratory condition

Essential oils	Exposure periods (hours)	LC ₅₀ ^a (µl)	LCL ^b	UCL ^b
<i>T. copicum</i> (Ajwain)	12 h	27.17	15.21	48.54
	24 h	22.48	14.48	34.91
	36 h	17.73	10.71	29.37
	48 h	12.36	04.99	30.60
	60 h	09.38	04.13	21.30
	72 h	07.24	02.19	23.89

Continued on next page

Table 2 continued

Essential oils	Exposure periods (hours)	LC ₅₀ ^a (μl)	LCL ^b	UCL ^b
<i>L. camara</i> (Lantana)	12 h	22.27	13.73	36.13
	24 h	18.34	10.79	31.19
	36 h	15.81	10.36	24.12
	48 h	12.28	07.08	21.29
	60 h	11.43	06.31	20.73
	72 h	08.72	03.62	21.01
<i>C. nardus</i> (Citronella)	12 h	32.63	20.46	52.04
	24 h	26.92	18.99	38.16
	36 h	20.95	14.44	30.40
	48 h	17.53	11.21	27.39
	60 h	13.44	06.96	25.96
	72 h	10.63	05.63	20.06
<i>C. zeylanicum</i> (Cinnamon)	12 h	21.91	11.87	40.45
	24 h	16.45	06.75	40.09
	36 h	13.49	06.25	29.14
	48 h	11.00	04.02	30.07
	60 h	09.11	02.73	30.51
	72 h	06.19	01.08	35.50

^a LC₅₀ represents lethal concentrations that cause 50% mortality

^b LCL and UCL represents lower and upper confidence levels

Many plant-derived materials such as monoterpenoids have fumigant action against a variety of insect pests attributed to their high volatility. Monoterpenoids (limonene, linalool, terpineol, carvacrol and myrcene) are the main insecticidal constituents of many essential oils effective against stored product insects [22, 23]. The findings of the present study is in accordance with the investigation by [24], two monoterpenes cinnamaldehyde and linalool were selected from different constituents of cinnamon essential oil and the results revealed higher toxicity to *C. maculatus* than adults of *S. oryzae*. Further the results are supported by [25] who reported that cinnamon oil provided the highest toxicity to adults and 10, 14 and 18 days old larvae, with LD₅₀ values of 0.03, 0.05, 0.088 and 0.09 mg cm⁻³ respectively in fumigation bioassay. Similar findings were also obtained by [26] that the insecticidal efficacy of *C. zeylanicum* and its two major constituents (Linalool and Cinnamaldehyde) against *C. maculatus* and *S. oryzae*. They unveiled that these two components of *C. zeylanicum* exhibited contact and fumigant toxicity against the adults of both the insect species. The toxic effects of cinnamon essential oil were attributed to its major constituent monoterpenes which are highly volatile and possess high fumigant toxicity. On the same note, [27] reported that the presence of toxic compounds such as 1, 2 naphthalenedione ethanone and borneol in cinnamon where cinnamaldehyde is the principle one. The results of the present investigation supported by previous findings [28] stated that cinnamon oil performed better against the pulse beetle, *C. chinensis* with the LC₅₀ values obtained at 24, 48, 72 and 96 h was 0.712 (0.597-0.859)%, 0.628 (0.506-0.764)%, 0.479 (0.332-0.602)% and 0.397 (0.234-0.511)% respectively. Cinnamon oil vapour exhibited 100 % mortality against *S. oryzae* adults when given at a dosage of 0.7 mg cm⁻² for 24 h [29].

3.2 Effect of fumigation of essential oils on the damage caused by test insects

The minimum loss of pulse grains was observed in *C. zeylanicum* treated at 60 % of 24 h LC₅₀ closely followed by *L. camara* 60 % of 24 h LC₅₀ and *T. copicum* 60 % of 24 h LC₅₀ with the per cent weight loss of 7.41, 8.27 and 12.77 per cent respectively. The maximum weight loss was observed in untreated control with 65 %. Among the treatments, the maximum weight loss was noticed in *C. nardus* 30 % of 24 h LC₅₀ recorded 27.50 % followed by *T. copicum* 30 % of 24 h LC₅₀ and *L. camara* 30 % of 24 h LC₅₀ with 26.26 % and 18.48 % respectively. The treatments, *C. zeylanicum* 60 % of 24 h LC₅₀ and *L. camara* 60 % of 24 h LC₅₀ were statistically on par with each other and also the treatments of *T. copicum* 30 % of 24 h LC₅₀ and *C. nardus* 30 % of 24 h LC₅₀ was witnessed with similar results statistically. The minimum weight loss was noticed in the treatment *C. zeylanicum* 60 % of 24 h LC₅₀ with the weight loss per cent of 9.68, this was followed by *T. copicum* 60 % of 24 h LC₅₀ with 11.50 % and 14.78 % in the treatment of *L. camara* 60 % of 24 h LC₅₀ against *S. oryzae*. The maximum weight loss was observed in the treatment *C. nardus* 30 % of 24 h LC₅₀ followed by *L. camara* 30% of 24 h LC₅₀ and *T. copicum* 30 % of 24 h LC₅₀ with the damage per cent of 33.57, 29.48 and 21.95 respectively. The *C. nardus* 60% of 24 h LC₅₀ and *C. zeylanicum* 30 % of 24 h LC₅₀ treatments were statistically on par with each other (Table 3). The weight loss of seeds/grains was found ranging from 7.41 % to 37.15%

in almost all the treatments and also against all the target insects. When comparing all the treatments, *C. zeylanicum* 60% of 24 h LC₅₀ provided minimum weight loss per cent and *C. nardus* 30% of 24 h LC₅₀ manifested poor results with maximum percent weight loss against target insects. The reduction in weight loss might be due to the fumigant effects of the essential oils tested, the volatile substances are mainly responsible for positive decrease in feeding of the respective food materials by the test insects.

Table 3. Effect of fumigation of essential oils on the damage caused by *C.chinensis* and *S. oryzae* in laboratory condition

Essential Oils	Treatments	Per cent weight loss (caused by one generation)	
		<i>C. chinensis</i>	<i>S. oryzae</i>
<i>T. copicum</i>	T1 - 30% of 24-h LC50	26.26 (30.82)c	21.95 (27.93)e
	T2 - 60% of 24-h LC50	12.77 (20.90)e	11.50 (19.82)h
<i>L. camara</i>	T3 - 30% of 24-h LC50	18.48 (25.45)d	29.48 (32.88)d
	T4 - 60% of 24-h LC50	08.27 (16.69)f	14.78 (22.61)g
<i>C. nardus</i>	T5 - 30% of 24-h LC50	27.50 (31.61)c	33.57 (35.41)c
	T6 - 60% of 24-h LC50	17.53 (24.74)d	18.76 (25.66)f
<i>C. zeylanicum</i>	T7 - 30% of 24-h LC50	12.60 (20.77)e	18.02 (25.11)f
	T8 - 60% of 24-h LC50	07.41 (15.75)f	09.68 (18.12)i
	T9 – Acetone	60.53 (51.08)b	62.27 (52.10)b
	T10 – Control	65.13 (53.81)a	67.86 (55.47)a
SE.d		1.12	0.58
CD (0.05)		2.33	1.21

*Mean of three replications

*DAT – Days after treatment

*Values in parenthesis are arc sine transformed

*Values with different alphabets differ significantly according to LSD

4 Conclusion

The essential oils obtained from naturally available plant species exhibited their potential fumigant effect against the target pests and also caused minimal damage to grains by them. From the results, it was noticed that as the exposure period to fumigation increases, mortality of test insect pests of grains also increased i.e. exposure period directly proportional to mortality of pests. However, further studies are required on the safety issues of essential oils against non-target organisms and to explore the mechanism of action against target pests. Furthermore, isolation and characterisation of the essential oil will also provide complete details of the compounds responsible for pesticidal activity and helpful in the preparation of easily usable formulations against stored produce pests. Hence, the use of essential oils of plant species may also be incorporated in the stored product pest management programme. It is used as a natural protectant in small scale storage so as to avoid the usage of synthetic chemicals. In India especially Tamil Nadu, it will be ideal if such organic way of protecting the grains in stores and supply of food grains through fair price shops is implemented on experimental basis as a public welfare measure.

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