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Fumigant Toxicity of Essential Oils from Some Common Spices against Pulse Beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae)

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Abstract: In the present study, the essential oil from seven common spices, *Anethum graveolens*, *Cuminum cyminum*, *Illicium verum*, *Myristica fragrans*, *Nigella sativa*, *Piper nigrum* and *Trachyspermum ammi* was isolated and its insecticidal, oviposition, egg hatching and developmental inhibitory activities were determined against pulse beetle, *Callosobruchus chinensis*. Essential oils were isolated by hydrodistillation method using Clevenger apparatus. These essential oils caused death of adults and larvae of *Callosobruchus chinensis* when fumigated. The 24-h LC_{50} values against the adults of the insect were 8.9 μl, 10.8 μl, 11.0 μl, 12.5 μl, 13.6 μl, 14.8 μl and 15.6 μl for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively. On the other hand, against larval stage these values were 6.4 μl, 7.9 μl, 8.9 μl, 11.1 μl, 11.7 μl, 12.2 μl and 13.5 μl for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* respectively. These essential oils reduced the oviposition potential, egg hatching rate, pupal formation and emergence of adults of F_1 progeny of the insect when fumigated with sublethal concentrations. These essential oils also caused chronic toxicity as the fumigated insects caused less damage to the stored grains. The essential oil of *N. sativa* was found most effective against all the different stages of the *Callosobruchus chinensis* followed by *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils. All the responses were found concentration-dependent. The toxic and developmental inhibitory effects may be due to suffocation and inhibition of various biosynthetic processes of the insects at different developmental stages.

Key words: Insecticide, essential oils, oviposition deterrence index, hatching inhibition rate, *Callosobruchus chinensis*

1 INTRODUCTION

Insect pests of stored grains have been damaging our economy by infesting agricultural stored products 10-40% annually[1]. In the present scenario, of all demands of human, food is of prime importance and because of increasing population pressure, the task of food production and protection is our priority. In such a critical situation, besides increasing our crop production, protection of agricultural products specially stored grains is quite necessary. In this regard, attempts to protect stored grains and other agricultural products from insect infestation, various synthetic insecticides have been used. But many insects have acquired resistance against most of these synthetic pesticides[2-4] and the uncontrolled use of these synthetic pesticides causes great problem for environment and consumers due to residual property[5]. Besides, the efficacy of these synthetic insecticides against storage pests varies greatly after treatment[6]. Thus, it is an urgent need to develop certain new alternatives that must be biodegradable, ecologically safe with no residual activity and adverse effect on other non-target animals. In this direction, many products of botanical origin have been evaluated for their toxic properties against different stored grain pests[7-10] especially in form of essential oils[11-15]. Besides crude essential oils, their constituents have also been evaluated for toxicity against stored product insect pests[16,17]. These essential oils are the complex mixture of volatile organic compounds produced as secondary metabolites whose functions are other than the nutrition.

Callosobruchus chinensis is a serious pest of stored

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gram and cowpea but also infests beans, lentil and other pulses. In India, it is responsible for 32-64% loss under storage conditions and the maximum damage occurs from April to October[18]. Only grubs are infective stages for the stored grains. These make holes in the grains and consume the inner part leaving empty kernel. The damaged grains become unsuitable for human consumption, production of sprout and also lose its market value. For the control of this pest, various synthetic pesticides in the form of fumigants, sprays and dusts have been used in the past. Some of these methods are not successful because they result in the high rate of migration of insects in the form of eggs, larva, pupa and adults during transportation from field to godowns[19]. From the last two decades, for the control of insect pests efforts have been made to develop new alternatives of synthetic insecticides in form of different plant products. Different workers have used different plant based agents to minimize the damage caused by this pest e.g. constituents of *Foeniculun vulgare* fruit have been shown to possess insecticidal activity[20]. Insecticidal nature of constituents of certain aromatic plants has been evaluated and mentioned that the insecticidal activity of these constituents was largely due to fumigant action[21]. Different extracts and pure compounds of *Capparis decidua* have been reported for toxic and oviposition inhibitory activities[9]. The efficacy of some vegetable seed oils of Cucurbitaceae family have been evaluated and proved that these oils successfully protect the infestations in legume pulse grains[22]. In the present study, I report laboratory studies on the insecticidal, oviposition, egg hatching and developmental inhibitory activities; and chronic toxicity of essential oils from common spices against pulse beetle, *Callosobruchus chinensis*.

2 EXPERIMENTAL

2.1 Essential oils

The dried fruits of dill (*Anethum graveolens*, Umbelliferae), cumin (*Cuminum cyminum*, Umbelliferae), star anise (*Illicium verum*, Mangoliacaea), mace (*Myristica fragrans*, Myrtiaceae), black cumin (*Nigella sativa*, Ranunculaceae), black pepper (*Piper nigrum*, Piperaceae) and Ajowan (*Trachyspermum ammi*, Umbelliferae) were purchased from local market of Gorakhpur, (U.P.), India. These were grounded by domestic mixer and powdered material was hydrodistilled in Clevenger apparatus continuously for 5 hours to yield essential oils. Oils were collected in glass containers and kept at 4℃ until their use.

2.2 Insects rearing

Pulse beetles, *Callosobruchus chinensis* was used to determine the insecticidal nature of essential oils. The insects were reared on cow-pea in laboratory at 30 ± 2℃, 75 ± 5% RH and a photoperiod of 10:14 (L:D) hours.

2.3 Toxicity assay

Glass vials (10 cm long and 3 cm diameter) with polystyrene cap were used for the toxicity assay against adults and larvae. For fumigation, one filter paper strip (1 cm^2) treated with essential oil was pasted on the inner side of cap. Ten grams of cow-pea grains were taken in the each vial and twenty adults were transferred to each vial and the open end of the vial was closed by the cap so that the oil treated filter paper remained inside the vial. For each type of essential oil, four different concentrations and for each concentration six replicates were used. Toxicity assay against larval stage was performed as previously. The vials were kept at 30 ± 2℃, 75 ± 5% RH and a photoperiod of 10:14 (L:D) hours. Mortality of the adults and larvae were recorded after every 24 hours of treatment up to 96 hour. In control, untreated filter paper was used.

2.4 Oviposition inhibitory assay

Oviposition inhibitory activities of essential oils were determined by fumigating newly emerged adults with the two sublethal concentrations viz. 30% and 60% of 24-h LC_{50}, of the essential oils as was done in the toxicity assay. Ten grams of cow-pea were placed at the bottom of the vial and twenty adults were transferred to the vial. Vials were kept at 30 ± 2℃, 75 ± 5% RH and a photoperiod of 10:14 (L:D) hours. For each concentration of oil and control, six replicates were used. After 96 hours of treatment, the number of eggs laid over the cow-pea grains was counted.

2.5 Ovicidal assay

Ovicidal activities were determined by fumigating eggs with different concentrations of essential oils as was done in toxicity assay. In each glass vial, 100 eggs were fumigated with 2 μl, 4 μl and 6 μl of essential oil for 24 hours. After fumigation, eggs were allowed to hatch. The number of larvae hatched from eggs was counted till 14 days of treatment. For each concentration of essential oil and control six replicates were set.

2.6 Developmental inhibitory effects

Developmental inhibitory activities of essential oils were determined by fumigation method. In each glass vial, 20 larvae were taken along with 2 grams of flour and ten grams of cow-pea grains. These larvae were fumigated with two sublethal concentrations viz. 30% and 60% of 24-h LC_{50} of essential oils for 24 hours. The survived larvae were then allowed to feed and grow in vial containing fresh flour and grains of cow-pea. For each concentration of oil, 20 treated larvae were taken. The number of pupa developed from treated larvae and the number of adults emerged from pupa was recorded. For each concentration of essential oil and control, six replicates were set.

2.7 Chronic toxicity assay

In this assay, adults were treated with two sublethal concentrations viz. 30% and 60% of 24-h LC_{50} of essential oils as was done in the toxicity assay. Insects surviving after treatment were taken and used immediately for this assay. 100 gram of cow-pea grains were taken in 500 ml flask and transferred 20 fumigated insects to it and reared them for one month. After this treatment, infected grains were counted. For each concentration of essential oil and control, six replicates were set.

2.8 Data analysis

LC_{50} was calculated by POLO programme and analysis of variance was performed to test the equality of regression coefficients[23,24].

3 RESULTS

3.1 Toxicity assay

Essential oils caused death of adults and larvae of *Callosobruchus chinensis* when fumigated. The 24-h LC_{50} values against the adults of the insect were 8.9 μl, 10.8 μl, 11.0 μl, 12.5 μl, 13.6 μl, 14.8 μl and 15.6 μl for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively (Table 1). On the other hand, against larval stage these values were 6.4 μl, 7.9 μl, 8.9 μl, 11.1 μl, 11.7 μl, 12.2 μl and 13.5 μl for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* essential oils respectively (Table 2). The 24-h LC_{50} value was found minimum for *N. sativa* followed by *C. cyminum*, *A. graveolens*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils.

3.2 Oviposition inhibitory assay

Essential oils inhibited the oviposition response of the insect when fumigated. In oviposition inhibitory assay, oviposition was reduced to 48.89% and 25.80%; 54.03% and 32.03%; 62.89% and 37.34%; 66.61% and 45.15%; 70.70% and 44.27%; 75.40% and 45.69%; and 78.96% and 55.29% of the control after treatment with 30% and 60% of 24-h LC_{50} of the *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively (Table 3). The maximum per cent oviposition deterrence index (%ODI ± SE) at highest concentration was observed in case of *N. sativa* (76.37 ± 0.45) followed by *A. graveolens* (64.73 ± 0.81), *C. cyminum* (59.46 ± 0.70), *I. verum* (55.31 ± 0.53), *P. nigrum* (48.87 ± 0.43), *M. fragrans* (37.21 ± 0.44) and *T. ammi* (28.88 ± 0.38) (Table 3). The efficacy in reducing the oviposition was found maximum for *N. sativa* followed by *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils (Table 3).

3.3 Developmental inhibitory effects

Essential oils inhibited egg hatching rate of the insect when fumigated with sublethal concentration. Egg hatching reduced to 51.51%, 33.62% and 27.24%; 58.44%, 44.03% and 31.93%; 63.75%, 49.48% and 36.57%; 67.27%, 53.45% and 42.06%; 71.82%, 58.63% and 44.79%; 76.91%, 61.37% and 50.48%; and 81.72%, 70.17% and 56.13% of the control for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively (Table 4). The maximum per cent inhibition rate (%HIR ± SE) at highest concentration was observed in case of *N. sativa* (62.76 ± 0.44), *A. graveolens* (57.07 ± 0.71), *C. cyminum* (53.43 ± 0.69), *I. verum* (47.93 ± 0.55), *P. nigrum* (46.21 ± 0.45), *M. fragrans* (40.52 ± 0.59) and *T. ammi* (38.87 ± 0.38) (Table 4). The efficacy in reducing the egg hatching rate was found maximum for *N. sativa* followed by *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils.

Transformation of the larva into pupa was retarded when fumigated with sublethal concentration of essential oils. Pupal formation was reduced to 47.89% and 29.82%; 53.68% and 33.76%; 59.56% and 38.51%; 63.68% and 40.65%; 69.88% and 45.35%; 72.44% and 48.51%; and 75.56% and 56.83% of the control for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively (Table 5).

Adult emerged from the pupa transformed from the fumigated larvae decreased in concentration-dependent manner. Adult emergence was reduced to 48.60% and 25.23%; 51.97% and 30.39%; 56.97% and 34.32%; 61.30% and 37.11%; 69.69% and 41.61%; 72.75% and 45.60%; 74.49% and 48.11% for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively (Table 5). The efficacy in reducing the per cent pupal formation and adult emergence was found maximum for *N. sativa* followed by *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils.

3.4 Chronic toxicity assay

In chronic toxicity assay, adults fumigated with sublethal concentration of the essential oils caused less damage to the grains. The per cent grain infestation was reduced to 51.82% and 83.66%; 47.23% and 74.52%; 42.09% and 69.82%; 39.81% and 66.23%; 36.54% and 61.20%; 33.87% and 57.30%; and 30.23% and 54.69% of the control for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively (Table 6). The efficacy in reducing the damage was maximum for *N. sativa* followed by *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans*, and *T. ammi* oils.

Table 1 LC_{50} Values of Different Essential Oils at Different Exposure Periods against Adults of *Callosobruchus chinensis*.

Essential oils	Exposure periods (hours)	LC_{50}[a]	UCL [b]	LCL [b]	t-ratio [c]	Heterog Eneity [c]	Slope Value [c]	g value [c]
N. sativa	24 h	8.9	9.6	8.2	3.74	0.29	2.19	0.28
	48 h	7.2	7.5	6.9	3.64	0.28	2.05	0.26
	72 h	5.9	6.3	5.5	3.89	0.29	2.16	0.27
	96 h	4.3	4.8	3.8	3.32	0.26	1.99	0.29
A. graveolens	24 h	10.8	11.6	10.2	3.28	0.28	1.62	0.36
	48 h	9.1	9.9	8.3	3.11	0.26	1.73	0.32
	72 h	8.2	8.9	7.5	2.99	0.27	1.72	0.35
	96 h	6.4	7.0	5.8	3.43	2.25	1.68	0.33
C. cyminum	24 h	11.0	11.8	10.2	4.21	0.26	1.65	0.21
	48 h	8.9	8.4	8.5	3.89	0.27	1.57	0.26
	72 h	7.4	8.1	6.7	3.76	0.25	1.87	0.23
	96 h	5.2	4.6	4.8	3.89	0.28	1.67	0.21
I. verum	24 h	12.5	13.3	11.7	3.76	0.30	2.03	0.26
	48 h	10.1	10.6	9.6	3.79	0.32	1.98	0.23
	72 h	8.3	8.7	7.9	3.61	0.29	2.02	0.26
	96 h	6.2	6.6	5.8	3.45	0.30	2.07	0.22
P. nigrum	24 h	13.6	14.5	12.7	2.97	0.34	1.92	0.19
	48 h	12.0	12.6	11.4	3.11	0.30	2.04	0.21
	72 h	10.8	11.2	10.4	3.08	0.32	2.09	0.17
	96 h	8.6	9.0	8.2	2.78	0.33	1.99	0.22
M. fragrans	24 h	14.8	15.5	14.1	2.99	0.37	1.95	0.29
	48 h	12.5	12.9	12.1	2.70	0.38	1.87	0.25
	72 h	9.4	9.9	8.9	2.67	0.35	1.99	0.27
	96 h	6.0	6.4	5.6	3.02	0.33	1.85	0.26
T. ammi	24 h	15.6	16.0	15.2	3.89	0.33	1.89	0.28
	48 h	12.5	12.9	12.1	3.45	0.37	1.79	0.29
	72 h	9.7	10.1	9.3	3.23	0.35	2.01	0.26
	96 h	8.3	8.6	8.0	3.19	0.33	1.99	0.28

[a.] LC_{50} represents lethal concentrations that cause 50% mortality

[b.] UCL and LCL represent upper and lower confidence levels

[c.] t-ratio, heterogeneity, slope values and g-values are significant at all probability levels (90, 95 and 99%)

4 DISCUSSION

In recent years use of plant products as pesticides is gaining importance as the synthetic insecticides pose environmental and health hazard. Earlier attempts to explore the toxicity of plant derivatives against *Callosobruchus chinensis* have been made by several scientific groups. The neem and sesame oils have reported to inhibit adult emergence completely[25]. The insecticidal nature of constituents of *Foeniculum vulgare* fruits have been studied and mentioned that the insecticidal activity of these constituents was largely due to fumigant action[20]. The dry ground leaves of *Chenopodium ambrosioides* inhibited F1 progeny production and adult emergence[26]. Different extracts and pure compounds of *Capparis decidua* caused toxicity and oviposition inhibitory activities[9]. Some vegetable seed oils of Cucurbitaceae family protect the infestations of the pest in legume pulse grains[22]. In the present study, the essential oils under investigation caused death of adults

Table 2 LC_{50} Values of Different Essential Oils at Different Exposure Periods against Larva of *Callosobruchus chinensis*.

Essential oils	Exposure periods (hours)	LC_{50} [a]	UCL [b]	LCL [b]	t-ratio [c]	Heterog Eneity [c]	Slope Values [c]	g value [c]
N. sativa	24 h	6.4	7.2	5.6	3.53	0.32	1.81	0.32
	48 h	4.7	5.3	4.1	3.34	0.29	1.92	0.35
	72 h	3.2	3.7	2.7	3.09	0.26	1.88	0.31
	96 h	1.9	2.4	1.4	3.11	0.31	1.91	0.36
A. graveolens	24 h	7.9	8.7	8.1	3.67	0.22	1.71	0.23
	48 h	6.8	7.7	5.9	3.53	0.29	1.98	0.25
	72 h	4.2	4.9	3.5	3.32	0.29	1.76	0.28
	96 h	2.5	3.1	1.9	3.78	0.26	1.78	0.24
C. cyminum	24 h	8.9	9.5	8.3	3.23	0.32	1.99	0.28
	48 h	7.9	8.6	7.2	3.43	0.31	2.01	0.24
	72 h	5.6	6.2	5.0	3.78	0.21	2.09	0.26
	96 h	3.7	4.4	3.0	3.34	0.29	2.17	0.25
I. verum	24 h	11.1	11.8	10.4	2.78	0.32	1.99	0.22
	48 h	9.0	9.7	8.3	3.54	0.35	1.98	0.25
	72 h	6.9	7.4	6.4	3.32	0.30	2.32	0.23
	96 h	4.3	5.1	3.5	2.67	0.31	2.39	0.27
P. nigrum	24 h	11.7	12.2	11.2	3.32	0.27	2.32	0.30
	48 h	8.7	9.3	8.1	3.78	0.30	2.11	0.28
	72 h	6.2	6.7	5.7	3.23	0.32	2.01	0.25
	96 h	4.9	5.4	4.4	3.89	0.29	2.19	0.30
M. fragrans	24 h	12.2	12.9	11.5	2.67	0.34	2.09	0.32
	48 h	8.4	9.0	7.8	2.56	0.33	1.99	0.28
	72 h	6.3	6.9	5.7	2.89	0.36	1.76	0.29
	96 h	4.1	4.8	3.4	3.34	0.32	1.67	0.27
T. ammi	24 h	13.5	14.1	12.9	3.23	0.31	1.93	0.29
	48 h	9.0	9.7	8.3	3.89	0.38	1.78	0.31
	72 h	6.9	7.4	6.4	3.01	0.32	2.32	0.29
	96 h	4.3	5.1	3.5	3.89	0.36	2.23	0.24

[a.] LC_{50} represents lethal concentrations that cause 50% mortality

[b.] UCL and LCL represent upper and lower confidence levels

[c.] t-ratio, heterogeneity, slope values and g-values are significant at all probability levels (90, 95 and 99%)

and larvae of *Callosobruchus chinensis* when fumigated. In the toxicity assay, the mortality rate was found to increase with an increase in concentration, and the LC_{50} values decreased at different graded exposure periods indicating that the response was concentration and time dependent. Low LC_{50} values of essential oils against larval stage clearly indicate that larval stage is more susceptible than the adult stage. The index of significance of potency estimation, g value, indicated that the mean value is within the limits of all probabilities (90, 95 and 99%) as it is less that 0.5. Value of t-ratio greater than 1.6 indicated that the regression was significant. The steep slope values indicated that even small increase in the concentration of the essential oil causes high mortality. Values of the heterogeneity factor less than 1.0 denoted that the model fits the data adequately. Oviposition response of the insect and the hatching of the eggs was inhibited significantly when fumigated with sublethal concentration of the essential oils.

Table 3 Effect of Fumigation of Essential Oils on Oviposition of *Callosobruchus chinensis*.

Essential oils	Treatment	Number of eggs laid per insect Mean ± SE	% eggs laid per insect Mean ± SE	% ODI [a] Mean ± SE	F-value [b] (at df 2 & 17)
N. sativa	30% of 24-h LC_{50}	11.08 ± 0.10	48.89 ± 0.54	42.80 ± 0.40	418.80
	60% of 24-h LC_{50}	5.05 ± 0.08	25.80 ± 0.42	76.37 ± 0.45	
A. graveolens	30% of 24-h LC_{50}	11.52 ± 0.11	54.03 ± 0.58	36.63 ± 0.43	344.49
	60% of 24-h LC_{50}	5.93 ± 0.13	32.03 ± 0.69	64.73 ± 0.81	
C. cyminum	30% of 24-h LC_{50}	12.05 ± 0.12	62.89 ± 0.64	32.49 ± 0.47	339.11
	60% of 24-h LC_{50}	6.93 ± 0.06	37.34 ± 0.43	59.46 ± 0.70	
I. verum	30% of 24-h LC_{50}	12.98 ± 0.09	66.61 ± 0.46	28.79 ± 0.33	295.93
	60% of 24-h LC_{50}	8.08 ± 0.10	45.15 ± 0.56	55.31 ± 0.53	
P. nigrum	30% of 24-h LC_{50}	13.50 ± 0.12	70.70 ± 0.62	25.99 ± 0.41	276.46
	60% of 24-h LC_{50}	8.23 ± 0.09	44.27 ± 0.49	48.87 ± 0.43	
M. fragrans	30% of 24-h LC_{50}	14.51 ± 0.11	75.40 ± 0.60	21.51 ± 0.42	245.31
	60% of 24-h LC_{50}	8.48 ± 0.11	45.69 ± 0.61	37.21 ± 0.44	
T. ammi	30% of 24-h LC_{50}	15.73 ± 0.08	78.96 ± 0.45	15.99 ± 0.29	215.11
	60% of 24-h LC_{50}	10.27 ± 0.08	55.29 ± 0.45	28.88 ± 0.38	

[a.] %ODI was calculated as 100(A-B)/(A+B), where A and B represent the number of eggs laid in the control and in the test respectively

[b.] F values were significant at all probability levels (90, 95 and 99%)

Transformation of the larva into pupa and adult emergence was retarded when fumigated with sublethal concentration of the essential oils. Number of adult emerged from the pupa transformed from the fumigated larvae decreased in concentration dependent manner. Fumigation with essential oils reduced per cent grain infestation caused by insect. F-values were highly significant for all stimuli at all probability levels (90, 95 and 99%) indicate that all the essential oils affect the oviposition behavior, egg hatchability and development of the insect significantly. Similarly, essential oils from *Tagetes minuta*, *Hyptis suaveolens*, *Ocimum canum* and *Ocimum basilicum* have caused mortality and complete inhibition of oviposition in *Callosobruchus maculatus*, another pest of cow pea[15]. Essential oil from *Artemisia annua* have ovicidal, larvicidal and developmental inhibitory activities against *Callosobruchus maculates*; and reduces egg hatching, pupal formation and adult emergence of the adults. All these findings clearly support the results of the present study. The mode of action of these essential oils has not yet been known but it may be due to suffocation and inhibition of various biosynthetic processes of the insect[28].

5 CONCLUSION

The findings of the present study indicate that the essential oils of *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* are toxic to the larva and adults of the *Callosobruchus chinensis*. After fumigation, these essential oils reduce oviposition, egg hatchability, pupal formation and adult emergence of the insect. These also prevent infestation rate in experimental condition suggesting chronic toxicity of the essential oils. Therefore, these essential oils may prove effective when used as protectant for infestation in pulse grains. Furthermore, isolation and characterization of the oil constituents will provide complete insight into the pesticidal activity and will help in the preparation of formulations against the pulse beetle, *Callosobruchus chinensis*.

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Table 4 Effect of Fumigation of Essential Oils on Egg Hatching Rate of *Callosobruchus chinensis*.

Essential oils	Treatment	% Egg Hatching [a] Mean ± SE	% HIR [b] Mean ± SE	F-value [c] (at df 3 & 23)
N. sativa	2 μl	51.51 ± 0.61	32.49 ± 0.61	339.29
	4 μl	33.62 ± 0.87	46.38 ± 0.87	
	6 μl	27.24 ± 0.44	62.76 ± 0.44	
A. graveolens	2 μl	58.44 ± 0.48	28.55 ± 0.48	316.12
	4 μl	44.03 ± 0.69	40.96 ± 0.69	
	6 μl	31.93 ± 0.71	57.07 ± 0.71	
C. cyminum	2 μl	63.75 ± 0.50	25.24 ± 0.50	307.05
	4 μl	49.48 ± 0.56	38.52 ± 0.56	
	6 μl	36.57 ± 0.69	53.43 ± 0.69	
I. verum	2 μl	67.27 ± 0.58	21.73 ± 0.58	246.36
	4 μl	53.45 ± 0.76	33.55 ± 0.76	
	6 μl	42.06 ± 0.55	47.93 ± 0.55	
P. nigrum	2 μl	71.82 ± 0.60	19.17 ± 0.60	233.39
	4 μl	58.63 ± 0.67	27.37 ± 0.67	
	6 μl	44.79 ± 0.45	46.21 ± 0.45	
M. fragrans	2 μl	76.91 ± 0.58	18.09 ± 0.58	226.94
	4 μl	61.37 ± 0.52	23.63 ± 0.52	
	6 μl	50.48 ± 0.59	40.52 ± 0.59	
T. ammi	2 μl	81.72 ± 0.66	17.27 ± 0.66	220.49
	4 μl	70.17 ± 0.52	23.83 ± 0.52	
	6 μl	56.13 ± 0.34	38.87 ± 0.38	

[a.] % Egg Hatching = (Total number of eggs hatch / Total eggs) × 100

[b.] % Hatching Inhibition Rate = [(Cn-Tn)/Cn] × 100, where, Cn = Number of larvae hatched from control and Tn = Number of larvae hatched from test

[c.] F values were significant at all probability levels (90, 95 and 99%)

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Table 5 Effect of Essential Oils on the Development of *Callosobruchus chinensis*.

Essential oils	Treatment	% Pupal formation [a] Mean ± SE	F-value [a] (at df 2 & 17)	% Adult emergence [c] Mean ± SE	F-value [a] (at df 2 & 17)
N. sativa	30% of 24-h LC_{50}	47.89 ± 0.72	491.32	48.60 ± 0.70	431.18
	60% of 24-h LC_{50}	29.82 ± 0.65		25.23 ± 0.47	
A. graveolens	30% of 24-h LC_{50}	53.68 ± 0.88	421.80	51.97 ± 0.70	356.67
	60% of 24-h LC_{50}	33.76 ± 0.94		30.39 ± 0.70	
C. cyminum	30% of 24-h LC_{50}	59.56 ± 0.94	325.46	56.97 ± 0.64	318.24
	60% of 24-h LC_{50}	38.51 ± 0.60		34.32 ± 1.08	
I. verum	30% of 24-h LC_{50}	63.68 ± 0.71	287.98	61.30 ± 1.08	291.08
	60% of 24-h LC_{50}	40.65 ± 0.53		37.11 ± 0.93	
P. nigrum	30% of 24-h LC_{50}	69.88 ± 0.67	234.86	69.69 ± 1.08	278.82
	60% of 24-h LC_{50}	45.35 ± 0.41		41.61 ± 1.13	
M. fragrans	30% of 24-h LC_{50}	72.44 ± 0.41	196.56	72.75 ± 0.66	259.84
	60% of 24-h LC_{50}	48.51 ± 0.60		45.60 ± 0.66	
T. ammi	30% of 24-h LC_{50}	75.56 ± 0.60	176.27	74.49 ± 0.47	219.66
	60% of 24-h LC_{50}	56.83 ± 0.87		48.11 ± 0.93	

[a.] F values were significant at all probability levels (90, 95 and 99%)

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Table 6 Effect of Fumigation of Essential Oils on Damage Caused by *Callosobruchus chinensis*.

Essential oil	Treatment	% grain infected Mean ± SE	F-value [a] (at df 2 & 17)
N. sativa	30% of 24-h LC_{50}	51.82 ± 0.71	659.63
	60% of 24-h LC_{50}	83.66 ± 0.31	
A. graveolens	30% of 24-h LC_{50}	47.23 ± 0.60	606.31
	60% of 24-h LC_{50}	74.52 ± 0.82	
C. cyminum	30% of 24-h LC_{50}	42.09 ± 0.63	567.36
	60% of 24-h LC_{50}	69.82 ± 0.56	
I. verum	30% of 24-h LC_{50}	39.81 ± 0.62	517.59
	60% of 24-h LC_{50}	66.23 ± 0.56	
P. nigrum	30% of 24-h LC_{50}	36.54 ± 0.49	448.48
	60% of 24-h LC_{50}	61.20 ± 0.64	
M. fragrans	30% of 24-h LC_{50}	33.87 ± 0.38	408.75
	60% of 24-h LC_{50}	57.30 ± 0.61	
T. ammi	30% of 24-h LC_{50}	30.23 ± 0.47	362.93
	60% of 24-h LC_{50}	54.69 ± 0.41	

[a.] F values were significant at all probability levels (90, 95 and 99%)

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