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Evaluation of some essential oils against pulse beetle (*Callosobruchus chinensis* L.) in pea seeds

Abstract

The present investigations on"Evaluation of some essentials oils against pulse beetle (Callosobruchus chinensis L.) in pea seeds" were carried out during 2014-15 in the Department of Seed Science and Technology, College of Horticulture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni–solan (H.P). The essentials oils of camphor, wild marigold, cone-bearing sage, eucalypts, lemongrass and sweet flag at 2.5ml/kg, 1.25ml/kg, 0.60ml/kg and 0.30ml/kg (v/w basis) were evaluated against C. chinensis L. infesting pea seeds. Among the six essentials oils, sweet flag possessed reasonably high and immediate toxicity resulting in 78.33 percent and 96.67 percent (2.5ml/kg and 1.25ml/kg doses resulted 100% mortality) mortality in 1 and 3days after treatment. After 7days, cent per cent mortality was observed in seeds coated with sweet flag essential oil followed by eucalypts (85.83%) cone - bearing sage (77.50%), camphor (74.17%), lemongrass (71.66%) and wild marigold (61.67%) in descending order. After 10-days of exposure, eucalypts essential oil also resulted in complete kill even at the lowest dose (0.30ml/kg). On day-15, mortality in control had substantially increased to 67.50 percent. Egg laying was minimum on sweet flag essential oil treated pea seeds (5.25 eggs/5females) on 7th–day of observations. In untreated pea seeds, increases in egg laying was negligible in 20–days observations (7th day 94.08- 20th day 101.92). Progeny development from the eggs laid on pea seed treated with sweet flag essentials oil was (1 beetle).

Keywords: *callosobruchus chinensis*, fecundity, mortality, pea seeds, essential oil

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Introduction

The successful production of any crop depends on the quality of seeds sown. Maintenance of high seed germination and vigour from harvest until planting is of utmost importance in a seed production programme. Pea (*Pisum sativum* L.) is an important vegetable crop of Himachal Pradesh. It is grown in an area of about 23900 ha with annual production of 271060 metric tones.[1] One of the major constraints for its production is the attack by insect- pests in the field as well as in storage. Several bruchid species attack cereals and pulses in the store and cause loss up to 10-15 per cent with a germination loss ranging from 50-92 per cent.[2] Losses due to insect infestation are the most serious problem in grain storage, particularly in the developing countries, where poor sanitation and use of inappropriate storage facilities encourage insect attack.[3,4] Stored product insects cause post-harvest losses up to 9 per cent in developed countries and more than 20 per cent in developing countries.[5] According to an estimate, the overall annual damage caused by stored grain insect pests accounts for 10-40 per cent worldwide annually.[6] In India, food grain losses during storage at the farm level approximate 10 per cent of the total production. Inspite of improved storage structures and modern chemical and physical control techniques employed for the safe storage of stored grains, 70-90 per cent of food grain is still stored for six months to a year at farmer's level in traditional storage structures.[7] In such a critical situation, protection of stored seed grains from insect infestation is quit essential.

Among the important insect pests that infest the pea seeds in storage is the pulse beetle, *Callosobruchus chinensis* L. (Bruchidae: Coleoptera) which causes substantial losses to the pulses in the storage[8–10] though the initial infestation occurs in the field itself. It is a cosmopolitan and serious pest of peas, mung, cowpeas and lentil and has also been reported attacking cotton seed, sorghum and maize.[11] Its active period is between March and October/November[2] and when such seeds harvested and stored, the pest population increases rapidly and results in total destruction within a short duration of 3-4months. Only grubs are damaging stages for the storage grains. These make holes in the grains which become unsuitable for human consumption, production of sprout and also lose its market value.[13] It causes weight loss, decreased germination potential and reduction in commercial value of seed.[14] Its damage generally starts in ripened pods in the field from where it is carried over to storage godowns. Adult females lay eggs on seeds and the emerging grubs bore in to seeds and feed on endosperm. Consequently seeds lose their viability and nutritional value. The life cycle is completed in 25-34days during summer, whereas, it takes 40-50days in winter.[15]

In order to keep stored seeds free from insect-pests infestations various synthetic pesticides are used. Although chemical insecticides are effective, but their indiscriminate use has led to residual toxicity, insecticide resistance, environmental pollution and adverse effects on food besides side effects on humans.[16–18] Methyl bromide and phosphine fumigants have been used for decades to control stored pests.[6,19] Growers are moving away from using methyl bromide as post-harvest fumigant because of its ozone-depleting nature. Ozone depleting nature of methyl bromide has led to restriction of its use, and the Montreal protocol of United Nations Environment Programme (UNEP) recommend the phasing out of methyl bromide by 2015 in developing countries.[20] Phosphine resistance is becoming more common[21–23] and is a matter of considerable concern. Therefore,

there is a need of some other alternatives to chemical pesticides and fumigants to protect stored grains from insect-pests infestations.

Material and methods

Raising of test insect culture

The pure culture of pulse beetle (*Callosobruchus chinensis* L.) was maintained under laboratory conditions throughout the year. For this purpose pea seeds cv. PB-89 seeds were heat sterilized at 550C for 4hours in an oven. These sterilized seeds were kept in sterilized jars of half kg capacity (Plate-1). In these jars ten pairs of freshly emerged adults of *C. chinensis* were released (Plate-2). The jars were covered with muslin cloth and kept in BOD incubator maintained at 27+10C temperature and 70+5% relative humidity for further multiplication of beetles.

Selection of essential oils: Six plant essential oils viz. Camphor (*Cinnamomum camphora* L.), Wild marigold (*Tegetes minuta* L.), Cone-bearing sage (*Meriandra strobilifera* B), Eucalyptus (*Eucalyptus sp*.), Lemongrass (*Cymbopogon citratus* L.), Sweet flag (*Acorus calamus* L.) were used for their insecticidal activity against *C. chinensis* and then on their effect on seed quality parameters (Plate-3). The plant material was collected locally, shade dried and essential oils were extracted with the help of clevenger apparatus by hydro-distillation (Plate-4) in the Department of Forest Products.

Evaluation of plant essential oils at different doses: Each essential oil was taken at different doses viz. 2.5ml/kg 1.25ml/Kg, 0.60ml/kg and 0.30ml/kg in separate plastic container of 250cc capacity containing 100g of sterilized seeds of pea with three replications. Contents were thoroughly mixed in plastic containers by vigorous shaking. In control no essential oil was mixed. Five pair of freshly emerged adults of pulse beetle were then released in each container. These containers were closed by muslin cloth tightly secured by rubber band. The experiment was carried out at room temperature (Plate-5). During the experimental period the average minimum and maximum temperature (0C) was 14.5°C and 26.8°C, respectively. There were seven treatments including control with three replications. The effect of treatment was studied on different biological parameters as per details given below:

Efficacy of different essential oils on adult mortality of *C. chinensis:* In order to determine the effect of plant essential oils on the life span of adults, the mortality of adult beetles released on treated seeds was recorded at different doses at 1, 3, 7, 10, and 15 days, till mortality of all the adults released in each treatment.

Effect of essential oils on fecundity and progeny development in *C. chinensis***:** The effects of different essential oils of all six plant species were recorded on fecundity of *C.chinensis* on day-7 and day-20 of release of adults and further progeny development after two months of release of adults. Egg laying data of progeny developed on treated seeds was recorded, and compared with untreated individuals.

Statistical Analysis

The data emanating from the above experiments was subjected to statistical analysis through two factor and three factor Completely Randomized Design after applying proper transformation. The significance of each treatment was calculated as suggested by Cochran and Cox.

Results

Data contained in Table 1 reveal that essential oils resulted in variable mortality of adults of C. *chinensis* with maximum mortality in sweet flag essential oil and minimum in untreated control.

The maximum mortality (78.33%) observed in seed coated with sweet flag essential oil was significantly superior over rest of the essential oils. Next best treatment was camphor (16.67%) which was statistically at par with lemongrass (16.67%), eucalypts (14.17%) and cone-bearing sage (15%). Mortality in pea seeds treated with wild marigold (8.33%) showed least mortality. All the treatments were superior over control. Mortality in pea seeds treated with sweet flag essential oil at 2.5ml/kg resulted 100 per cent mortality which was statistically at par with its 1.25ml/kg (100.00%). Pea seeds treated with camphor essential oil at 1.25ml/kg, cone-bearing sage (0.60 ml/kg), wild marigold (2.5ml/kg), eucalypts (0.60ml/kg) recorded equal mortality (13.33% kill). Mortality of pulse beetle significantly decreases with decrease in doses (2.5ml/kg:34.29; 0.30ml/kg: 8.57% kill).

Among various essential oils sweet flag gave best results (96.67% overall kill) which was statistically superior over camphor (43.33%) and lemongrass (35%). Mortality in pea seeds treated with cone-bearing sage (25.83%), eucalypts (21.67%) and wild marigold (18.33%) was statistically at par with each other and were significantly superior over control (4.16%). Mortality in pea seeds treated with 1.25ml/kg dose of lemongrass (33.33%) was at par with its 0.60ml/kg and 0.30ml/kg doses (0.60ml/kg :26.67%; 0.30ml/kg :16.67%) and 1.25ml/kg, 0.60ml/kg doses of eucalypts (1.25ml/kg : 26.67% ;0.60ml/kg :20%), 1.25ml/kg and 0.60ml/kg doses of cone-bearing sage (1.25ml/kg :26.66% ; 0.60ml/kg :23.33%), 2.5 ml/kg, 1.25ml/kg and 0.60ml/kg of wild marigold (2.5ml/kg :23.33%; 1.25ml/kg :20.00%; 0.60ml/kg :16.67%). Mortality recorded in pea seeds treated with essential oils was significantly superior over control (4.16%).

Highest mortality was observed in pea seeds coated with sweet flag essential oil (100%). The next best treatment was eucalypts (85.83%) followed by cone-bearing sage (77.50%) and camphor (74.17%) which was statistically at par with each other. Lemongrass essential oil resulted 71.67 per cent mortality which was significantly superior over wild marigold (61.67%) and both were significantly superior over control (11.66%). Mortality in pea seeds treated with eucalypts at 2.5ml/kg dose (96.67%) was statistically at par with its 1.25ml/kg dose (86.67%), lemongrass at 2.5ml/kg (93.33%), cone-bearing sage at 1.25ml/kg dose (93.33%) and 2.5ml/kg , 1.25ml/kg and 0.60ml/kg dose of camphor (2.5ml/kg: 93.33; 1.25ml/kg :86.66%; 0.60ml/kg :83.33%) (Table 2).

Mortality recorded in pea seeds treated with sweet flag and eucalypts was equal (both 100% kill) and both were statistically at par with camphor (96.67%). Next best treatment was lemongrass (88.33%) which was statistically at par with cone-bearing sage (87.50%). Lowest mortality was observed in pea seeds treated with wild marigold (78.33%) which was significantly superior over control (44.17%). Cent per cent pulse beetle mortality was observed at all dose of sweet flag and eucalypts essential oils and 2.5ml/kg and 1.25ml/kg dose of camphor (both 100%) , 2.5ml/kg , 1.25ml/kg doses of cone-bearing sage (both 100%) and 2.5ml/kg dose of lemongrass (100%). Mortality response (96.67%) with0.60ml/kg dose of camphor was statistically at par with its 0.30ml/kg dose (90%), 2.5ml/kg , 1.25ml/kg dose of wild marigold (2.5ml/kg :93.33%; 1.25ml/kg :90.0%),

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cone-bearing sage at 0.60ml/kg (93.33%) and lemongrass at 2.5ml/kg and 1.25ml/kg doses (1.25 ml/ kg :90%; 0.60ml/kg :83.33%). Pea seeds treated with wild marigold caused lowest mortality (78.33%), which was significantly superior over control (44.17%).

All the treatments were superior over control and mortality in treatments such as camphor (100%), eucalypts (100%), lemongrass (96.67%) and wild marigold (95%) were statistically at par with sweet f lag essential oil as well as with each other. Next best treatment was wild marigold (95%) and cone-bearing sage (93.33%) which was statistically at par with each other. Lemongrass at 0.30ml/kg dose (90%) was statistically at par with 0.30 ml/ kg of wild marigold (83.33%) and cone-bearing sage (80.33%). Mortality recorded at 0.60ml/kg dose of lemongrass as well as wild marigold at same dose were equally effective (96.67% mortality). Overall result shows that mortality of pulse beetle decreases with decrease in dose (2.5ml/kg: 95.24; 0.30ml/kg :88.10%).

Effect of essential oils on fecundity of C. chinensis at different intervals and doses

Data contained in Table 3 revealed that on day 7 minimum number of eggs (5.25eggs/5females) was laid by 5 pairs of *C. chinensis* in sweet flag essential oil coated seeds followed by lemongrass (7.75eggs/5females), wild marigold (33.16eggs/5female), cone-bearing sage (34.41eggs/5females), camphor (40.33eggs/5females) and eucalypts (67.75eggs/5females), all the essential oils were statistically different from each other.

Eucalypts proved to be least effective (67.75eggs/5 females) but was superior to untreated control (94.08eggs/5 females). The number of eggs laid in control was very high (94.08 eggs/5 females). Even the best proved essential oil of sweet flag differed non significantly with respect to the doses used and hence at par with each other. Overall the egg laying by 5 pairs of beetles were dose dependent as the dose increases the egg laying decreases (54.86eggs/5 females at 0.30ml/kg and 27.33 eggs/5 females at 2.5ml/kg).

The minimum number of eggs (8.17eggs/females) were laid by 5 pairs of beetles on seeds coated with sweet flag essential oil. Next best treatment was lemongrass (10.08 eggs/females) which was statistically superior over rest of essential oils. Among other treatments, egg laying recorded with wild marigold treated pea seeds (38.00 eggs/5females) was significantly at par with cone-bearing sage (38.08eggs/5females) and differed significantly with rest of the essential oils. There was significant reduction in egg laying from 0.30ml/kg to 2.5ml/kg dose in all essential oils. Comparison of oviposition taking place on treated seeds during first seven days with the eggs laid in next 13 days reveals that there was no significant increase in oviposition in treated and control lots and whatsoever oviposition occurred, that remained restricted to first week of adult life.

Table 1A Effect of pea seed treatment with different doses of essential oils of some plant species on mortality of C. chinensis beetles at different days after treatment

Plant species	Day-1				Mean	Day-3				Mean	Day-7				Mean
	2.5	1.25	0.6	0.3		2.5	1.25	0.6	0.3		2.5	1.25	0.6	0.3	
Camphor	43.33	13.33	6.67	3.33	16.67	73.33	60	33.33	6.67		93.33	86.67	83.33	33.33	74.17
	-41.07	-21.14	-12.28	-6.14		-63.93	-50.85	-34.93	-12.28	43.33	-81.14	-72.79	-70.07	-35.22	-63.79
Wild Marigold	13.33	10	6.67	3.33	8.33	23.33	20	16.67	13.33	18.33	76.67	73.33	53.33	43.33	61.67
	-21.14	-15	-12.29	-6.14	-13.29	-28.78	-26.07	-23.86	-17.71	-24.09	-65.86	-59.22	-47.01	-41.15	-53.26
Cone-bearing sage	20	16.67	13.33	10	15	36.67	26.67	23.33	16.67	25.83	100	93.33	73.33	43.33	77.5
	-26.57	-23.35	-17.21	-15	-22.92	-30.99	-28.78	-28.77	-19.92	-29.22	-90	-77.71	-59	-41.07	-66.91
Eucalypts	23.33	16.67	13.33	3.33	14.17	30	26.67	20	10	21.67	96.67	86.67	76.67	73.33	85.83
	-28.78	-23.35	-17.21	-6.14	-19.95	-37.22	-30.99	-26.07	-15.05	-26.26	-83.85	-72.29	-69.37	-63.86	-69.79
Lemongrass	40	16.67	6.67	3.33	16.67	63.33	33.33	26.67	16.67	35	93.33	76.67	63.33	53.33	71.66
	-39.15	-23.36	-8.86	-6.14	-19.39	-52.78	-34.93	-30.29	-23.86	-35.45	-81.14	-61.22	-53.07	-46.93	-60.57
Sweet flag	100	100	76.67	36.67	78.33	100	100	96.67	90	96.67	100	100	100	100	100
	-90	-90	-61.22	-37.22	-69.58	-90	-90	-83.86	-78.93	-85.68	-90	-90	-90	-90	-90
Control	0	0	0	0	0	3.33	3.33	3.33	6.667	4.16	13.33	10	13.33	10	11.66
	0	0	0	0	0	-6.14	-6.14	-6.14	-12.29	-7.67	-21.14	-18.43	-21.14	-18.43	-19.78
Mean	34.29	24.76	17.62	8.57	21.31	47.14	38.57	31.43	22.86	35	81.91	75.24	67.62	50.95	68.93
	-35.24	-28.35	-20.15	-10.58	-23.58	-44.53	-38.56	-33.4	-25.7	-35.55	-73.28	-63.94	-57.65	-47.49	-60.59

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Table 1B Effect of pea seed treatment with different doses of essential oils of some plant species on mortality of C. chinensis beetles at different days after treatment

Treatment	Day									
	Day-10					Day-15				
	Dose (ml/kg)									
	2.5	1.25	0.6	0.3	Mean	2.5	1.25	0.6	0.3	Mean
Camphor	100	100	96.67	90	96.67	100	100	100	100	100
	-90	-90	-83.86	-78.93	-85.68	-90	-90	-90	-90	-90
	93.33	90	70	60	78.33	100	100	96.67	83.33	95
	-77.71	-75	-57.28	-50.86	-69.61	-90	-90	-83.85	-70.07	-83.46
	100	100	93.33	56.67	87.5	100	100	93.33	80.33	93.33
	-90	-90	-77.71	-48.94	-76.65	-90	-90	-77.71	-67.86	-80.27
Wild marigold	100	100	100	100	100	100	100	100	100	100
	-90	-90	-90	-90	-90	-90	-90	-90	-90	-90
	100	90	83.33	80	88.33	100	100	96.67	90	96.67
	-90	-71.57	-70.07	-63.93	-76.87	-90	-90	-83.85	-74.99	-84.69
Cone-bearing sage	100	100	100	100	100	100	100	100	100	100
	-90	-90	-90	-90	-90	-90	-90	-90	-90	-90
	43.33	43.33	46.67	43.33	44.17	66.67	66.67	73.33	63.33	67.5
	-41.05	-41.05	-41.25	-41.05	-41.11	-54.76	-54.76	-58.98	-52.78	-55.31
Eucalypts	90.95	89.05	84.29	75.71	84.99	95.24	95.24	94.29	88.1	93.33
	-81.25	-78.22	-75.39	-66.23	-75.27	-84.97	-84.97	-82.09	-75.88	-81.96
	100	100	96.67	90	96.67	100	100	100	100	100
	-90	-90	-83.86	-78.93	-85.68	-90	-90	-90	-90	-90
Lemongrass	93.33	90	70	60	78.33	100	100	96.67	83.33	95
	-77.71	-75	-57.28	-50.86	-69.61	-90	-90	-83.85	-70.07	-83.46
	100	100	93.33	56.67	87.5	100	100	93.33	80.33	93.33
	-90	-90	-77.71	-48.94	-76.65	-90	-90	-77.71	-67.86	-80.27
Sweet flag	100	100	100	100	100	100	100	100	100	100
	-90	-90	-90	-90	-90	-90	-90	-90	-90	-90
	100	90	83.33	80	88.33	100	100	96.67	90	96.67
	-90	-71.57	-70.07	-63.93	-76.87	-90	-90	-83.85	-74.99	-84.69
Control	100	100	100	100	100	100	100	100	100	100
	-90	-90	-90	-90	-90	-90	-90	-90	-90	-90
	43.33	43.33	46.67	43.33	44.17	66.67	66.67	73.33	63.33	67.5
	-41.05	-41.05	-41.25	-41.05	-41.11	-54.76	-54.76	-58.98	-52.78	-55.31
m Mean	90.95	89.05	84.29	75.71	84.99	95.24	95.24	94.29	88.1	93.33
	-81.25	-78.22	-75.39	-66.23	-75.27	-84.97	-84.97	-82.09	-75.88	-81.96
	100	100	96.67	90	96.67	100	100	100	100	100
	-90	-90	-83.86	-78.93	-85.68	-90	-90	-90	-90	-90

Figure in parenthesis are arc sine transformed values CD (p=0.05) Day : 2.72
Dose X Day : 5.43
Treatment X Day: 7.19
Treatment X Dose X day : 14.38

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Table 2 Effect of essential oils on oviposition by C. chinensis on treated pea seeds.

Treatments	Number of eggs laid/5 pairs of beetle at different days and doses									
	Day									
	Day-7					Day-20				
	Dose (ml/kg)									
	2.5	1.25	0.6	0.3	Mean	2.5	1.25	0.6	0.3	Mean
Camphor	6.67	32.67	47	75	40.33	9	36.33	49.33	78.33	43.25
	-2.74	-5.65	-6.87	-8.69	-5.99	-3.16	-6.11	-7.09	-8.91	-6.32
Wild Marigold	17.33	29	37	49.33	33.16	23.33	35.33	40.67	52.67	38
	-4.19	-5.48	-6.14	-7.08	-5.72	-4.93	-6.03	-6.46	-7.33	-6.19
Cone-bearing sage	22	31.33	36.67	47.67	34.41	24.33	36	42.67	49.33	38.08
	-4.75	-5.69	-6.09	-6.95	-5.87	-5.03	-6.08	-6.61	-7.09	6.20)
Eucalypts	47	59	72.33	92.67	67.75	49.67	62.33	73	93.33	69.58
	-6.89	-7.72	-8.51	-9.67	-8.2	-7.19	-7.96	-8.6	-9.71	-8.35
Lemongrass	3.33	6.67	8.33	12.67		5.33	9	11.67	14.33	
	-2.08	-2.32	-2.61	-3.05	7.75	-2.56	-3.16	-3.56	-3.92	10.08
					-2.89					-3.29
Sweet flag	2		6	8.33		4.67	7	9.33	11.67	
	-1.71	4.67	-2.44	-2.07	5.25	-2.38	-2.83	-3.21	-3.56	8.17
		-2.32			-2.42					-2.99
Control		88.33	96.67	98.33		98.67	100.33	102	106.67	
	93	-9.43	9.87)	-9.95	94.08	-9.97	-10.07	-10.15	-10.38	101.92
	-9.66				-9.73					-10.14
Mean	27.33	35.95	43.43	54.86	40.39	30.71	41.91	46.95	58.05	44.16
	-4.57	-5.58	-6.16	-7.4	-5.83	-5.02	-6.03	-6.53	-7.27	-6.16

Figure in parentheses are square root transformed values

CD (p=0.05)

Day : 0.04

Treatment X Day : 0.10

Dose X day : 0.08

Treatment X dose X day : NS

Data presented in Table 3 reveal that 60 days after release of 5 pairs of *C. chinensis* on pea seeds treated with essential oils, there was reduction in progeny produced by them. Minimum adult emergence (0.94 beetles) was recorded in sweet flag essential oil which was statistically different from rest of the essential oils. Lemongrass treated pea seeds produced 3.42 beetles which was superior over rest of essential oils. Seeds treated with cone-bearing sage, wild marigold and camphor essential oils produced 25.58, 26.67 and 27.34 beetles which were statistically at par with each other. Progeny produced in camphor (27.34 beetles) was also low as compared to control where the progeny of 72.66 beetles was obtained. Least effective treatment was eucalypts where the progeny produced was 47.42 beetles. Progeny production was significantly decreased from 40.19 to 18.57 beetles with the increase in dose of essential oils. Sweet flag at 2.5ml/kg and 1.25ml/kg dose completely restricted adult emergence of beetles. The progeny developed (2.67 beetles) in sweet flag at 0.30ml/kg dose was statistically at par with 2.5 ml/kg, 1.25ml/kg and 0.60ml/kg doses of lemongrass (2.5ml/kg :1.34; 1.25ml/kg : 1.67 ; 0.60ml/kg

:2.33 beetles). Eucalypts at 2.5ml/kg and cone-bearing sage at 0.60ml/kg dose were able to restrict progeny production to 29.67 beetles and both were equally effective. On the other hand, in control as many as 72.66 beetles were formed.

Table 3 Effect of essential oils on number of beetles/ 5 pairsdeveloped after 60-days of treatment of pea seeds at different dose of essential oils

Treatment	*Mean number of beetles / 5 pair developed at indicated dose				
	Dose (ml/kg)				
	2.5	1.25	0.6	0.3	Mean
Camphor	5	17.33	36.67	50.74	27.34
	-2.31	-4.49	-6.09	-7.13	-5
Wild Marigold	11.33	25.33	30.33	39.67	26.67
	-3.43	-5.08	-5.54	-6.3	-5.11
Cone-bearing sage	12.33	25.33	29.67	34.67	25.58
	-3.62	-5.08	-5.49	-5.91	-5.09
Eucalypts	29.67	32	56.33	71.67	47.42
	-5.47	-5.69	-7.53	-8.49	-6.8
Lemongrass	1.33	1.67	2.33	8.33	3.42
	-1.34	-1.46	-1.67	-2.96	-1.86
Sweet flag	0	0	1.09	2.67	0.94
	-0.71	-0.71	-1.04	-1.76	-1.06
Control	70.33	74	71.67	74.65	72.66
	-8.43	-8.64	-8.49	-8.65	-8.55
Mean	18.57	25.19		40.19	30.66
	-3.61	-4.45	32.62	-5.89	-4.78
			-5.18		

Figures in parenthesis are √x+0.5 transformed values
Treatment: (0.19)
Dose : (0.25)

Treatment X Dose: (0.49)

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Conflict of interest

The author declares no conflict of interest.

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