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Gitte ME
M.Sc. Scholar, Department of Agricultural Entomology, College of Agriculture, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Sawant VP
Junior Entomologist, Regional Agriculture Research Institute, Karjat, Raigad, Maharashtra, India

Kale SN
Assistant Professor, Department of Agricultural Entomology, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Rajemahadik VA
Assistant Professor, Department of Agronomy, College of Agriculture, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Dethe SR
M.Sc. Scholar, Department of Entomology, College of Agriculture, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Corresponding Author:
Gitte ME
M.Sc. Scholar, Department of Agricultural Entomology, College of Agriculture, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Effect of essential oils against pulse beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) under laboratory conditions

Gitte ME, Sawant VP, Kale SN, Rajemahadik VA and Dethe SR

Abstract
The laboratory experiment on "effect of different essential oils against pulse beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) under laboratory condition," was carried out at College of Agriculture, Dapoli Dr. B. S. Konkan Krishi Vidyapeeth Dapoli, Dist. Ratnagiri (M.S.) during 2021-22. From the investigation an effect of Tea tree oil, Rosemary oil, Lemongrass oil and Geranium oil at concentration of 200 and 400µl/kg against pulse beetle was evaluated. The Lemongrass oil at 400 µl/kg and was found most effective to control pulse beetle also Rosemary oils at 400 µl/kg, Lemongrass oil at 200 µl/kg and Rosemary oil at 200 µl/kg found significant to pulse beetle. In the treatments with Tea tree oil at 200 µl/kg and Geranium oil at 200 µl/kg was less effective as compared to the essential oil treatments. All the treatments were significantly better over control.

Keywords: pulse beetle, essential oils, *Callosobruchus maculatus*, treatments

1. Introduction
Pulses are the important source of proteins, vitamins and minerals and so contributes significantly to the nutritional security of the country as well as play important role in enhancing the soil fertility by symbiotic nitrogen fixation. Status of India with respect to global pulse production constitutes 25 per cent of global production and 27 per cent world consumption and importer 14 per cent of global imports. The Union Ministry of Agriculture in its first advance estimate of production for 2020-21 was 9.31 MT as against target of 10.66 MT for 2020-21 for pulse crops In term of total pulses production, gram share about 40 per cent which is highest among all the pulses followed by Arhar at 15 to 20 per cent and Black gram and Moong at around 8 to 10 per cent respectively. In India, top five pulses producing states are Madhya Pradesh, Maharashtra, Rajasthan, Utter Pradesh and Karnataka. The productivity of pulses in India range around 764 kg/ha. (Vikaspedia.in. 2019) [19].
Important constraint in production and storage of pulses is stored-insect pests. It was reported around 55 - 60 per cent weight loss of stored pulses and 46 to 66 per cent loss in protein content by infestation with the insect pests. Feeding by the insect larva and adults account the major portion of the crop losses annually which causes the economic loss of millions of rupees. Among the several important insect pests of stored grains, bruchids i.e. pulse beetle, *Callosobruchus* spp. is major pest during storage of pulses. The pulse beetle is the most widespread and destructive major insect pest of economically important leguminous grains such as green gram, chickpea, black gram, peas, cowpea, lentil and pigeon pea (Aslam *et al*. 2002) [2]. They are present in all the tropical and subtropical climates and attack a wide range of grain legume species (Southgate, 1978). The pulse beetle, *Callosobruchus* spp. is the important pest of stored pulse grains in storage causing 40 to 50 per cent losses during storage (Gosh *et al.* 2003) [25]. Several species of bruchids in the genus *Callosobruchus* are known to damage grains of legumes up to 93.3 per cent during storage. Among five well known species of *Callosobruchus* from India, three *viz. C. maculatus*, *C. chinensis* and *C. analis* are important pests of stored pulses (Raina, 1970) [14]. The germination loss due to the attack of storage pests on cereals and pulse grains ranges from 3 to 37 per cent and 4 to 88 per cent respectively (Adugna *et al.* 2003) [1]. One of the major pests of stored pulse is *C. maculatus* infesting seeds by feeding on its inner protein thus causing nutrient loss for human consumption as well as seed loss its germination (Epidi *et al.* 2008) [4]. Food and agricultural organization reported that 8.5 per cent of grain loss occurs during post-harvest handling and storage in India.

Essential oils *viz*. lemongrass oil, rosemary oil, tea tree oil, juniper oil, geranium oil, eucalyptus oil, etc. could be used as grain protectants. Based on the prior context, the primary objectives of the current work is to assess and developmental parameters of pulse beetle against specific essential oils, in order to identify the lead for developing a botanical formulation for seed treatment.

The management of pulse beetle is also important, therefore the present study was carried out to know the effect of essential oils against pulse beetle infesting chickpea.

2. Materials and Methods

2.1 Location

Department of Agricultural Entomology, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli Dist. Ratnagiri (Maharashtra)

2.2 Experimental details

Statistically designed laboratory experiment was laid out during year 2021-22 to evaluate efficacy of various essential oils against pulse beetle, *Callosobruchus maculatus* Fab. (Coleoptera: Chrysomelidae) on chickpea pulse. The details of the proposed experiment are as follows.

2.3 Treatment details

Table 1: Treatment details to study of developmental parameter:

Sr. No.	Treatments	Details of Treatments	Dose (µl/kg of seed)
1.	T_1	Tea tree oil	200µl
2.	T_2	Tea tree oil	400µl
3.	T_3	Rosemary oil	200µl
4.	T_4	Rosemary oil	400µl
5.	T_5	Lemongrass oil	200µl
6.	T_6	Lemongrass oil	400µl
7.	T_7	Geranium oil	200µl
8.	T_8	Geranium oil	400µl
9.	T_9	Untreated control	-

2.4 Method of recording observations

This experiment was conducted on chickpea seeds under laboratory conditions using four essential oils such as Tea tree oil, Rosemary oil, Lemongrass oil and Geranium oil. In order to evaluate the efficacy of different essential oils as a seed protectant against the pulse beetle, *C. maculatus*, experiment was carried out in Completely Randomized Design with nine treatments including untreated control.

Calculated dose of essential oil was collected with the help of micro-pipette and inserted in the center and then mixed thoroughly with 100g of chickpea seeds. The experiment was replicated thrice. Untreated seeds was taken as control. Five pairs of adults were released in plastic bottle containing 100g of treated seeds. Bottles were closed with lids tightly and kept in the laboratory at ambient temperature and humidity.

Test insects were allowed to lay the eggs up to death. Efficacy of essential oils on developmental parameters *viz*. fecundity, developmental period, adult emergence, adult longevity, weight loss, germination percentage were recorded.

The total number of adults (F1 adult emergence) emerged in each treatments were counted up to last emergence of the adults and emerged adults were discarded from the treatments bottles.

To study the effect of various oil treatments, after removing the emerged adults from the treatment bottles, per cent reduction in weight was recorded with electronic balance. The per cent weight loss was recorded by removing the dead insects, using the following formula:

$$\text{Per cent weight loss} = \frac{\text{Initial wt. (g)} - \text{Final wt. (g)}}{\text{Initial wt. of grains (g)}} \times 100$$

To observe the effect of test materials used for treatment, germination test of the treated chickpea pulses and untreated (Control) was carried out separately after 2 month of seed treatment. 50 chickpea seeds per replication per treatment were taken. The seed germination tests was carried out employing rolled paper towel test at room temperature. The germinated seeds were counted and recorded after 4 to 5 days.

$$\text{Per cent (\%) Germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds taken}} \times 100$$

2.5 Statistical methods

The data obtained were subjected to Completely Randomized Design and data statistically analysed.

3. Results and Discussion

The results of the experiment are presented and discussed under the subheading given below. The observation of efficacy on developmental parameter *viz*. fecundity, developmental period, adult emergence, adult longevity, weight loss, germination test etc. were recorded. The results of the study were statistically analysed, presented and discussed under the following sub-heading.

3.1 Effect of different essential oils on fecundity of female pulse beetle, *C. maculatus* in chickpea seeds [No Choice Test]

Table 2: Effect of different essential oils on number of eggs laid by *C. maculatus* on chickpea [No Choice Test]

Sr. No.	Treatments	Dose (µl/100g)	Mean no. of eggs laid by female *C. maculatus*
1	T_1- Tea tree oil	20µl	103.67(10.19)*
2	T_2- Tea tree oil	40µl	83.33(9.17)
3	T_3- Rosemary oil	20µl	54.00(7.41)
4	T_4- Rosemary oil	40µl	34.67(5.97)
5	T_5- Lemongrass oil	20µl	44.00(6.69)
6	T_6- Lemongrass oil	40µl	30.00(5.56)
7	T_7- Geranium oil	20µl	94.00(9.74)
8	T_8- Geranium oil	40µl	79.00(8.94)
9	T_9- Untreated control	-	224.00(14.97)
	S.E(m±)		0.36
	C.D. at 5%		1.1

*Figures in the parenthesis are square root transformed values with $\sqrt{x+0.5}$

The data on the average number of eggs laid by *C. maculatus* on chickpea grains treated with different oils presented in Table 2 revealed that mean number of eggs laid by female pulse beetle, *C. maculatus* on chickpea treated with different oils were ranging Between 34.67 to 224.00. Lemongrass oil @ 40µl/100g of seeds and Rosemary oil @ 40µl/100g of seeds were observed to be most effective in reducing egg laying by pulse beetle. The highest number of eggs were laid in untreated control. All the treatments with different essential oils were found superior over untreated control. Among the treatments of essential oils, maximum egg laying (103.67)

was observed in the treatment T1, Tea Tree oil @ 20µl/100g seeds. Thus, fecundity not only varied with the treatments, but also along with different concentrations of essential oils. The result obtained coherence with Hedjal *et al.* (2013) [7, 8] found that essential oils significantly affected the longevity, fecundity, and emergence in *C. maculatus* in contact tests at a dose of 50 µl essential oil/50g of seeds. Similarly, Sahoo *et al*. (2013) [17] observed that, the fecundity of female *C. maculatus* with minimum number of eggs (10.70) was recorded at dose 0.25 ml/100g neem oil treated with seeds, showing the effectiveness of oils response to fecundity. Whereas, Sabbour (2019) [16] performed an experiment on effectiveness of Nano-rosemary oils against the biological activity of *C. maculatus* and *C. chinensis*. And the results revealed that number of eggs laid per female of *C. maculatus* were significantly decreased to 6.4 ± 9.89 eggs/female after Nano-rosemary treatments as compared to 299.9 ± 9.89 eggs per female in the control condition.

3.2 Effect of different essential oils on the developmental period of pulse beetle, *C. maculatus* in chickpea [No Choice Test]: The observations of mean developmental period mentioned in Table 3, showed that among the various treatments with essential oils, the developmental period varied from 24.67 to 37.00 days. Prolonged developmental period was observed in the treatment T6, Lemongrass oil @ 40µl/100g chickpea seeds (37.00 days) which was at par with the treatment T4, Rosemary oil @ 40µl/100g seeds (36.33 days). Minimum developmental period was observed in Untreated control T9 i.e. 24.67 days.

Table 3: Effect of different essential oils on total developmental period of *C. maculatus* [No Choice Test]

Sr. No.	Treatments	Dose (µl/100g)	Total developmental period (in days)
1	T_1- Tea tree oil	20µl	27.00
2	T_2- Tea tree oil	40µl	28.33
3	T_3- Rosemary oil	20µl	33.00
4	T_4- Rosemary oil	40µl	36.33
5	T_5- Lemongrass oil	20µl	34.00
6	T_6- Lemongrass oil	40µl	37.00
7	T_7- Geranium oil	20µl	27.67
8	T_8- Geranium oil	40µl	30.00
9	T_9- Untreated control	-	24.67
	S.E(m±)		0.86
	C.D. at 5%		2.59

The present research finding are on same line with the findings of Naik and Dumbre (1984) [12] who observed the effect of some non-edible oils such as neem, karanj, castor and undi and some edible oils such as groundnut, safflower, coconut, mustard and niger in stored cowpea on *C. maculatus*. Similarly, Sawant (2001) [18] studied efficacy of different oils was observed on *C. maculatus* in green gram. Results from the experiment revealed that the no adult emergence in green gram seeds treated with castor oil, neem oil and mustard oil and groundnut oil at 1.0 ml/100g of seeds while in neem oil, it was observed that the developmental period of *C. maculatus* was prolonged as compared with untreated control. Namdev *et al* (2014) [13] who conducted a laboratory experiment to investigate the bio-efficacy of natural oils *viz*., groundnut, sunflower, mustard, mahua, neem seeds, linseed, sesamum, taramira and soyabean oil against the pulse beetle, *C. maculatus*. These oil applied @ 2 ml and 3 ml per kg of seeds with three replications. Both the doses of indigenous oils significantly extended the developmental period. The neem oil increased the developmental period (14.3) at 3 ml per kg of seed dose it was followed by taramira and mustard oil.

3.3 Effect of different essential oils on adult emergence of pulse beetle, *C. maculatus* in chickpea [No Choice Test]

The data depicted in Table 4 clearly showed that number of adults emerged under No Choice Test found to be least in treatment T6 Lemongrass oil @ 40µl/100g of chickpea seeds i.e. 20.00 adults. While highest adult emergence was observed in treatment T9 untreated control (197.00). Kumar *et al.* (2017) [11] conducted an experiment on the pulse beetle, *Callosobruchus chinensis* (L.), using nine different plant-based essential oils were evaluated for adult emergence of *C. chinensis*. All the treatments reduced adult emergence as compared to the untreated control. Whereas, Rathod *et al.* (2019) [15] conducted an experiment to check the efficacy of botanicals against pulse beetle in stored green gram. All the botanicals significantly reduced adult emergence than the untreated control.

Table 4: Effect of different essential oils on the number of adults emerged of *C. maculatus* in chickpea [No Choice Test]

Sr. No.	Treatments	Dose (µl/100g)	Mean no. of adults emerged of *C. maculatus*
1	T_1- Tea tree oil	20µl	68.67(8.34)*
2	T_2- Tea tree oil	40µl	59.33(7.76)
3	T_3- Rosemary oil	20µl	41.67(6.53)
4	T_4- Rosemary oil	40µl	25.00(5.09)
5	T_5- Lemongrass oil	20µl	33.67(5.88)
6	T_6- Lemongrass oil	40µl	20.00(4.55)
7	T_7- Geranium oil	20µl	74.33(8.67)
8	T_8- Geranium oil	40µl	58.00(7.68)
9	T_9- Untreated control	-	197.00(14.03)
	S.E(m±)		0.33
	C.D. at 5%		1.01

*Figures in the parenthesis are square root transformed values with $\sqrt{x} + 0.5$

3.4 Effect of different essential oils on female longevity of *C. maculatus* in chickpea [No Choice Test]

The data pertained in the Table 5 clearly indicated that, lowest mean female longevity was observed in treatment T6, Lemongrass oil @ 40µl/100g seeds (9.00 days) whereas highest mean female longevity was observed in T9, Untreated control i.e. 13.00 days. Lemongrass oil and Rosemary oil each @ 40µl/100g seeds concentration were found most effective in reducing the female longevity significantly. The efficacy of essential oils was checked for female longevity of pulse beetle. It was observed that essential oils found effective in reduction of female longevity of adult pulse beetle. The result obtained are on similar line with the result obtained by the, Douiri *et al.* (2013) [3] evaluated the fumigant ability of *Allium sativum* essential oils against *C. maculatus* in chickpea. Garlic essential oils affected adult longevity. The adult longevity of treated pulses was 1 - 3 days and that of controls was 2 - 13 days. Whereas, Hafez *et al.* (2014) [6] evaluated bio efficacy of two vegetable oils, *viz*. *Ethyl oleate* and *Acorus calamus*, against the pulse beetle, *C. maculatus* in mung bean. *A. calamus* oil was found more toxic to the adults at all the doses tested in comparison with *Ethyl oleate* oil. Adult longevity of male and female were 6.0 ± 0.37 days (5 to 7 days) and 7.0 ± 0.41days (6 to 8 days) respectively in control.

Table 5: Effect of different essential oils on female longevity of *C. maculatus* in chickpea [No Choice Test]

Sr. No.	Treatments	Dose (µl/100g)	Mean of female longevity of *C. maculatus* in chickpea
1	T1- Tea tree oil	20µl	12.00
2	T2- Tea tree oil	40µl	11.33
3	T3- Rosemary oil	20µl	10.00
4	T4- Rosemary oil	40µl	9.33
5	T5- Lemongrass oil	20µl	9.67
6	T6- Lemongrass oil	40µl	9.00
7	T7- Geranium oil	20µl	11.67
8	T8- Geranium oil	40µl	11.00
9	T9- Untreated control	-	13.00
	S.E(m±)		0.77
	C.D. at 5%		2.32

3.5 Effect of different essential oils on male longevity of pulse beetle, *C. maculatus* in chickpea [No Choice Test]

The observations on efficacy of essential oils on male longevity mentioned in Table 6 indicated that, minimum male longevity was observed in treatment T6, Lemongrass oil @ 40µl/100g of chickpea seeds while maximum male longevity was observed in untreated control. Treatments with different essential oils significantly affected the male longevity. The present findings are in agreement with Hafez *et al.* (2014) [6] as mentioned above.

Table 6: Effect of different essential oils on male longevity of pulse beetle, *C. maculatus* in chickpea [No Choice Test]

Sr. No.	Treatments	Dose (µl/100g)	Mean of male longevity of *C. maculatus* in chickpea
1	T_1- Tea tree oil	20µl	8.00
2	T_2- Tea tree oil	40µl	7.00
3	T_3- Rosemary oil	20µl	6.33
4	T_4- Rosemary oil	40µl	5.67
5	T_5- Lemongrass oil	20µl	6.00
6	T_6- Lemongrass oil	40µl	5.33
7	T_7- Geranium oil	20µl	7.67
8	T_8- Geranium oil	40µl	6.67
9	T_9- Untreated control	-	9.00
	S.E(m±)		0.45
	C.D. at 5%		1.37

3.6 Effect of different essential oils on per cent weight loss caused due to *C. maculatus* infestation in chickpea [No Choice Test]

Mean per cent weight loss caused by the pulse beetle in chickpea seeds was ranging between 1.46 to 11.28%. The lowest weight loss was recorded in the treatment T6, Lemongrass oil @ 40µl/100g seeds and Rosemary oil @ 40µl/100g seeds. Maximum average weight loss was observed in untreated control.

The present findings are in agreement with Kathirvelu *et al.* (2020) [10] who evaluated the fumigant toxicity of essential oils, *C. zeylanicum* against adults of test insects *viz.*, *C. chinensis*, and *S. oryzae*. The minimum grain weight loss was observed in *C. zeylanicum* treated at 60 per cent of 24 hrs LC50, with 7.41 per cent weight loss. Similarly, Hedjal *et al.* (2013) [7, 8] found that essential oils significantly affected the longevity, fecundity, and emergence of *Callosobruchus maculatus* (Coleoptera: Bruchidae) at a dose of 50 µl essential oil/50gm seed. There was no secondary effect on *Vigna unguiculata* seeds treated with the highest doses (50 and 75µl of essential oils), as evidenced by weight and germination capacity.

Table 7: Effect of different essential oils on per cent weight loss by *C. maculatus* in chickpea [No Choice Test]

Sr. No.	Treatments	Dose (µl/100g)	Mean per cent weight loss caused by *C. maculatus* in chickpea
1	T_1- Tea tree oil	20µl	6.41(14.62)*
2	T_2- Tea tree oil	40µl	4.61(12.37)
3	T_3- Rosemary oil	20µl	3.33(10.49)
4	T_4- Rosemary oil	40µl	2.30(8.64)
5	T_5- Lemongrass oil	20µl	2.81(9.50)
6	T_6- Lemongrass oil	40µl	1.46(6.78)
7	T_7- Geranium oil	20µl	4.85(12.66)
8	T_8- Geranium oil	40µl	3.83(11.24)
9	T_9- Untreated control	-	11.28(19.56)
	S.E(m±)		0.90
	C.D. at 5%		2.69

*Figures in the parenthesis are arc sine transformed values

3.7 Effect of different essential oils on germination percentage of chickpea [No Choice Test]

The data obtained from the Table 8 indicated that there was no adverse effect of essential oils on germination capacity of chickpea seeds. The non-significant effect of essential oils were recorded on germination percentage of treated chickpea

seeds with different essential oils. The present findings on germination percentage of chickpea are in agreement with the findings of Huseyin Cetin and Melek Gudek (2017) [9] who studied the fumigant effect of *Rosmarinus officinalis* L. essential oil against adults of *C. maculatus*. The results revealed that the highest applied dose (60µl/l−1air) and the longest exposed time (72 h) have no negative effect on chickpea germination. Paranagama *et al.* (2003) studied effect of the essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Cinnamomum zeylanicum* against Angoumois grain moth *S. Cerealella*. The results revealed that per cent grain damage was minimum in *C. citratus* and *C. nardus* and observed that the percentage seed germination differed non-significantly with the control and caused reduced seed germination in paddy seeds treated with *C. citrates.*

Table 8: Effect of different essential oils on germination percentage of chickpea [No Choice Test]

Sr. No.	Treatments	Dose (µl/100g)	Mean of germination percentage in chickpea pulse
1	T_1- Tea tree oil	20µl	95.00(77.12)*
2	T_2- Tea tree oil	40µl	94.00(75.95)
3	T_3- Rosemary oil	20µl	93.33(75.07)
4	T_4- Rosemary oil	40µl	92.00(73.65)
5	T_5- Lemongrass oil	20µl	92.67(74.34)
6	T_6- Lemongrass oil	40µl	92.67(74.32)
7	T_7- Geranium oil	20µl	94.67(76.66)
8	T_8- Geranium oil	40µl	93.33(75.07)
9	T_9- Untreated control	-	95.33(77.58)
	S.E(m±)		0.92
	C.D. at 5%		NS

*Figures in the parenthesis are arc sine transformed values

4. Conclusion

The overall results revealed that, Pulse beetle, *Callosobruchus maculatus* (Fab.) was highly host specific. Chickpea was the preferred and suitable host for growth and development of *C. maculatus*. Based on all the parameters observed of pulse beetle, *C. maculatus*, it was concluded that the developmental period was extended due to the treatments with essential oil as well as fecundity was also remarkably reduced as compared with untreated control. The rate of adult emergence was significantly minimize as against untreated control. However, due to treatment with different essential oils, the adult longevity was significantly affected as it was found maximum in case of female bruchids than male pulse beetle, *C. maculatus* (Fab.). There was non-significant effect recorded on germination percentage of chickpea treated with different essential oils.

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