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Studies on effect of essential oils on quality characters of pea seeds (*Pisum sativum* L.) damaged by *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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Abstract
The experiment was conducted during 2014 in the month of July- August in the Department of Seed Science and Technology. 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.) and sweet flag (*Acorus calamus* L.) were used to coat the seeds @ 2.5,1.25,0.60 and 0.30 per cent concentrations in four replications. Minimum seed damage (0.33%) and weight loss (0.23%) was recorded with sweetflag essential oil. Seed damage and weight loss was nil in sweetflag essential oil at 2.5 per cent concentration and 1.25 per cent. Maximum seed germination was in sweetflag essential oil (92.75%).Maximum seed vigour index-I (1665.19) and seed vigour index-II (2115.74) was with sweet flag essential oil followed by lemon grasss. Thus, sweetflag (*Acorus calamus* L.) essential oil was best in protecting peas seeds against *C. chinensis*.

Keywords: Essential oil, Callososbruchus chinensis, pea seeds. quality character

1. Introduction

A number of insect-pests attack the stored grains, seeds and other products. Among the important insect pests of stored grain, the pulse beetle, *Callosobruchus chinensis* L. (Bruchidae: Coleoptera), causes substantial losses to the pulses in the storage [1, 2] though the initial infestation occurs in the field itself. It causes weight loss, decreased germination potential and reduction in commercial value of the seed [3, 4]. It is a serious pest of pea, mung bean, cowpea and lentil and has also been reported attacking cotton seed, sorghum and maize [5]. The use of synthetic organic pesticides for the control of insect-pests of stored seeds has led to the development of resistance, toxic residues in food grains, besides being costly. The use of plant products as grain protectants is an age old practice [6] and appears to be quite safe and promising [7, 5]. Therefore, there is a need of some other alternative of chemical pesticides and fumigants to protect stored seed grains from insect-pests infestations. Plant essential oils and its constituents have been used as an alternative of synthetic pesticides possessing insecticidal, ovicidal, repellent, ovipositional activities and fumigant against various stored grain insect-pests [8-10]. Therefore, the present study aims to find the ecofriendly approaches particularly essential oils against *C. chinensis*.

2. Materials and Methods

2.1. Raising of insect culture: The pure culture of *C. chinensis* was raised on pea seeds and maintained under controlled conditions at 27 ±1 ^{0}C and 70% R.H. The freshly harvested seeds of pea seeds were sterilized in oven at 55 ^{0} C for 4 hours [11]. The sterilized grains were put in half kg capacity glass jars and 5 pairs of freshly emerged *C. chinensis* adults were released in the jars. The jars were tightly covered with muslin cloth and were kept in BOD incubator for raising the culture.

2.2. Plant material: Six plant essential oils i.e. Camphor (*Cinnamomumcamphora 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 against *C. chinensis* and their effect on seed quality parameters.

The plant materials were collected locally, shade dried and essential oils were extracted with the help of clevenger apparatus by hydro-distillation. Each oil @ 2.5.1.25, 0.60 and 0.30 ml/kg were thoroughly mixed by vigorously shaking in different plastic containers filled with seed. Five pairs of the newly emerged adults of *C. chinensis* were released in each plastic container which was covered with muslin cloth and was tightened with rubber band. The treatments, including control,were maintained in four replications. All the adults were allowed to remain in the container till their natural mortality under room temperature. After storage period of 2-months data on seed damage, seed weight loss, seed germination and seed vigour index-I and seed vigour index-II was recorded. The per cent seed damage was calculated by adopting the procedure given by Adams and Schulten [12]. Loss in seed weight was calculated by taking initial weight and final weight of the seed and per cent weight loss was calculated. The per cent seed germination was calculated by taking 100 seeds from each container. The seeds were sandwiched between towel paper [13]. The paper towel was then kept in seed germinator chamber at 25 ± 1^{0}C. The germination percentage was calculated as per the ISTA procedure [14]. Seed Vigour Index- I was calculated by using the method given by Abdul Baki and Anderson [15]. Seed Vigour Index- II was calculated by using the formula: Germination (%) X Seedling dry weight (mg).

Statistical analysis

The data emanating from the above experiments was subjected to statistical analysis through two factor Completely Randomized Design after proper transformation and significance of each treatment was calculated [16].

Result and Discussion

It is evident from Table 1 that all the tested essential oils provided significant protection to pea seeds from the damage by *C. chinensis.* Sweet flag was most effective allowing only 0.33 per cent damage followed by lemongrass (3.90%). No damage was recorded in pea seeds treated with sweet flag essential oil at 2.5 ml/kg and 1.25 ml/kg doses. Next effective treatments were wild marigold (6.81%), camphor (7.86%), cone-bearing sage (7.94%) and eucalypts (11.74%) in increasing order. Reduction in seed damage may be due to lower oviposition and high ovicidal activity of sweet flag essential oil, thereby inhibiting the adult development of beetles. The present finding was in conformity with the findings of Su [17] who reported that seeds of cowpea seeds (*Vigna unguiculata*) treated with essential oil of sweet flag inhibited the adult development and gave cent per cent protection of seeds from pulse beetle.

Sweet flag and lemongrass essential oils minimized the weight loss in treated pea seeds to 0.23-2.05 per cent, respectively as against 20.35 per cent in control (Table 2). Among all treatments, sweet flag even at lowest dose was effective in minimizing weight loss due to *C. chinensis*. Other effective treatments were camphor (7.82%), cone-bearing sage (15.18%), wild marigold (15.34%) and eucalypts (17.30%). In the present study, the lower weight loss in acorus treated seeds may be due to insecticidal action of acorus ingredients which caused high mortality of adults thereby less egg laying and ultimately lower seed damage and weight loss. The present findings corroborate the findings of Schmidit and Streloke [18] who reported the reduction in feeding and reduced weight loss by *Prostephanus truncates* when maize grains were treated with sweet flag and beta-asarone. The present findings also corroborate with the findings of Raja and William [19] who reported reduced weight loss of cowpea seeds treated with lemongrass essential oil.

Data contained in Table 3 revealed that seed germination in various treatments ranged from 34.92-92.75 per cent as against 19.75 per cent in control. Oils of sweet flag and lemongrass were the best (92.75 and 85.58%, respectively). Effect on seed germination by other essential oils treated pea seeds were recorded as camphor (78.33%), cone-bearing sage (43.75%), eucalypts (41.25%) and wild marigold (39.42%) in decending order. Thus seed germination of stored pea was improved due to protection from damage caused by *C. chinensis* provided by these essential oils. None of the treatments apparently reduced seed germination indicating that these essential oils can be used safely for the control of *C. chinensis*. The present study finds support from work of Yadav [20] who reported oil emulsion of sweet flag had rapid knockdown action of *C. chinensis* and was safe for seed germination.

Seed vigour index-I in various treatments ranged from 685.30 to 1665.19. Maximum seed vigour index-I was recorded in pea seeds treated with sweet flag essential oil followed by lemongrass essential oil (Table 4). Minimum seed vigour index-I was recorded in pea seeds treated with wild marigold (688.30) but statistically it was superior over control (374.57). Maximum seed vigour index-II recorded with pea seeds treated with sweet flag essential oil (2115.74) followed by lemongrass with 1681.86 seed vigour index-II (Table 5). Minimum seed vigour index-II was recorded in wild marigold essential oil treated pea seeds (799.55) as compared to control (144.14). The high seed vigour index-I and seed vigour index-II recorded with pea seeds treated with sweet flag and lemongrass essential oil may be due to reduced damage to pea seeds by *C. chinensis.* The present findings were in accordance with the findings of Vishwamitra *et. al.* [21] who reported the increase in seedling vigour index of pigeon pea seeds treated with eucalypts essential oil.

Table 1: Effect of essential oils on pea seed damage caused by *C. chinensis*

Treatment	*Mean seed damage (%)				
	Dose (ml/kg)				
	2.5	1.25	0.60	0.30	Mean
Camphor	3.67 (2.01)	4.80 (2.30)	9.52 (3.16)	13.43 (3.71)	7.86 (2.80)
Wild Marigold	4.18 (2.14)	5.81 (2.51)	7.38 (2.85)	9.86 (3.22)	6.81 (2.68)
Cone-bearing sage	4.66 (2.26)	7.37 (2.80)	8.74 (3.04)	11.00 (3.39)	7.94 (2.87)
Eucalypts	9.02 (3.08)	11.98 (3.43)	14.30 (3.86)	17.65 (4.23)	11.74 (3.65)
Lemongrass	1.15 (1.28)	1.72 (1.49)	2.73 (1.79)	9.99 (3.22)	3.90 (1.95)
Sweet flag	0.00 (0.71)	0.00 (0.71)	0.60 (1.07)	0.70 (1.08)	0.33 (0.89)
Control	20.64 (4.55)	19.81 (4.50)	20.61 (4.59)	23.10 (4.86)	20.04 (4.63)
Mean	5.62 (2.29)	7.07 (2.53)	8.76 (2.91)	11.89 (3.38)	8.37 (2.78)

* Mean of three replications

Figures in parenthesis are $\sqrt{x+0.5}$ transformed values
CD (p=0.05)
Treatment : (0.11)
Dose : (0.15)
Treatment X Dose : (0.29)

Table 2: Effect of essential oils on weight loss caused by *C. chinensis*

Treatment	*Mean weight loss (%)				
	Dose (ml/kg)				
	2.5	**1.25**	**0.60**	**0.30**	**Mean**
Camphor	2.42 (1.67)	4.91 (2.32)	11.78 (3.50)	12.19 (3.56)	7.82 (2.76)
Wild Marigold	9.38 (3.11)	11.87 (3.52)	19.84 (4.51)	20.26 (4.56)	15.34 (3.92)
Cone-bearing sage	11.38 (3.44)	12.41 (3.59)	18.38 (4.34)	18.54 (4.36)	15.18 (3.93)
Eucalypts	14.08 (3.81)	17.44 (4.23)	18.73 (4.38)	18.96 (4.41)	17.30 (4.21)
Lemongrass	1.57 (1.41)	1.69 (1.48)	1.92 (1.53)	3.01 (1.86)	2.05 (1.57)
Sweet flag	0.00 (0.71)	0.00 (0.71)	0.39 (0.94)	0.52 (1.01)	0.23 (0.84)
Control	20.43 (4.57)	19.80 (4.50)	20.71 (4.60)	20.47 (4.58)	20.35 (4.56)
Mean	8.47 (2.68)	9.73 (2.91)	13.09 (3.40)	13.37 (3.48)	11.17 (3.12)

*Mean of three replications

Figures in parenthesis are $\sqrt{x+0.5}$ transformed values
CD (p= 0.05)
Treatment : (0.16)
Dose : (0.18)
Treatment X Dose : (0.36)

Table 3: Effect of essential oils on seed germination caused by *C. chinensis*

Treatment	*Mean seed germination(%)				
	Dose (ml/kg)				
	2.5	**1.25**	**0.60**	**0.30**	**Mean**
Camphor	89.33 (71.03)	88.33 (70.10)	68.33 (55.82)	67.33 (55.18)	78.33 (63.03)
Wild Marigold	50.33 (45.18)	44.67 (41.89)	34.67 (36.04)	28.00 (31.71)	39.42 (38.72)
Cone-bearing sage	61.33 (51.61)	55.67 (48.33)	34.00 (35.63)	24.00 (29.22)	43.75 (41.24)
Eucalypts	55.33 (48.07)	50.67 (45.35)	30.00 (33.09)	29.00 (32.49)	41.25 (39.79)
Lemongrass	93.67 (75.53)	89.00 (70.71)	84.33 (66.83)	75.33 (60.25)	85.58 (68.33)
Sweet flag	95.00 (77.09)	92.67 (74.29)	92.00 (73.56)	91.33 (72.87)	92.75 (74.45)
Control	21.33 (27.49)	20.33 (26.79)	18.33 (25.33)	19.00 (25.81)	19.75 (26.36)
Mean	66.61 (56.57)	63.04 (53.92)	51.67 (46.62)	47.71 (43.94)	57.25 (50.26)

* Mean of three replications

* Figure in parenthesis are arc sine transformed values
Treatment : (3.03)
Dose : (2.29)
Treatment X Dose : (6.06)

Table 4: Effect of essential oils on seed vigour index-I caused by *C. chinensis*

Treatment	*Mean seed vigour index- I				
	Dose (ml/kg)				
	2.5	**1.25**	**0.60**	**0.30**	**Mean**
Camphor	1409.83	1269.83	1113.04	982.34	1193.76
Wild Marigold	821.50	714.23	634.55	570.91	685.30
Cone-bearing sage	785.92	720.27	712.28	637.88	714.09
Eucalypts	946.62	865.33	826.06	736.73	843.69
Lemongrass	1765.93	1580.97	1351.70	1064.54	1440.78
Sweet flag	1997.20	1803.90	1499.02	1360.65	1665.19
Control	374.11	385.21	359.82	379.13	374.57
Mean	1157.30	1048.53	928.07	818.88	988.19

* Mean of three replications
CD (p=0.05)
Treatment : (46.68)
Dose : (61.75)
Treatment X Dose : (123.50)

Table 5: Effect of essential oils on seed vigour index-II caused by *C. chinensis*

Treatment	*Mean Seed Vigour Index-II				
	Dose (ml/kg)				
	2.5	1.25	0.60	0.30	Mean
Camphor	20,08.29	17,72.84	12,63.69	10,53.40	15,24.56
Wild marigold	12,31.80	7,11.27	7,09.93	5,45.25	7,99.55
Cone-bearing sage	13,38.47	13,01.76	10,03.03	9,70.62	11,53.46
Eucalypts	13,71.16	10,69.14	10,30.64	9,19.41	10,97.59
Lemongrass	26,22.40	16,86.04	14,06.70	10,12.31	16,81.86
Sweet flag	24,09.51	23,01.12	19,51.98	18,00.32	21,15.74
Control	1,73.35	1,57.33	1,37.42	1,08.47	1,44.14
Mean	15,93.56	12,85.67	10,71.91	9,15.68	

*Mean of three replications
CD (p=0.05)
Treatment : (122.34)
Dose : (161.84)
Treatment X Dose : (323.69)

Conclusion

The present study revealed that out of 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.) and sweet flag (*Acorus calamus* L.) tested sweetflag (*Acorus calamus* L.) essential oil followed by lemon grass (*Cymbopogon citratus* L.) was found to be best in protecting peas seeds against *C. chinensis*.

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