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Efficacy of plant powders against pulse beetle, *Callosobruchus chinensis*, L. infesting pea seed

Kamal Deep Kaur, SC Verma and PL Sharma

Abstract
Seven plant powders *viz*. neem (*Azadirachta indica* J.), Camphor (*Cinnamomum camphora* L.), sweet flag (*Acorus calamus* L.), lemongrass (*Cymbopogon citratus* L.), eucalyptus (*Eucalyptus* sp.), wild marigold (*Tegetes minuta* L.) and drek (*Melia azadirachta* L.) were used to coat the seeds @ 5, 3, and 1g /100g seeds. There were eight treatments including control. Sweet flag resulted in 64.49,84.44 and 98.89 per cent mortality of the pulse beetle, *Callosobruchus chinensis* at the highest dose (5 g/100 g seeds) after 1,3 and 7 days of treatment, respectively. Neem was other highly effective product that accounted for 18.89, 42.22 and 63.33 per cent mortality of the beetle after the respective treatment intervals. Egg laying by females after one week of treatment was also the least in sweet flag followed by neem and eucalyptus treatment (6.33,10.67 and 28.33 eggs/5 females, respectively, at the lowest dose of 1g) as against 97.0 eggs/5 females in untreated control. The progeny development and survival was minimum (3.72,6.89 and 17.89 adults/ 5females) on pea seeds treated with sweet flag, neem and eucalyptus at the tested doses compared to 103.17 adults in untreated control.

Keywords: Plant powders, *Callosobruchus chinensis*, pea seeds, mortality, fecundity, and progeny development

Introduction
Pea (*Pisum sativum* L.) is an important off season vegetable crop of Himachal Pradesh and is grown over an area of 49800 ha with a production of 481100 metric tonnes [1]. A number of insect-pests attack the stored grains, seeds and other products. Among the important insect pests of stored grains, the pulse beetle, *Callosobruchus chinensis* L. (Bruchidae: Coleoptera), causes substantial losses to the pulses in the storage though the initial infestation occurs in the field itself [2]. According to an estimate, the overall annual damage caused by stored grain insect pests accounts for 10-40 per cent worldwide annually [3]. It causes weight loss, decreased germination potential and reduction in the commercial value of the seed [4, 5, 6]. In order to keep stored seed grains free from insect-pests infestation various synthetic pesticides are used [7]. 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, pose risk to human health and destruction of the balance of the ecosystem [8, 9, 10] besides being costly. Therefore, there is a need of some other alternative of chemical pesticides and fumigants to protect stored seed grains from insect-pests infestations. The use of botanical insecticides is now receiving increased attention due to their safety and effectiveness [11, 12, 13]. Botanicals can be used to keep the stored grains free from pulse beetle attack and for long term storability and quality parameters of stored grains. Various locally available plant products have been tried recently with good degree of success against a number of stored grain insect pests [14, 15, 16, 17, 18, 19]. Therefore, use of botanical pesticides is considered to be the most viable and environmentally safe approach to offset ever increasing danger caused by conventional pesticides [20, 21]. Therefore, the present study was carried out for plant products toxicity, effect on oviposition, progeny development and survival of *C. chinensis* infesting pea seed in storage. So that locally available botanicals can be used to keep the stored grains free from pulse beetle attack and for long term storability of pea seeds.

Materials and Methods
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 [22]. 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.

Plant material: Seven plant powders *viz*. neem (*Azadirachta indica* J.), camphor (*Cinnamomum camphora* L.), sweet flag (*Acorus calamus* L.), lemongrass (*Cymbopogon citratus* L.), eucalyptus (*Eucalyptus sp*.), wild marigold (*Tegetes minuta* L.) and drek (*Melia azadirachta* L.) were evaluated for their insecticidal activity against *C. Chinensis*. The plant materials were collected locally, shade dried and plant powders were made with the help of grinder. Plant powders were taken at different doses *viz*., 5, 3, and 1g per 100g seeds in separate plastic container of 250 cc capacity containing 100 g of sterilized seeds of pea with three replications. Contents were thoroughly mixed in plastic containers by vigorous shaking. In control no plant powder 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. During the experimental period the average minimum and maximum temperature was 14.5 and 30.5(ºC), respectively and humidity was 57.7 per cent. There were eight treatments including control with three replications. All the adults were allowed to remain in the container till their natural mortality under room temperature.

Efficacy of plant powders against the beetles

In order to determine the efficacy of plant powders against the pulse beetles, the mortality of adult beetles released on treated seeds was recorded at different intervals i.e. 1, 3, 7, 10 and 15 days.

Fecundity and progeny development: The effects of different plant powders on fecundity of *C. chinensis* were recorded on 7 and 20 day of release of adults and further progeny development after two months of release of adults. Egg laying data and progeny developed on treated seeds were compared with untreated individual (control).

Results and Discussion

Data contained in Table 1 reveal that among seven plant powders, sweet flag had the highest toxicity and irrespective of dose, resulted in 64.45 per cent mortality within one day of treatment, followed by neem (18.89%) which was statistically at par with eucalyptus (14.44%). Within 3 days, the mortality increased to 96.67 and 80 per cent at 5 and 3 g/100g dose of the sweet flag powder, respectively. Whereas, the mortality in neem treatment was 42.22 per cent and in eucalyptus was 35.56 per cent, both being statistically at par with each other.

After 7-days of treatment, highest mortality (98.89%) was observed with sweet flag powder followed by neem (67.78%), eucalyptus (63.33%), camphor (56.67%), lemongrass (52.22%), drek (46.67%) in decreasing order. After 10- days of exposure, cent per cent beetle mortality was recorded with pea seeds treated with sweet flag (100 %). Next best treatment was neem (91.11%) which was statistically at par with eucalyptus (87.78%), the later was statistically at par with camphor (83.33%). Mortality recorded with camphor (83.33%) was statistically at par with lemongrass (78.89%) whereas mortality recorded with drek (73.33%) was statistically at par with wild marigold (67.78%). Complete mortality of beetle was recorded at 5 g, 3 g and 1 g doses of sweet flag and 5 g dose of neem and eucalyptus powder. Camphor at 3g, eucalyptus at 1 g and wild marigold at 5 g caused equal mortality (80.00%). Eucalyptus at 3 g and drek at 5 g dose caused equal mortality (83.33%). Mortality of pulse beetle at 5 g dose was 85.42 per cent which decreased to 74.17 and 70.00 per cent with 3 and 1 g doses of plant powders, respectively.

After 15-days of exposure, treatments such as neem, sweet flag and eucalyptus caused 100.00 per cent mortality which was statistically at par with camphor (98.89%). Whereas drek caused 94.44 per cent mortality which differed statistically with wild marigold (87.78%) and statistically at par with lemongrass (92.22%). Overall results showed that mortality of pulse beetle decreased with decrease in dose (5g: 94.58%; 3g: 92.92%; 1g: 89.17%).

In the present study, on day-7 observation, mortality in control was 6.67 per cent, which substantially increased to 63.33 per cent on day-15 consequently complete mortality was observed in lots of seed treated with sweet flag, eucalyptus and neem. Thus additional mortality that had occurred in seed treated with plant powders after 15 days of treatment was mainly due to natural mortality of beetles released in treated pea seeds. The mortality of adult beetles of *C. chinensis* depends on type of plant powder and its dose used. Irrespective of plant powders, there was dose dependent mortality and as dose decreased from 5g/100g to 1g/100g there was significant decrease in mortality (61.25%, 54.50 % and 46.58%). Thus, out of seven plant powders, sweet flag at 5g/100g dose (96.67% kill) proved best and was significantly superior to its lower dose (3g/100g and 1g/100g) with 89.33 and 82.67 per cent mortality. The present findings corroborate the findings of Govindan and Nelson [23] who reported the efficacy of sweet flag powder against the adults of *C. chinensis.* Similar to present study, Shukla [24] also reported that sweet flag rhizome powder caused 100 per cent mortality of *C. chinensis*.

Table 1: Cumulative mortality response of *C. chinensis* beetles exposed to pea seeds treated with plant powders

Treatment	*Mean Mortality (%) of beetles on different days and doses											
	Days											
	Day-1				Day-3				Day-7			
	Dose (g/100g seed)											
	1	3	5	Mean	1	3	5	Mean	1	3	5	Mean
Neem	10.00 (18.43)	20.00 (26.55)	26.67 (30.98)	18.89 (25.32)	30.00 (33.20)	43.33 (41.14)	53.33 (46.90)	42.22 (40.41)	60.00 (50.75)	63.33 (52.76)	80.00 (63.41)	67.78 (55.64)
Camphor	3.33(6.14)	13.33 (21.14)	16.67 (23.84)	11.11 (17.04)	13.33 (21.14)	30.00 (33.20)	36.67 (37.21)	26.67 (30.52)	40.00 (39.22)	60.00 (50.75)	70.00 (56.77)	56.67 (48.98)
Sweet flag	40.00 (39.22)	66.67 (54.76)	86.67 (68.83)	64.45 (54.27)	76.67 (61.20)	80.00 (63.41)	96.67 (83.82)	84.44 (69.48)	96.67 (83.82)	100.00 (89.96)	100.00 (89.96)	98.89 (87.91)
Lemongrass	3.33 (6.14)	10.00 (18.43)	13.33 (21.14)	8.89 (15.24)	16.67 (23.77)	26.67 (30.98)	33.33 (35.21)	25.56 (30.01)	36.67 (37.21)	53.33 (46.90)	66.67 (54.76)	52.22 (46.29)
Eucalyptus	6.67	16.67	20.00	14.44	23.33	40.00	43.33	35.56	46.67	66.67	76.67	63.33

	(12.29)	(23.84)	(26.55)	(20.89)	(28.77)	(39.22)	(41.14)	(36.38)	(43.06)	(54.76)	(61.20)	(53.01)
Wild Marigold	0.00 (0.00)	3.33 (6.14)	6.67 (12.29)	3.33 (6.14)	6.67 (12.29)	20.00 (26.55)	30.00 (33.20)	18.89 (24.01)	30.00 (33.20)	46.67 (43.06)	53.33 (46.90)	43.33 (41.05)
Drek	0.00 (0.00)	6.67 (12.29)	10.00 (18.43)	5.56 (10.24)	10.00 (18.43)	23.33 (28.77)	26.67 (30.98)	20.00 (26.06)	33.33 (35.21)	50.00 (44.98)	56.67 (48.83)	46.67 (43.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.67 (12.29)	6.67 (12.29)	6.67 (12.29)	6.67 (12.29)
Mean	7.92 (10.28)	17.08 (20.39)	22.50 (25.26)	15.83 (15.43)	22.08 (24.86)	32.92 (32.91)	40.00 (38.56)	31.67 (32.11)	43.75 (41.84)	55.83 (49.43)	63.75 (54.29)	54.45 (48.52)

Treatment	***Mean Mortality (%) of beetles in the indicated days and doses**							
	Day							
	Day-10				**Day-15**			
	Dose (g/100g seed)							
	1	**3**	**5**	**Mean**	**1**	**3**	**5**	**Mean**
Neem	83.33 (66.12)	90.00 (71.54)	100.00 (89.96)	91.11 (75.87)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Camphor	73.33 (58.98)	80.00 (63.41)	96.67 (83.82)	83.33 (68.74)	96.67 (83.82)	100.00 (89.96)	100.00 (89.96)	98.89 (87.91)
Sweet flag	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Lemongrass	70.00 (56.77)	73.33 (58.98)	93.33 (77.68)	78.89 (64.48)	83.33 (66.12)	93.33 (77.68)	100.00 (89.96)	92.22 (77.92)
Eucalyptus	80.00 (63.41)	83.33 (66.12)	100.00 (89.96)	87.78 (73.16)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Wild Marigold	56.67 (48.83)	66.67 (54.76)	80.00 (63.41)	67.78 (55.67)	80.00 (63.41)	90.00 (74.97)	93.33 (77.68)	87.78 (72.02)
Drek	66.67 (54.76)	70.00 (56.97)	83.33 (66.62)	73.33 (59.38)	90.00 (71.54)	93.33 (77.68)	100.00 (89.96)	94.44 (79.73)
Control	30.00 (33.20)	30.00 (33.20)	30.00 (33.20)	30.00 (33.20)	63.33 (52.76)	63.33 (52.76)	63.33 (52.76)	63.33 (52.76)
Mean	70.00 (59.00)	74.17 (61.84)	85.42 (74.33)	76.53 (65.06)	89.17 (75.94)	92.50 (80.37)	94.58 (83.77)	92.08 (80.02)

*Mean of three replications
Figure in parenthesis are arc sine transformed values
CD (p=0.05)

Treatment × Day	:	(4.60)
Dose × Day	:	(2.82)
Day	:	(1.63)
Treatment × Dose × Day	:	(7.98)

Effect of plant products on oviposition

Data presented in Table 2 revealed that on day 7 minimum number of eggs (3.11 eggs/ 5 females) was laid by 5 pairs of *C. chinensis* in sweet flag plant powder followed by neem (5.89 eggs/5 females), eucalyptus (16.22 eggs/5 females), camphor (20.33 eggs/5 females), lemongrass (23.78 eggs/ 5 females), drek (26.34 eggs/5 females) and wild marigold (46.33 eggs/5 females), all the plant powders were statistically different from each other. Wild marigold was least effective (46.33 eggs/5 females) but was superior to untreated control (99.67 eggs/5 females). The best plant powders i.e. sweet flag at 5 g (0.67 eggs/ 5 females) differed significantly with its lower doses i.e. 3 and 1 g respectively. Overall the egg laying by 5 pairs of beetles were dose dependent as the dose increases the egg laying decreases (20.09 eggs/5 females at 5 g, 29.13 eggs/5 females at 3 g and 41.29 eggs/5 females at 1 g dose). Whereas 20-days after release on comparison of oviposition on treated seeds during first seven days with the egg laid in next 13 days reveals that there was no significant increase in oviposition in treated and control lot and indicating that what so ever oviposition has occurred, that remained to first week of adult life. In the present investigations, sweet flag rhizome powder treated pea seeds recorded minimum number of eggs followed by neem. In untreated pea seeds, increase in egg laying was negligible in 20 day observation, thus most of beetles have laid their eggs within a week of release and similar trend was noticed in treatments with plant powders. Sweet flag and neem was the best in preventing egg laying and other treatments were not as effective as sweet flag and neem. The reduction in egg laying by sweet flag and neem may be due to high toxicity of beta-asarone content of sweet flag and azadirachtin in neem, which may lead to reduction in oviposition of pulse beetle. The present investigation was in accordance with the findings of Shukla [24] who reported the bioefficacy of sweet flag powder with 100 per cent ovicidal activity and completely inhibiting adult emergence against pulse beetle infesting stored chickpea seeds. The results of present investigation corroborate the results of Gupta [25] who reported the efficacy of neem leaf powder in reducing oviposition against *C. maculatus* on green gram seeds. Similarly, Devi and Devi [26] reported the reduction in oviposition of *C. chinensis* in gram treated with neem powder.

Table 2: Effect of plant powders on oviposition by *C. chinensis* on treated pea seeds.

Treatment	Mean number of eggs laid/5 pairs of beetle at different days and dose							Mean
	Day							
	Day-7				Day-20			
	Dose (g/ 100g seed)							
	1	3	5	Mean	1	3	5	
Neem	10.67 (3.42)	5.33 (2.52)	1.67 (1.63)	5.89 (2.52)	13.33 (3.79)	7.00 (2.83)	3.33 (2.08)	7.89 (2.90)
Camphor	31.67 (5.72)	19.33 (4.51)	10.67 (3.42)	20.56 (4.55)	36.33 (6.11)	23.33 (4.93)	12.33 (3.65)	24.00 (4.90)
Sweet flag	6.33 (2.71)	2.33 (1.82)	0.67 (1.28)	3.11 (1.93)	7.67 (2.94)	3.67 (2.16)	1.67 (1.63)	4.33 (2.24)
Lemongrass	36.67 (6.14)	23.67 (4.93)	11.33 (3.51)	23.78 (4.86)	43.33 (6.66)	25.67 (5.16)	12.67 (3.70)	27.22 (5.17)
Eucalyptus	28.33 (5.42)	14.67 (3.95)	6.33 (2.71)	16.44 (4.03)	33.33 (5.86)	16.67 (4.20)	8.00 (3.00)	19.33 (4.35)
Wild Marigold	71.67 (8.52)	46.33 (6.88)	20.33 (4.62)	46.11 (6.67)	77.33 (8.85)	49.33 (7.10)	22.67 (4.86)	49.78 (6.94)
Drek	43.67 (6.68)	25.00 (5.10)	11.67 (3.56)	26.78 (5.11)	49.67 (7.12)	26.33 (5.23)	14.67 (3.96)	30.22 (5.43)
Control	97.00 (9.90)	97.00 (9.90)	97.00 (9.90)	97.00 (9.90)	109.33 (10.50)	109.33 (10.50)	109.33(10.50)	109.33 (10.50)
Mean	40.75 (6.06)	29.17 (4.95)	19.96 (3.83)	40.60 (4.95)	46.29 (6.48)	32.67 (5.26)	23.08 (4.17)	33.99 (5.30)

*Mean of three replications

Figure in parenthesis are $\sqrt{x+1}$ *transformed values*

CD (p=0.05)

Day : (0.03)
Treatment × Day : (0.08)
Dose × Day : (0.05)
Treatment × Dose × Day : (0.15)

Effect of treatment on progeny development

Data presented in Table 3 reveals that 60 days after release of 5 pairs of *C. chinensis* on pea seeds treated with plant powders, there was reduction in progeny produced by them. Minimum adult emergence (0.44 beetles) was recorded with sweet flag powder which was statistically different from rest of the plant powders. Neem treated pea seeds produced 1.66 beetles which was superior over rest of the plant powders. Seeds treated with camphor, lemongrass, eucalyptus, wild marigold and drek produced 15.89, 17.11, 5.44, 33.11 and 16.44 beetles. Progeny development significantly reduced from 31.83 beetles to 12.63 beetles with the increase in dose of plant powders. Sweet flag at 5 g and 3 g dose completely restricted adult emergence of beetles. Progeny developed (1.33 beetles) with sweet flag at 1 g dose was statistically at par with neem (1.33 beetles) at 3 g dose. Camphor and lemongrass at 1 g and wild marigold at 3 g were able to restrict progeny development to 31.67, 32.67 and 33.33 beetles and were statistically at par with each other. Maximum beetle production was observed with wild marigold at 1 g (57.33 beetles). In the present findings, number of adults emerged on sweet flag and neem leaf powder treated pea seeds was low (0.44 and 1.66 beetles/5 females). No adult emergence was observed in pea seeds treated with sweet flag powder at 3g/100g and 5g/100g dose. Neem at 5g/100g dose (0.33 beetles/ 5 females) was also effective in reducing adult emergence. The present findings also corroborate the findings of Govindan and Nelson [23] who reported lower adult emergence of *C. maculatus* in seeds treated with sweet flag powder. Similar to present findings Shukla [23] reported no adult emergence of *C. chinensis* in seeds treated with sweet flag powder. Neem leaf powder (*A. indica*) applied @ 1.5mg/100g seeds of mungbean decreased total progeny by 38 per cent [27].

Table 3: Effect of plant powders on number of beetles/5 pairs developed after 60-days of treatment of pea seeds at different doses of plant powders.

Treatment	Mean number of beetles/5 pair developed at indicated doses			Mean
	Dose (g/ 100g seed)			
	1	3	5	
Neem	3.33 (2.08)	1.33 (1.52)	0.33 (1.38)	1.66 (1.58)
Camphor	31.67 (5.72)	13.67 (3.83)	2.33 (1.82)	15.89 (3.78)
Sweet flag	1.33 (1.52)	0.00 (1.00)	0.00 (1.00)	0.44 (1.17)
Lemongrass	32.67 (5.80)	14 (3.87)	4.67 (2.38)	17.11 (4.02)
Eucalyptus	12.33 (3.64)	3.00 (1.99)	1.00 (1.38)	5.44 (2.34)
Wild Marigold	57.33 (7.64)	32.33 (5.77)	9.67 (3.26)	33.11 (5.56)
Drek	38.00 (6.24)	6.33 (2.71)	5.00 (2.41)	16.44 (3.79)
Control	78.00 (8.85)	78.00 (8.85)	78.00 (8.85)	78.00 (8.85)
Mean	31.83 (5.19)	18.58 (3.69)	12.63 (2.78)	21.04 (3.89)

*Mean of three replications

Figures in parenthesis are $\sqrt{x+1}$ transformed values

Treatment : (0.23)
Dose : (0.38)
Treatment × Dose : (0.66)

Conclusion

Among seven plant powders sweet flag resulted in 64.49,84.44 and 98.89 per cent mortality of the pulse beetle, *C. chinensis* at the highest dose (5 g/100 g seeds) after 1,3 and 7 days of treatment, respectively. Egg laying by females as well as progeny development and survival of *C. chinensis* was minimum on pea seeds treated with sweet flag.

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