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Biology of pulse beetle, *Callosobruchus maculatus* (F.) and its response to botanicals in stored pigeonpea, *Cajanus* cajan (L.) grains

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ABSTRACT

Biology of pulse beetle (*Callosobruchus maculatus* F.) was studied on pigeonpea grains during 2014-15 in laboratory conditions. The adult beetle was oval in shape and reddish-brown in colour, with dark stripes on each side of dorsal abdomen with average fecundity of 74.8±1.8 eggs per female. The average incubation period was 4.2±0.2 days with hatching 98.2±0.3 percent. Average larval-pupal period, oviposition, post-oviposition period, total life period and adult life span were 21.3±0.3, 8.2±0.5, 2.8±0.5, 33.3 ±2.4 and 12.0±2.1 days, respectively. The effect of nine botanicals *viz.*, *neem* oil, mustard oil, groundnut oil, turmeric powder and their mixture and surface protectants *viz.*, neem seed kernel powder, saw dust, sand, dung cake ash and wheat husk were used on pigeonpea seed against *Callosobruchus maculatus*. The highest mortality (84-100%) was observed by *neem* oil @ 10ml/kg and lowest (3.33%) by turmeric powder @ 3.5g/kg seeds, after 135 days of storage. *Neem* oil @ 10ml/kg was completely inhibited the oviposition, adult emergence and seed damage. All the oils and inert materials prevented egg laying, reduced population build up of beetles and minimized the seed damage as compare to control.

Key words: Biology, *Callosobruchus maculatus*, Pulse beetle, Pigeonpea, botanicals.

INTRODUCTION

Pulses can be stored for considerable periods of times after harvest but during storage they are attacked by store grain pest. The bruchid *Callosobruchus maculatus* (F.) is one of the major store grain pest of pulses capable of attacking wide range of legumes *viz*., green gram, black gram, chick pea and pigeonpea (Sharma, 1984) and causes 50 per cent damage during storage within 3 to 4 months (Caswell, 1981). Biology of pulse beetle in different pulses were reported by Chander and Ghosh (2006), Van Hius and De Rooy (1998), Creadland (1987) and Chandrakantha and Mathavans (1986). The post-harvest losses and quality deterioration caused by storage pests are major problems throughout the world. Although synthetic insecticides have been successfully used to protect stored grains pest infestations, their indiscriminate and frequent uses have created serious problems (Sighamony *et al*., 1980) like, residues in food grains (Fishwick, 1988), environmental pollution (WMO, 1995), and development of resistant strains (Yusof and Ho, 1992). Finding safe alternatives to synthetic insecticides to protect stored grains and grain products from insect infestations are highly desirable. Botanicals insecticides are more safe than synthetic insecticides. Recently, attention have been given by using of plant products or plant derived compounds as promising alternative to synthetic insecticides in controlling insect pests of stored products (Rajapakse and Ratnasekera, 2009). The present investigation was carried out on biology of pulse beetle, *Callosobruchus maculatus* and its response to botanicals on pigeonpea grains in laboratory at 29-30^{0}C and 65-70 per cent RH.

MATERIALS AND METHODS

To study the biology of *C. maculatus*, was conducted in the laboratory at the Department of Agriculture, OPJS University Churu, in completely randomized block design with 3 replications. For this purpose, *C. maculatus* (F.) was reared in the laboratory at room temperature (29±1^{0}C) for stock culture. Three plastic containers (each 9 x 4 cm) were taken and each was filled with 50 g conditioned grains. Five virgin pairs of newly emerged adults (0-24 h old) of *C. maculatus* were isolated from stock culture using key of sex differentiation (Raina, 1970) and introduced into each of the plastic containers. The mouth of the plastic container was wrapped with a muslin cloth to allow aeration and to prevent escape of the beetles. The data on different parameters *viz.*, fecundity, oviposition & post-oviposition period and adult longevity were recorded replication-wise. From each replication, 30 grains each with one freshly laid egg on it (others were removed with the help of a needle) were picked up and kept in glass vial. Observations were made daily for recording the incubation period, hatchability, larval-pupal period, adult emerged and total life period**.** Dead adults were removed daily.

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Eleven grain protectants, *viz*., *neem* oil @ 10ml/kg seed, mustard oil @ 7.5ml/kg seed, groundnut oil @ 7.5ml/kg seed, turmeric powder @ 3.5g/kg seed, mustard oil @ 3.75ml + turmeric powder @ 1.75g/kg seed, groundnut oil @ 3.75ml + turmeric powder @ 1.75g/kg seed, saw dust, sand, dung cake ash and wheat husk with 7cm covering, were used. Each of the grain protectants was mixed thoroughly in 750g of pigeonpea seeds (var. *Manak)* by shaking well manually for five minutes in a jar (2 kg capacity) and untreated seeds were taken as control. These treated seeds were used for conducting two experiments (500 numbers of grains for first and 250g for second). Each treatment was replicated thrice for each of the two experiments. Experiment1: From the above, 500 numbers of grains of treated seeds were taken in plastic jars (15 X 8 cm) under normal storage condition. Five pairs freshly emerged pulse beetle (0-24 h old) were released in each plastic jars. Mortality of adult and numbers of eggs laid by *C. maculatus* were recorded after 1, 45, 90 and 135 days of storage. Experiment 2: Ten pairs of freshly emerged pulse beetle (0-24 h old) were released in each plastic jars (2 kg capacity) containing 250g treated seeds with different protectants. Number of adult emerged and per cent seed damage were recorded after 45, 90 and 135 days by collecting a sample of 1000 number of seeds randomly from each replication. The statistical analysis was done as per procedure suggested by Sharma and Rathore (2006).

RESULTS AND DISCUSSION

Egg and larval period: The female beetle started egg laying after 24h of emergence. Maximum eggs were laid on first day of oviposition and it decreased subsequently with the passing of time. In general the egg laying lasted for eight days. The egg was small, shiny and oval to spindle-shaped, glued singly on the grain. Freshly laid eggs were transculant smooth, which became yellowish later on. Average fecundity was 74.8±1.8 eggs per female. Egg hatched within 4-5 days with an average of 4.2±0.2 days and viability was 98.2±0.3 per cent (Table 1). These results are supported the findings of Chandra and Ghosh (2006) and Varma and Anandhi (2010).

The larvae passed through at least four instars which are were creamy and somewhat C-shaped. The larval- pupal period of *C. maculatus* varied from 20 to 23 days with average of 21.3±0.3 days (Table 1). Chandra and Ghosh (2006) observed that larval-pupal period of *C. maculatus* on different pulses which are closely related to the present findings. The variations in larval-pupal period of *C. maculatus* on pulses reported by different scientists may be due to variation of temperature, relative humidity and deference of pulse used.

Total life, oviposition, post oviposition and adult period: The total life period was calculated from the day of oviposition to adult emergence. It was range from 27-39 days with an average of 33.3±2.4 days. The oviposition and post-oviposition period of *C. maculatus* ranged from 8-9 and 2-3 days with an average of 8.2±0.5 and 2.8±0.5 days, respectively (Table 1). Where, Varma and Anandhi (2010) observed that the post-oviposition period of *C. chinensis* was 2.2 days with a range of 1-3 days in green gram.

The adult beetle was oval in shape and reddish-brown in colour, with dark stripes on each side of dorsal abdomen. The adult male was smaller and more rounds shaped than the female. On an average, the adults lived for 12.0±2.1 (9-15) days. Similar results were reported by Varma and Anandhi (2010) that the adult longevity of *C. chinensis* was 11.0 days with a range of 9-12 days in green gram. The minor variations might be due to variation of temperature, relative humidity and different pulse used. On an average the adult emergence was 51.0±1.2 per cent with the male: female ratio was 1:0.86 (Table 1), which revealed that the males dominated numerically over females.

Adult mortality: All grain protectants except saw dust, wheat husk and *neem* seed kernel powder proved to be more effective for adult mortality of *C. maculatus* as compared to the control for storage intervals of 1, 45, 90, and 135 days (Table 2). After one day of storage, *neem* oil @ 10ml/kg was observed most effective grain protectant. The same trend was observed after 45, 90 and 135 days of storage than the other protectants. After 45 days of storage, maximum adult mortality (96.67%) was recorded in seeds treated with *neem*

Table 1: Biology of pulse beetle *Callosobruchus maculatus* under laboratory conditions.

Insect Stages	Range	Average ±SE
Fecundity/female (no. of eggs)*	72-78	74.8±1.8
Incubation period (days)**	4-5	4.2±0.2
Total hatchability of eggs (%) **	-	98.2±0.3
Larval-pupal period (days)**	20-23	21.3±0.3
Adult longevity (days)*	9-15	12.0±2.1
Oviposition period (days)*	8-9	8.2±0.5
Post-oviposition period (days)*	2-3	2.8±0.5
Total life period (days)**	27-39	33.3±2.4
Adult emergence (%) **	-	51.0±1.2
Sex ratio (Male: Female)**	-	1: 0.86

* n=15 ; **n=90

Table 2: Effect of grain protectants on mortality and egg laid by pulse beetle, *Callosobruchus maculatus* in pigeonpea, *Cajanus cajan* seeds at different storage intervals.

Treatment & Dosages	*Per cent Adult mortality at				#Average no. of eggs laid by 5 pairs/500 seeds at			
	1 (DAS)	45 (DAS)	90 (DAS)	135 (DAS)	1 (DAS)	45 (DAS)	90DAS	135DAS
Neem seed kernel powder	23.33	16.67	6.67	0.00	29.67	33.00	33.00	35.00
@ 20g/kg	(29.12)	(24.72)	(13.96)	(4.05)	(5.46)	(5.78)	(5.78)	(5.95)
Neem oil @ 10ml/kg	100.00	96.67	86.96	84.00	0.00	0.00	2.33	3.67
	(90.00)	(84.02)	(69.83)	(67.00)	(0.71)	(0.71)	(1.67)	(2.04)
Mustard oil @ 7.5ml/kg	26.67	23.33	16.67	13.33	7.67	12.33	13.678	14.33
	(31.32)	(29.12)	(24.72)	(21.58)	(2.84)	(3.57)	(3.75)	(3.83)
Groundnut oil @ 7.5ml/kg	20.66	13.33	6.66	6.66	9.67	11.67	12.00	13.00
	(27.00)	(21.58)	(13.96)	(13.96)	(3.18)	(3.46)	(3.52)	(3.66)
Turmeric powder @ 3.5g/kg	13.33	10.00	10.00	3.33	46.33	50.66	54.67	66.00
	(21.58)	(18.91)	(18.91)	(9.00)	(6.82)	(7.16)	(7.42)	(8.14)
Mustard oil @ 3.75ml/kg +	23.33	20.00	10.00	10.00	3.00	3.67	5.00	6.00
Turmeric powder @ 1.75g/kg	(29.12)	(26.45)	(18.91)	(18.91)	(1.86)	(2.04)	(2.63)	(2.30)
Groundnut oil @ 3.75ml/kg +	16.67	16.66	13.33	13.33	2.66	3.66	4.33	5.00
Turmeric powder @ 1.75g/kg	(24.72)	(24.72)	(21.58)	(21.58)	(1.77)	(2.04)	(2.63)	(2.30)
Saw dust 7 cm covering	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(4.05)	(4.05)	(4.05)	(4.05)	(0.71)	(0.71)	(0.71)	(0.71)
Sand 7 cm covering	13.33	6.67	3.33	3.33	0.00	0.00	0.00	0.00
	(21.58)	(13.96)	(9.00)	(9.00)	(5.75	(5.95)	(5.95)	(5.78)
Dung cake ash 7 cm covering	93.33	75.66	63.33	56.67	0.00	0.00	0.00	0.00
	(78.03)	(63.41)	(53.07)	(49.14)	(0.71)	(0.71)	(0.71)	(0.71)
Wheat husk 7 cm covering	6.66	3.33	3.33	3.33	33.00	35.00	35.00	38.00
	(13.96)	(9.00)	(9.00)	(9.00)	(5.78)	(5.95)	(5.95)	(5.78)
Untreated Control	0.00	0.00	0.00	0.00	101.33	127.66	128.67	131.33
	(4.05)	(4.05)	(4.05)	(4.05)	(10.08)	(11.31)	(11.36)	(11.47)
C.D. at 5 %	(0.98)	(8.94)	(8.37)	(7.08)	(0.63)	(0.58)	(0.69)	(0.47)

DAS = Days after storage; *Figures in the parenthesis are Angular transformed values; #Figures in the parenthesis are $\sqrt{n+1}$ tansformed values

oil @ 10ml/kg and proved most effective grain protectant. Whereas, the remaining treatments were found significantly inferior to above treatment. After 90 days of storage, same trend of adult mortality was observed in all the treatments. Wherein, adult mortality was recorded from 0 to 86.96 per cent in grain protectants as compared to 0.0 per cent in untreated control. After 135 days of storage, almost same trend was observed with a maximum (84.00%) adult mortality in *neem* oil @ 10ml/kg and minimum (0.0%) adult mortality was recorded in *neem* seed kernel powder and saw dust and it was at par with sand, wheat husk and turmeric powder (3.33%) followed by groundnut oil @ 7.5ml/kg, mustard oil @ 3.75ml + turmeric powder @ 1.75g/kg (10.00%), mustard oil @ 7.5ml and groundnut oil @ 3.75ml + turmeric powder @ 1.75g/kg (13.33%).

The results of the present study indicate that *neem* oil @ 10ml/kg and dung cake ash 7cm were most effective treatments on the basis of adult mortality and saw dust was the least effective (Table 2) compare to control. These findings are in conformity with that of Raghvani and Kapadia (2003); they reported 100 per cent adult mortality with *neem* oil at 10 ml/kg seed in pigeonpea against *C. maculatus* up to six months, where sesamum and groundnut oil @ 10ml/kg seeds gave good protection up to four months of storage. Bhargava and Meena (2002), reported that 76.8 and 73.4 per cent adult mortality of *C. chinensis* with mustard oil at 1ml/100 g and groundnut oil at 1ml/100g of cowpea seeds after 3 days of release of beetles. Where, Venkatasham *et al*. (2014); found that, after one month of storage groundnut oil at 5ml/kg and black pepper at 5gm/kg seed were best protectants.

Number of eggs laid: All the grain protectants except turmeric powder, wheat husk and *neem* seed kernel powder was observed significantly lesser number of eggs/500 seeds as compared to untreated control after 1, 45, 90 and 135 days of storage (Table 2). However, saw dust, sand, dung cake ash and *neem* oil @ 10ml/kg was found to be most effective group of grain protectants. After one day of storage, the minimum number of eggs (0 to 46.33/500 seeds) laid by 5 females in different treatments as compared to untreated control (101.33 eggs/500 seeds). Amongst the grain protectants, it was observed that the maximum (46.33) eggs were laid in the treatment with turmeric powder and minimum (0.0) eggs laid in treatments of *neem* oil @ 10ml/kg seeds which proved as the most effective treatment in reducing the number of eggs laid after one day of storage. After 45 days of storage, it was observed that the, minimum (0.0) eggs were found in saw dust, sand, dung cake ash and *neem*

oil @ 10ml/kg, which proved to be the most effective treatments than the other treatments. Whereas, after 90 days of storage, the number of eggs laid ranged from 0 to 54.67 eggs/500 seeds which differed significantly from untreated control (128.67 eggs/500 seeds). After 135 days of storage, a minimum number of (0 eggs) eggs were recorded in saw dust, sand and dung cake ash and these were followed by *neem* oil (3.67 eggs), groundnut oil + turmeric powder (5.00 eggs), mustard oil + turmeric powder (6.00 eggs) and these treatments proved to have anti-ovipositional effect. The maximum numbers of eggs (66.00 eggs) was in turmeric powder than other treatments.

The results of the present study indicate that on the basis of number of eggs laid per 5 females, seed treatment with *neem* oil, saw dust, sand and dung cake ash were equally and most effective and followed by turmeric powder and wheat husk (38.00 eggs) were the least effective (Table 2). Similar results in respect to *neem* oil, groundnut oil and mustard oil, were reported by Naik and Dumbre (1984) where minimum number of eggs laid by *C. maculatus* when cowpea seeds were treated with *neem* oil @ 1.0 per cent concentration as compared to groundnut oil @ 7.5 per cent and mustard oil @ 7.5 per cent. These results are also in line of Bhatnagar *et al*. (2001) observed that *neem* oil was most effective as oviposition deterrent as compared to groundnut and mustard oil. Similar findings were reported by Bhargava and Meena, 2002 with groundnut oil @ 0.1ml/kg seeds.

Number of adult emerged and seed damage: On the basis of number of adult emergence and per cent seed damage, all the grain protectants except *neem* seed kernel powder, turmeric powder, and wheat husk were highly effective and no adult were emerged from sand, dung cake ash and *neem* oil treated seeds after a storage intervals of 45, 90 and 135 days (Table 3). The next order of grain protectants was recorded as saw dust, mustard oil + turmeric powder, mustard oil and groundnut oil, respectively. *Neem* seed kernel powder, wheat husk and turmeric powder were least effective as compared to other grain protectants after 45, 90 and 135 days of storage. After 45 days of storage, no adult were emerged from saw dust, sand and dung cake ash, *neem* oil and mustard oil + turmeric powder and per cent seed damage were significantly better than all other treatments. The maximum number of adult (18.33 adults/1000 grains) and maximum per cent seed damage (10.10%) was observed in the treatment of wheat husk (7cm covering). The same trend was observed after 90 and 135 days of storage.

The results of the present study revealed that treatments with different grain protectants had significant effect on emergence of *C. maculatus* adults. All the

Table 3: Effect of grain protectants on adult emergence and per cent seed damage by *Callosobruchus maculatus* in pigeonpea, *Cajanus cajan* grains at different storage intervals.

Treatment & Dosages	*Number of adult emerged/ 1000 grains			#Per cent Seed damage at		
	45 (DAS)	90 (DAS)	135 (DAS)	45 (DAS)	90 (DAS)	135 (DAS)
Neem seed kernel powder @ 20g/kg	6.00	65.67	77.33	0.33	4.53	7.20
	(2.91)	(8.13)	(8.81)	(5.23)	(12.96)	(16.02)
Neem oil @ 10ml/kg	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(4.05)	(4.05)	(4.05)
Mustard oil @ 7.5ml/kg	1.67	2.33	3.00	0.17	0.33	1.43
	(1.39)	(1.68)	(1.86)	(5.68)	(5.23)	(7.97)
Groundnut oil @ 7.5ml/kg	3.67	4.00	4.67	0.20	0.23	0.93
	(2.03)	(2.22)	(2.26)	(4.79)	(4.91)	(6.87)
Turmeric powder @ 3.5g/kg	4.00	10.33	17.33	0.27	1.53	2.13
	(2.22)	(3.29)	(4.21)	(5.01)	(8.19)	(9.34)
Mustard oil @ 3.75ml/kg + Turmeric	0.00	3.00	6.00	0.00	0.00	0.03
powder @ 1.75g/kg	(0.71)	(1.86)	(2.91)	(4.05)	(4.05)	(4.18)
Groundnut oil @ 3.75ml/kg + Turmeric	1.67	4.67	10.33	0.13	0.27	0.73
powder @ 1.75g/kg	(1.39)	(2.26)	(3.29)	(4.56)	(5.01)	(6.37)
Saw dust 7 cm covering	0.00	0.00	0.33	0.00	0.00	0.00
	(0.71)	(0.71)	(0.88)	(4.05)	(4.05)	(4.05)
Sand 7 cm covering	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(4.05)	(4.05)	(4.05)
Dung cake ash 7 cm covering	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(4.05)	(4.05)	(0.71)	(4.05)
Wheat husk 7 cm covering	18.33	77.33	105.67	2.13	6.10	10.10
	(4.33)	(8.81)	(10.30)	(9.34)	(14.88)	(18.99)
Untreated Control	65.67	103.66	192.00	6.10	19.03	23.36
	(8.13)	(10.10)	(13.89)	(14.88)	(26.33)	(32.44)
C.D. at 5 %	(0.96)	(0.77)	(0.69)	(0.65)	(0.87)	(0.98)

DAS = Days after storage; #Figures in the parenthesis are Angular transformed values; *Figures in the parenthesis are $\sqrt{n+1}$ transformed values.

treatments were significantly better than the control. Saw dust, sand, dung cake ash, *neem* oil, mustard oil and groundnut oil resulted in lesser adult emergence, where as groundnut oil + turmeric powder and mustard oil + turmeric powder were also equieffective and it was maximum in untreated control. The results of the present investigation with *neem* oil and sand are similar to Ramangoudar *et al*. (2000) who reported that no adults of *C. chinensis* emerged and zero per cent seed damage when horse gram (*Macrotyloma uniflorum* Lam. verde) seeds were treated with *neem* oil @ 5ml/kg and sandy soil as 2.5 cm thick layer over seeds after 4 months of treatments. The results in respect of *neem* oil are also similar to those of Bhatnagar *et al*. (2001). Similar results also found by Ratnasekera and Rajapakse (2012) who reported reduction of adult emergence (11.2, 3.2 and 2.1%) and seed damage as increasing doses of *neem* oil @ 0.5, 1.5 and 3.0ml/kg seeds of cowpea. Similar results was also found by Lal and Raj (2012), who reported that the adult emergence and seed damage was zero per cent in the treatment with *neem* oil @ 3ml/kg pigeonpea seeds. Venkatasham *et al*. (2014); found that, even after three month of storage groundnut oil @ 5ml/kg and black pepper @ 5gm/kg seed showed zero per cent damage and weight loss proving it to be best protectant, followed by mustard oil.

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