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Vulnerability of popular mungbean varieties of South India to the predominant pulse storage pest, *Callosobruchus maculatus* (F.)

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Abstract
Mungbean serves as an excellent source of high quality protein in the form of dry edible seeds and fresh sprouts. The most devastating primary storage pest of mungbean is bruchids that make the seeds unsuitable for consumption and agricultural uses. Studies were carried out on the biology and extent of damage caused by a pair of *Callosobruchus maculatus* in five mungbean varieties *viz.,* CO 6, CO 7, CO 8, VBN 2 and VBN 3 under laboratory conditions which almost resembled the storage environment. Significant differences were observed among the varieties in fecundity, adult emergence, mean developmental period, seed damage and weight loss. Based on the percent adult emergence and mean developmental period, CO 7, CO 8 and VBN 3 were categorized as susceptible varieties and CO 6 and VBN 2 were categorized as highly susceptible varieties. Number of eggs laid per female varied from 54.4 – 69.0 eggs with significantly lower hatching percentage in VBN 3. Adult emergence was also significantly lower in VBN 3 (94.31%) with significantly higher mean developmental period (25.11 days) and low female to male ratio of 0.78. Percent seed damage was lowest in CO 8 (51.8%) and percent weight loss is lowest in VBN 3 (37.13%). These observations would be helpful in choosing desirable donor in back cross breeding programme to design a resistant genotype for pulse beetle, *C. maculatus.*

Keywords: *Callosobruchus maculatus*, biology, mungbean, susceptible, damage

Introduction
Pulses are the main protein source of an Indian diet. In India, pulses are grown on an area of around 29.99 million hectares with an annual production of 25.23 million tonnes. About 2-3 million tonnes of pulses are imported annually to meet the domestic consumption requirement. The average productivity of the country and the world are 841 and 1023 kg/ha, respectively (MoA & FW, GOI, 2018) [13]. The main reason for this huge gap in yield is that 87% of the country's pulses are grown under rainfed condition and farmers are not affordable to invest plant protection inputs to the crop. As reported by Mannan and Tarannum (2011) [12], storage pests accounted for 26.3% yield loss in pulses, being one of the major biotic constraints. Among storage pests, the bruchids (*Callosobruchus* spp.) play a major role in reducing the economic value of the pulses. Among the various species of bruchids, cowpea weevil (*Callosobruchus maculatus* (F.)) and adzuki bean weevil (*Callosobruchus chinensis* (Linn.)) are the most destructive during transportation and storage (Talekar 1988) [28]. Even if the initial infestation is little, the population will mushroom when seeds are stored for several months because of the short life cycle and high reproductive rate of the pest.
Among pulses, mungbean (*Vigna radiata*) is of special mention, being a self-pollinated diploid legume, widely cultivated in tropical, subtropical and temperate zones of Asia. Matured raw mungbean seeds are highly nutritive with calcium, magnesium, phosphorus, potassium, sodium, zinc and vitamins in addition to the high protein content of 24%. The dried mungbean seeds can be consumed after cooking or as fresh sprouts or can be converted into flour. In mungbean, both *C. maculatus* and *C. chinensis* caused 7–73% yield loss, depending upon the genotype and the morphological and biochemical attributes of the seeds (Sarwar, 2012) [20]. Despite being a serious pest in stored pulses, little is known about their biology and its damage potential in the existing popular varieties of pulses. Therefore a study on the biology and the extent of damage caused by pulse beetle on the ruling varieties of mungbean in Tamil Nadu

was conducted so that a cost effective pest management strategy can be devised to safeguard the stored pulse against this pest. The data thus generated would give an understanding in choosing the desirable parent in breeding programme meant for designing a resistant genotype of mungbean against *C. maculatus.*

Materials and Methods

Investigation on comparative biology of pulse beetle *C. maculatus* and the extent of damage on different varieties of mungbean was carried out at Agricultural Research Station, Bhavanisagar during the year 2018-19.

The culture of the test insect was maintained in the storage laboratory, Agricultural Research Station, Bhavanisagar in plastic containers (20 × 10 cm), kept in a BOD-incubator maintained at 27 ± 1°C temperature and 65 ± 5 per cent relative humidity. To raise the culture in laboratory, bruchids adults were collected from the local grain market and species of *C. maculatus* was carefully separated by using stereo zoom microscope on the basis of morphological traits *viz.*, antennal type, male and female being serrate in *C. maculatus* and male being pectinate and female serrate in *C. chinensis*. Adult male and female of *C. maculatus* were differentiated by means of readily observable morphological traits *viz*., size and shape of the abdomen. The abdomen of males was shorter with the dorsal side of terminal segments curved sharply downwards when compared with females. Also the posterior dorsal abdomen of females consisted of dark stripes on each side that is not present in males (Beck *et al*., 2013) [3]. The adults were then maintained using 100 g disinfected mungbean seeds.

The experiments were carried out using five varieties of mungbean *viz*., CO 6, CO 7, CO 8, VBN 2 and VBN 3. One pair of freshly emerged adults of *C. maculatus* was taken out from stock culture and were released in a plastic jar containing 100 mungbean seeds and replicated five times for each genotype. The jars were covered with muslin cloth on the top and tied with rubber bands. The jars were suitably labeled and kept in BOD-incubator at a temperature of 27±1ºC and 65±5 relative humidity. The infested seeds were examined daily under stereozoom microscope. The following observations *viz.*, duration of egg, larval and pupal period and emergence of adults were recorded following the procedures enumerated by Singh and Pandey (2001) [24]. The duration of egg-stage was arrived based on the transparency of egg shell. Appearance of black spot on the egg indicated that the eggs were about to hatch. The whitish appearance of the egg shell (due to the deposition of frass by the hatched grub while entering inside the seed) indicated the successful grub penetration into the seed. The appearance of capped exit hole (window of dark spot) on the seed surface indicated the transformation of grub to pupal stage. Twenty days after insect introduction, the number of damaged seeds was recorded daily. The seeds of the varieties with exit holes after adult emergence were categorized as damaged whereas varieties without exit holes were categorized as undamaged seeds. The observation were recorded based on the following biological and damage assessment parameters

Biological parameters

1. **Number of eggs laid:** Total number of eggs laid were counted in each genotype for seven days after adult release.
2. **Egg period:** Each egg was observed daily for egg hatch indicated by the change of colour of eggs from translucent to white and were marked as hatched for two weeks.
3. **Hatching percentage:** Number of eggs hatched/ number of eggs laid × 100
4. **Grub period:** Each seed was observed daily after egg hatch up to the formation of exit hole on the seed coat indicated by a dark circular spot.
5. **Pupal period:** Each seed was observed daily for adult emergence till the number of adults equaled number of grubs pupated or till five weeks whichever is earlier and the period (days) required for the pupa to emerge as adults was calculated.
6. **Number of adults emerged:** Total number of adults emerged were counted for five weeks.
7. **Adult emergence percentage:** (Number of adults emerged/ number of eggs laid) × 100
8. **Female to male ratio:** Number of females / Number of males
9. **Mean developmental period (Howe, 1971) [7]:** (MDP = $D_1A_1 + D_2A_2 + D_3A_3 + \ldots\ldots + D_n A_n$ /Total number of adults emerged; where D_1 = day at which the adults started emerging (1st day) and A_1 = number of adults emerged on D_1^{th} day)
10. **Susceptibility Index:** [log (percent adult emergence) / mean developmental period] × 100

Table 1: Resistance category based on susceptibility index (Howe, 1971[7] and Sulehrie *et al*., 2003) [27]

Category	Susceptibility index
Resistant	<0.05
Moderately resistant	0.051-0.060
Moderately susceptible	0.061-0.070
Susceptible	0.071-0.080
Highly susceptible	>0.081

Damage assessment parameters

- Percent weight loss (Khattak *et al*., 1987) [11]: [(Initial weight – Final weight / Initial weight] × 100
- Percent seed damage: (Number of seeds damaged/ Number of seeds taken) × 100
- The data on the biology of *C. maculatus* in different varieties of mungbean were subjected to square root transformation in case of number values and angular transformation in case of percent values and analyzed by adopting Completely Randomized Design as suggested by Panse and Sukhatme (1985) [14]. Analysis of variance (ANOVA) was carried out using AGRES package. The treatment means were compared using Least Square Deviation (LSD) at p = 0.05 level of significance.

Results and Discussion

To know the level of resistance against pulse beetle in the ruling varieties of mungbean *viz*., CO 6, CO 7, CO 8, VBN 2 and VBN 3, no choice test was performed using the laboratoryculture of *C. maculatus.* The biological parameters *viz*., fecundity, egg period, hatching percentage, grub period, pupal period, adult emergence percentage and male to female ratio were recorded for the calculation of resistance parameters such as mean development period and susceptibility index. Based on the susceptibity index, the varieties were categorized as susceptible and highly susceptible. Damage assessment parameters *viz*., number of seeds damaged (with holes), initial seed weight, seed weight after adult emergence were observed to calculate the percent seed damage and percent weight loss.

Fecundity

The number of eggs laid by a single female for six days on five different varieties of mungbean is presented in Table 1. Maximum number of eggs was laid on first day of adult release in CO 6 and VBN 2 while on second day in other varieties *viz.,* CO 7, CO 8 and VBN 3. Number of eggs laid diminished on subsequent days with no eggs being laid on 6th day in CO 6 and 7th day on other varieties. Similar results were obtained by Bashir *et al*. (2014) [2] where highest fecundity of *C. maculatus* was observed on 1st and 2nd days of adult release. Kazemi *et al.* (2009) [10] also reported maximum number of eggs on first day of oviposition of *C.maculatus*.

C. maculatus invariably laid eggs on all the five varieties of mugbean. The total number of eggs laid by a single female of *C. maculatus* ranged from 54.4 to 69.0 (Table 1). Maximum number of eggs was laid in seeds of VBN 2 (69.0) which was significantly higher than other varieties. Least number of eggs was laid in CO 8 (54.4) which was on par with CO 6 (54.6) but significantly lower when compared with other varieties. The other varieties *viz.,* CO 7 and VBN 3 showed a total fecundity of 64.2 and 59.8 per female respectively. Thanthianga and Mitchell (1990) [29] reported fecundity of 69-76 eggs in beans by a single unfed female of *C. maculatus* whereas 102-125 eggs by a female fed with sucrose and honey. Kavitha *et al.* (2018) [9] reported fecundity ranging from 14.00 to 73.17 eggs in resistant and susceptible mungbean accessions infested with two pairs of *C. chinensis*. Hence it is inferred that the female which was capable of laying more than hundred eggs when additional nutrition was supplied, laid only around half of the full potential under laboratory conditions depending on the type of seed material available for oviposition

Table 2: Fecundity of *C. maculatus* on different varieties of mungbean

S. No.	Varieties	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Total fecundity
1.	CO 6	20.8 ± 1.59^{b}	19.8 ± 1.83^{b}	5.8 ± 0.58^{e}	6.2 ± 0.66^{c}	2.0 ± 0.71^{d}	-	54.6± 0.20^{d}
2.	CO 7	4.2 ± 0.59^{d}	23.2 ± 2.08^{a}	16.2 ± 0.80^{a}	13.0 ± 1.41^{a}	5.8 ± 0.80^{c}	1.8 ± 0.58^{b}	64.2 ± 0.63^{b}
3.	CO 8	7.0 ± 0.55^{c}	23.2 ± 2.96^{a}	7.0 ± 0.55^{d}	7.0 ± 0.71^{b}	8.8 ± 0.58^{b}	1.4 ± 0.51^{c}	54.4 ± 0.64^{d}
4.	VBN 2	25.8 ± 2.87^{a}	16.0 ± 1.41^{c}	8.8 ± 0.86^{c}	5.2 ± 0.58^{d}	9.4 ± 0.75^{b}	3.8 ± 0.20^{a}	69.0 ± 0.30^{a}
5.	VBN 3	7.00 ± 0.71^{c}	19.2 ± 1.46^{b}	11.4 ± 1.33^{b}	6.00 ± 0.54^{c}	12.2 ± 0.58^{a}	4.0 ± 0.32^{a}	59.8 ± 0.52^{c}

Values are mean ± SE of five replicates; Mean values followed by different letters in same column are significantly different at 5% level by LSD

Egg period

Egg deposited on CO 7, VBN 2 and VBN 3 took 2.6 days to hatch whereas those deposited on CO 6 and CO 8 took 2.4 days (Table 2). Radha and Susheela (2014) [18] reported three days incubation period for *C. maculatus* in mungbean, cowpea and blackgram. Similar observation was also reported by Sarkar and Bhattacharyya (2015) [19] in mungbean infested with *C. chinensis* where the incubation period ranged from 2.11 to 5.30 days.

Hatching percentage

Egg hatch was identified by daily observing the difference in the colour of eggs from translucent to white. CO 6 exhibited highest hatching percentage of 99.32% and was found to be on par with VBN 2 (98.74%) and CO 7 (97.61%) (Table 2). VBN 3 exhibited the lowest hatching percentage of 96.57% and was on par with CO 8 (96.98%). Radha and Susheela (2014) [18] and Sharma *et al.* (2016) [23] recorded 97.25% and 98.1% hatchability of *C. maculatus* in mungbean respectively. Hatching percentage ranged from 80-100% as reported by Sarkar and Bhattacharyya (2015) [19] in mungbean infested with *C. chinensis.* In a crowded population, all the eggs laid by the pest would not hatch, mainly due to competition created by population density (multiple eggs per seed) and even if hatched would result in developmental malformation. In the present study due to more availability of seeds the pest laid almost one egg per seed hence the competition was avoided and the hatching percentage was above 90%.

Grub and pupal period

The grub period was calculated in days from egg hatch to the appearance of black circular translucent exit hole carved out by the pupating grub visible in the outer skin of the seed which indicated the formation of pupa. The pupal period was worked out in days from the day of appearance of exit hole to the day of adult emergence. The maximum grub period of 14.6 days was recorded from CO 7 and VBN 3 which was significantly more when compared with CO 8 (14.2 days) (Table 2). CO 6 exhibited the minimum grub period of 13.6 days which was on par with VBN 2 (13.8 days). The maximum pupal period was observed in CO 8 (7 days) followed by CO 7 (6.8 days), VBN 3 (6.8 days) and VBN 2 (6.6 days). CO 6 exhibited significantly lower pupal period of 6.4 days. Similar results were obtained by Devi and Devi (2014) [5] who reported

5-7 days of pupal period in *C. maculatus* on mungbean. Sharma *et al*. (2016) [23] reported the

grub-pupal period of *C. maculatus* as 19-21 days on mungbean. Grub and pupal period of

18 days in *C. maculatus* was reported by Radha and Susheela (2014) [18]. Patel *et al*. (2005) [15], Varma and Anandhi (2010) [30], Sarkar and Bhattacharyya (2015) [19] and Hosamani *et al*. (2018) [6] also reported a grub + pupal period of 17 – 19 days, 13 – 20 days, 15 – 20 days and 20 days respectively in mungbean by *C. chinensis.*

Adult emergence percentage

The adult emergence percentage in CO 6 (98.17%) was highest among the varieties followed by VBN 2 (97.10%), CO 7 (96.57%) and CO 8 (95.22%) (Table 2). VBN 3 (94.31%) recorded the lowest adult emergence percentage of 94.31% and was on par with CO 8 (95.22%). Kashiwaba *et al*. (2003) [8] reported 90% adult emergence in mungbean infested with *C. analis*. The adult

emergence percentage of 69.23, 84.5 and 21.34-84.05 were reported by Bashir *et al*. (2014) [2], Radha and Susheela (2014) [18] and Soumia *et al*. (2017) [26] respectively in mungbean infested with *C. maculatus*. The adult emergence percentage ranging from 74.3 to 90.4 was reported by Ponnusamy *et al*. (2014) [17] in mungbean infested with *C. chinensis*. Chakraborty and Mondal (2016) [4] reported the maximum adult (*C. chinensis*) emergence (68.3%) during April-May and minimum (25.6%) during Aug-Sep and stated that temperature and relative humidity played a major role in determining the length of life cycle of pulse beetle.

Mean developmental period

Significantly minimum mean developmental period was exhibited by the eggs deposited in CO 6 (24.02 days) and was on par with VBN 2 (24.25 days) (Table 2). *C. maculatus* eggs took 25.11 days to emerge as adults in VBN 3 which was on par with those laid in CO 7 (24.97 days) and CO 8 (24.83 days). Similar results with mean development period ranging from 21-32.21 days were reported for *C. maculatus* in mungbean by Radha and Susheela (2014) [18] and Soumia *et al*. (2017) [26], in cowpea by Verma *et al*. (2018) [31], for *C. analis* by Soumia *et al*. (2015) [25] and for *C. chinensis* by Ponnusamy *et al*. (2014) [17] and Pawara *et al*. (2019) [16]. Sekar and Nalini (2017) [21] reported higher mean developmental period ranging from 32.50 days to 36 gays for *C. chinensis* in mungbean varieties CO 6, CO 7, CO 8 and VBN 2 and lowest of 24.50 days in VBN 3. The delay in the life cycle of bruchids among the same host material might be due to the temperature variant of the culture environment as enumerated by Ponnusamy *et al*. (2014) [17] and Verma *et al*. (2018) [31] where the life cycle got prolonged for 60-85 days below 20 ℃ and no development at or above 42 ℃.

Susceptibility index

Susceptibility index worked out based on mean developmental period and percentage of adult emergence ranged from 0.079-0.083 (Table 2). CO 7, CO 8 and VBN 3 with susceptibility index of 0.079, 0.080 and 0.079 respectively came under susceptible category and CO 6 and VBN 2 with susceptibility index of 0.083 and 0.082, respectively, came under highly susceptible category. Susceptibility index for different genotypes infested with bruchids were reported by Ponnusamy *et al*. (2014) [17], Badii *et al*. (2013) [1] and Soumia *et al*. (2015; 2017) [25; 26]. Sekar and Nalini (2017) [21] reported mungbean variety VBN 3 as susceptible and CO 6, CO7, CO 8 and VBN 2 as moderately susceptible to *C. chinensis.*

Female to male ratio

The maximum female to male ratio of 0.84 was observed in CO 6 and CO 8 followed by 0.81 in CO 7 and VBN2 whereas the minimum ratio of 0.78 was recorded in VBN 3 (Table 2). Lesser number of females was reported by Chakraborty and Mondal (2016) [4] than males of *C. chinensis* (0.96) and *C. maculatus* (0.67) by Sharma *et al*. (2016) [23]. Bashir *et al*. (2014) [2] also reported a male to female ratio of 2:1 for pulse beetle reared in mungbean.

Table 3: Growth and developmental parameters of *Callosobruchus maculatus* on different varieties of mungbean

S. No.	Variety	No. of eggs laid in 100 seeds	Egg period (days)	Hatching %	Grub period (days)	Pupal period (days)	No. of adults emerged	Adult emergence (%)	Mean develop-mental period (days)	No. of adults emerged		Female to male ratio	Susceptibility Index	Resistance class
										Male	Female			
1.	CO 6	54.6± 0.20^{d}	2.4 ± 0.32^{b}	99.32^{a}	13.6 ± 0.20^{c}	6.4 ± 0.32^{d}	53.6 ± 0.42^{d}	98.17^{a}	24.02 ± 0.22^{b}	29.2 ± 0.33^{d}	24.4 ± 0.12^{d}	0.083^{a}	0.84^{a}	HS
2.	CO 7	64.2 ±0.63^{b}	2.6 ± 0.45^{a}	97.61^{abc}	14.6 ± 0.32^{a}	6.8 ± 0.45^{b}	62.0 ± 0.58^{b}	96.57^{ab}	24.97 ± 0.17^{a}	34.2 ± 0.08^{b}	27.8 ± 0.23^{b}	0.079^{b}	0.81^{b}	S
3.	CO 8	54.4 ±0.64^{d}	2.4 ± 0.32^{b}	96.98^{bc}	14.2 ± 0.37^{b}	7.0 ± 0.32^{a}	51.8 ± 0.17^{e}	95.22^{b}	24.83 ± 0.13^{a}	28.2 ± 0.32^{e}	23.6 ± 0.28^{e}	0.080^{b}	0.84^{a}	S
4.	VBN 2	69.0 ±0.30^{a}	2.6 ± 0.20^{a}	98.74^{ab}	13.8 ± 0.37^{c}	6.6 ± 0.45^{c}	66.8 ± 0.27^{a}	97.10^{ab}	24.25 ± 0.04^{b}	37.0 ± 0.05^{a}	29.8 ± 0.07^{a}	0.082^{a}	0.81^{b}	HS
5.	VBN 3	59.8 ±0.52^{c}	2.6 ± 0.37^{a}	96.57^{c}	14.6 ± 0.45^{a}	6.8 ± 0.32^{b}	56.4 ± 0.45^{c}	94.31^{b}	25.11 ± 0.12^{a}	31.6 ± 0.10^{c}	24.8 ± 0.11^{c}	0.079^{b}	0.78^{c}	S
	Mean	60.45	2.52	97.91	14.15	6.72	58.22	96.29	24.65	32.05	26.08	0.08	0.82	-
	S Ed	0.59	0.02	0.96	0.13	0.08	0.44	1.25	0.23	0.38	0.14	0.001	0.01	-
	CD (p=0.05)	1.26	0.05	2.04	0.29	0.16	0.94	2.64	0.49	0.80	0.29	0.001	0.02	-

HS = Highly susceptible; S = Susceptible

Values are mean ± SE of five replicates; Mean values followed by different letters in same column are significantly different at 5% level by LSD

Table 4: Seed damage caused by *Callasobruchus maculatus* on different varieties of mungbean

S. No.	Variety	Initial weight of 100 seeds (g)	% of seed damage	Final weight of seeds (g)	% weight loss of seeds
1.	CO 6	3.83 ± 0.03^{c}	53.6 ± 0.42^{d}	2.35 ± 0.04^{b}	38.64 ± 0.05^{b}
2.	CO 7	4.25 ± 0.05^{a}	62.0 ± 0.58^{b}	2.54 ± 0.05^{a}	40.24 ± 0.03^{a}
3.	CO 8	3.71 ± 0.04^{d}	51.8 ± 0.17^{e}	2.32 ± 0.04^{b}	37.80 ± 0.05^{b}
4.	VBN 2	3.73 ± 0.06^{d}	66.8 ± 0.27^{a}	2.20 ± 0.04^{c}	41.02 ± 0.04^{a}
5.	VBN 3	4.04 ± 0.05^{b}	56.4 ± 0.45^{c}	2.54 ± 0.04^{a}	37.13 ± 0.06^{c}
	Mean	3.91	58.22	2.39	38.98
	S Ed	0.04	0.44	0.03	0.53
	CD (p=0.05)	0.07	0.94	0.06	1.13

Values are mean ± SE of five replicates; Mean values followed by different letters in same column are significantly different at 5% level by LSD

Seed damage

Seed damage inflicted by the progeny of a pair of adults in mungbean varieties ranged from 51.8 to 66.8% (Table 3). CO 8 exhibited the lowest damage of 51.8% and was significantly lower than CO 6 (53.6%), VBN 3 (56.4%), CO 7 (62%) and VBN 2 (66.8%). Percent weight loss of seeds ranged from 37.13 to 41.02%. The lowest weight loss percent observed in VBN 3 (37.13%) followed by CO 8 (37.80%), CO 6 (38.64%), CO 7 (40.24%) and VBN 2 (41.02%), all being significantly different from each other. Seram *et al*. (2016) [22] reported 91.2% seed damage in 50 seeds inflicted by one pair of *C. maculatus.* This is in correspondence with the results of the present investigation where observations were made on 100 seeds infested by a single pair of *C. maculatus.*

Conclusion

The studies on the biology and extent of damage of *C. maculatus* on mungbean varieties CO 6, CO 7, CO 8, VBN 2 and VBN 3 revealed that all the varieties are susceptible to the pest with difference in the intensity of susceptibility. VBN 3, CO 8 and CO 7 were identified as susceptible with a comparatively and significantly lower hatching percentage, adult emergence percentage, weight loss percentage and significantly higher mean developmental period. The other varieties CO 6 and VBN 2 were found to fall in highly susceptible category with significantly higher values for the above parameters expect mean developmental period which was lesser. These inferences might be helpful in the selection of the suitable parent used for backcrossing in developing a recombinant genotype with both the desirable characters of growth, yield and consumer preference and also with resistance against the most prevalent and economically important pest of pulse.

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