Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(5): 975-980

Received: 18-07-2018
Accepted: 19-08-2018

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Development of pulse bruchid, *Callosobruchus chinensis* (L.), on different genotypes of greengram under no choice storage conditions

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Abstract
A total of 12 greengram genotypes were screened for resistance to pulse bruchid, *Callosobruchus chinensis*, under no-choice artificial infestation conditions. There were significant differences among the genotypes in number of eggs laid, adult emergence, seed weight loss, development period and the growth index of *C. chinensis* on greengram. The number of eggs laid, adult emergence by pulse bruchid and seed weight loss after artificial infestation was initially low but gradually increased with the increase in storage period. The pooled mean data over 8 months showed that PM-5 was found comparatively resistant to the pulse bruchid as the number of eggs laid (14.00 / 100 seed), adult emergence (17.44%) and weight loss (0.98%) were significantly less when compared to the other genotypes. WGG-42 recorded a significantly higher number of eggs (73.17 / 100 seed), adult emergence (63.20%) and weight loss (29.21%). The development period of pulse beetle on PM-5 (33.83 days) was also longer compared to WGG-42 (25.81 days) indicating the presence of some antibiotic constituents. Of the 12 genotypes screened, five genotypes were categorized based on growth index as resistant, one as moderately susceptible and the remaining six as highly susceptible to *C. chinensis.*

Keywords: Greengram, pulse bruchid, development, no choice test, artificial storage

1. Introduction
Pulses are the important sources of nutrients such as carbohydrates, proteins, fats and vitamins [5]. Among the pulses, *Vigna radiata* (L.) Wilczek is the third most important pulse crop cultivated throughout India. About 70 per cent of the world's production of greengram is from India, which is cultivated annually in an area of 3.83 million hectares with a total production and average productivity of 1.60 million tonnes and 418 kg ha^{-1}, respectively. The total area in Andhra Pradesh is 2.12 lakh hectares contributing to an annual production of 1.37 lakh tonnes with an average productivity of 646 kg ha^{-1} [2].
The most common and destructive pest of greengram in storage is pulse bruchid, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). It is a field-to-store pest as its infestation on pulses often begins in the field itself as adults lay eggs on mature pods [8] and when such seed is harvested and stored, the pest population increases rapidly and results in total destruction within a short period of 3-4 months [15]. The infestation causes considerable losses both in terms of quality as well as quantity. Both the grubs and adults cause damage and endosperm is eaten by the grubs leaving only the thin outer covering or thin film of seed coat. But the relative preference of bruchid varies from variety to variety. Some are highly susceptible while some others are resistant due to some inherent biochemical and physical factors. Keeping the above facts in view, the present study was carried out on the development of *C. chinensis* under no-choice storage conditions on different genotypes of greengram.

2. Material and Methods
The present study was conducted under laboratory conditions at Regional Agricultural Research Station (RARS), Lam, Guntur and Department of Seed Science and Technology, Advanced Post Graduate Centre, Lam, Guntur for a period of 8 months from August, 2017 to March, 2018. Twelve greengram genotypes *i.e.*, GGG-1, LGG-407, LGG-450, LGG-460, LGG-574, LGG-586, LGG-595, LGG-607, LGG-610, PM-5, TM-92-2 and WGG-42 collected from the Regional Agricultural Research Station, Lam, Guntur were used in the study.

Infestation free, sound and healthy greengram seed of each genotype were shade dried for three days to attain the uniformity in moisture content (9%) of different genotypes of greengram.

2.1 Insect Culture and maintenance
The culture of pulse beetle (*Callosobruchus chinensis*) was obtained from the stock culture at Regional Agricultural Research Station, Lam, Guntur. This culture was maintained in plastic jars of one liter capacity containing greengram seed. The mouth of the jar was covered with muslin cloth and fastened tightly with the help of a rubber band. Freshly emerged insects from the culture were used for the experiment.

2.2 No choice test
100 g seed of each greengram variety was taken in a plastic container into which two pairs of freshly emerged bruchids were released. After collecting the adults from stock culture, they were kept in a deep freezer for a few minutes in order to inactivate counting and sexing. After introducing the bruchids into each jar, the mouth of the jar was secured with perforated lids. The experiment was laid out in a Completely Randomized Design with three replications under ambient conditions. After three days, the adults were removed and data on various biological parameters *i.e.* number of eggs laid, adult emergence, seed weight loss, mean development period and growth index were recorded at monthly interval for eight months.

2.3 Observations recorded
2.3.1 Number of Eggs: After three days, the insects were removed and the number of eggs laid on the 100 seed in each replication was counted with the help of magnifying glass and the mean number of eggs laid was calculated.

2.3.2 Adult emergence (%): Adult emergence (%) was calculated by using the formula given below [7]:

$$\text{Adult emergence (\%)} = \frac{\text{Number of adults emerged}}{\text{Number of eggs laid}} \times 100$$

2.3.3 Seed weight loss (%): The number and weight of damaged and undamaged grains of composite sample of 100 seed were taken from each replication in each genotype at final observation. Percentage weight loss was calculated by using the count and weight method by using the formula given below [1]:

$$\text{Seed weight loss (\%)} = \frac{(Wu \times Nd)-(Wd \times Nu)}{Wu \times (Nd+Nu)} \times 100$$

Where, Wu = Weight of undamaged grains
Nu = Number of undamaged grains
Wd = Weight of damaged grains
Nd = Number of damaged grains

2.3.4 Mean development period (days): Mean development period (MDP) is the time taken in days for 50 per cent of the adults to emerge. It was estimated by using the formula given below [7]:

$$\text{Mean Development Period (days)} = \frac{D_1A_1+D_2A_2+D_3A_3+\ldots\ldots D_nA_n}{\text{Total number of adults emerged}} \text{ X } 100$$

Where
D_1– Day at which the adults started emerging (First day)
A_1– Number of adults emerged on the day D_1
A_n–Number of adults emerged on the day D_n

2.3.5 Growth index of pulse bruchid: Growth Index (GI) of pulse bruchid was computed by using the formula given below [9]:

$$\text{Growth Index of pulse bruchid} = \frac{\text{Adult emergence (\%)}}{\text{Mean development period}}$$

The greengram genotypes used in the present study were classified [10] based on the growth index into resistant (0.00-1.60), moderately resistant (1.60-1.65), moderately susceptible (1.65-1.70), susceptible (1.70-1.75) and highly susceptible (> 1.75) genotypes.

3. Statistical Analysis
The data collected for 8 months was pooled and subjected to appropriate transformations wherever necessary and analyzed by adopting Completely Randomized Design [13]. Data obtained was subjected to analysis of variance (ANOVA) procedure using OP STAT software. The treatment means were compared using Duncan's multiple range test (P<0.05) using SPSS software (version 6.0) at 1% and 5% level of significance.

4. Results and Discussion
The results revealed that twelve greengram genotypes screened under no-choice laboratory conditions displayed significant variation in the expression of resistance to *C. chinensis*. There were significant differences between the genotypes in terms of number of eggs laid, adult emergence, seed weight loss, mean development period and growth index of *C. chinensis* on greengram under no choice conditions.

4.1 Number of eggs / 100 seed: The pooled data on number of eggs laid by pulse bruchid is presented in Table 1. The egg count significantly differed among the genotypes throughout the storage period *i.e* at different months. The egg laying by pulse bruchid after artificial infestation was initially low but gradually increased with increase in storage period. The mean number of eggs varied widely from 6.69 in first month to 92.19 per 100 seed in the eighth month (Fig. 1)

The pooled data over 8 months showed that the greengram genotype, PM-5 is the least preferred variety by pulse bruchid which recorded a significantly lowest number of eggs (14.00 number / 100 seed). The variety, WGG-42 was found to be a highly preferred host for egg laying by pulse bruchid with the significantly highest number of eggs (73.17 number / 100 seed) when compared to all the other varieties (Table 1).

Oviposition is a paramount behavior exhibited by an insect for continuation of its race and establishment of their population. The ovipositional preference of bruchid seems to be governed by several biotic and ecological factors. There is an appreciable variation in ovipositional preference on different accessions which could be attributed to physico-chemical properties of seed. Earlier studies in pigeonpea also indicated differential response of oviposition by bruchid in different varieties [21]. In another study in pea using 20 varieties, Rachna was found to be least preferable for egg laying by the bruchids [19]. On the other hand, the oviposition by *C. chinensis* had no significant difference on candidate varieties in chickpea [17].

4.2 Adult emergence (%)

The adult emergence of pulse bruchid was initially low but gradually increased with the duration of storage with significant differences among the genotypes. The mean adult emergence varied widely from first month (38.67%) to eighth month of storage (52.64%) (Fig. 2) among the different varieties.

The pooled data over 8 months indicated significant differences among the greengram genotypes in adult emergence of pulse bruchid. PM-5 was the least preferred variety by pulse bruchid which recorded significantly lowest adult emergence (17.44%) over all the other entries. The variety, WGG-42 was found to be a highly preferred variety by pulse bruchid with highest adult emergence (63.20%) among the different varieties (Table 1).

Earlier reports in cowpea [11] stated that the adult emergence varied significantly among the varieties. There were significant differences in the percentage of adults emerged between different pulses [14].

4.3 Seed weight loss (%)

The seed weight loss was initially low but gradually increased with increase in period of storage among all the varieties. The mean weight loss ranged from 1.33% during first month to 27.10% in eighth month of storage (Fig. 3) which might be due to relative increase in the population of insects. There were significant differences in seed weight loss among the genotypes irrespective of storage period.

The pooled data showed that PM-5 recorded significantly lowest weight loss (0.98%) and hence considered as least preferred variety over all the other entries. The variety, WGG-42 recorded highest weight loss (29.21%) and was categorized as highly preferred by pulse bruchid among all the entries (Table 1).

Similar results were reported in cowpea [16] revealing that the weight loss was significantly less in CP-17, while Local variety which permitted highest number of larvae to feed and develop, recorded significantly higher weight loss of grains. The weight loss of seed is due to internal feeding habit of pulse bruchid which might cause damage to the major portion of the cotyledon, consequently leading to reduction in weight. The maggots, just after hatching bored into the seed and internal feeding is reflected as loss in seed weight [6].

4.4 Mean development period (days)

Significant variation was observed in the mean development period of *C. chinensis* on different genotypes of greengram. The mean development period of bruchid ranged from 25.81 - 33.83 days among the different entries (Table 1). The longest mean development period was found in PM-5 (33.83 days) followed by GGG-1 (32.72 days) and LGG-607 (32.39 days), which were found to be on par with each other. Shortest mean development period (25.81 days) was recorded on WGG-42 followed by LGG-586 (26.56 days) and LGG-450 (26.78 days) which indicated that these varieties favoured the development of bruchid, thus facilitating to complete more number of generations in a given period of time (Table 1).

The results are in close proximity with [12, 3, 18] who reported that the development period was significantly longer in resistant varieties of cowpea than the susceptible varieties. Further, they also noted that it was prolonged by 8 to 10 days on least preferred cowpea varieties.

4.5 Growth index

The experimental results indicated that the growth index of *C. chinensis* significantly differed among the different varieties of greengram. The highest growth index was observed on WGG-42 (2.83) which might be due to highest oviposition and adult emergence and thus can be considered as most preferred variety for development of pulse bruchid. The lowest growth index was recorded on PM-5 (1.05). The next best genotypes with lower growth indices were LGG-607 (1.06), LGG-610 (1.06), and GGG-1 (1.23) (Table 1).

In the present study, growth index was calculated based on the adult emergence and developmental period of the pulse beetle and was taken as a criteria for categorization of varieties as given by [10]. Out of 12 greengram genotypes used in the present investigation, five genotypes *viz.*, PM-5 (1.05), LGG-610 (1.06), LGG-607 (1.06), GGG-1 (1.23) and LGG-595 (1.48) were classified as resistant entries,while only one genotype, LGG-460 (1.68), was categorized as moderately susceptible and the remaining six genotypes *viz.*, LGG-574 (1.76), TM-92-2 (1.78), LGG-407 (1.95), LGG-450 (2.08), LGG-586 (2.17) and WGG-42 (2.83) were grouped as highly susceptible genotypes (Table 2).

The present findings are concurrent with [3, 20, 18] who differentiated the various cultivars of cowpea into most susceptible and least susceptible groups on the basis of growth indices. They observed higher growth index values on susceptible varieties of various pulses. The growth index of *C. chinensis* on certain varieties of chickpea ranged from 1.59 to 1.83 [4].

Table 1: Growth parameters of pulse bruchid on different genotypes of greengram under no choice conditions

Genotypes	Pooled mean for 8 months			Mean development period (days)	Growth index
	Number of eggs / 100 seed*	Adult emergence (%)**	Weight loss (%)**		
GGG-1	24.96 (5.09)^{g}	40.23 (39.35)^{e}	6.01 (14.18)^{f}	32.72^{a}	1.23^{f}
LGG-407	52.33 (7.30)^{c}	55.67 (48.24)^{b}	19.89 (26.48)^{c}	28.56^{d}	1.95^{c}
LGG-450	54.33 (7.44)^{c}	55.80 (48.31)^{b}	17.66 (24.84)^{d}	26.78^{e}	2.08^{b}
LGG-460	37.54 (6.21)^{e}	50.47 (45.25)^{c}	9.73 (18.16)^{e}	30.06^{cd}	1.68^{d}
LGG-574	44.25 (6.73)^{d}	51.03 (45.57)^{c}	10.07 (18.49)^{e}	29.00^{d}	1.76^{d}
LGG-586	61.88 (7.93)^{b}	57.64 (49.38)^{b}	22.96 (28.62)^{b}	26.56^{e}	2.17^{b}
LGG-595	31.79 (5.73)^{f}	45.93 (42.64)^{d}	6.29 (14.52)^{f}	31.06^{bc}	1.48^{e}
LGG-607	31.17 (5.65)^{f}	34.49 (35.95)^{f}	5.80 (13.93)^{f}	32.39^{ab}	1.06^{g}
LGG-610	21.88 (4.78)^{g}	31.34 (34.03)^{g}	3.27 (10.40)^{g}	29.45^{cd}	1.06^{g}
PM-5	14.00 (3.87)^{h}	17.44 (24.67)^{h}	0.98 (5.65)^{h}	33.83^{a}	1.05^{h}
TM-92-2	43.54 (6.67)^{d}	51.31 (45.73)^{c}	10.69 (19.03)^{e}	28.89^{d}	1.78^{d}
WGG-42	73.17 (8.61)^{a}	63.20 (52.64)^{a}	29.21 (32.70)^{a}	25.81^{e}	2.83^{a}

Mean	40.90 (6.33)	46.15 (42.65)	11.88 (18.92)	29.59	1.60
F-test	Sig.	Sig.	Sig.	Sig.	Sig.
S Em ±	0.11	0.65	0.35	0.54	0.04
C. D. (5%)	0.31	1.89	1.01	1.58	0.11
C.V.%	2.93	2.63	3.16	3.16	4.14

* Values in the parentheses are square root transformed values
** Values in the parentheses are angular transformed values
Means in the same coloumn showing similar alphabets are not significantly different

Table 2: Categorization of greengram genotypes based on growth index of *C. chinensis*

Category	Growth index range	Number of greengram genotypes	Greengram genotypes
Resistant	0 – 1.60	5	PM-5, LGG-610, LGG-607, GGG-1 and LGG-595
Moderately resistant	1.60 – 1.65	-	-
Moderately susceptible	1.65 – 1.70	1	LGG-460
Susceptible	1.70– 1.75	-	-
Highly susceptible	> 1.75	6	LGG-574, TM-92-2, LGG-407, LGG-450, LGG-586 and WGG-42

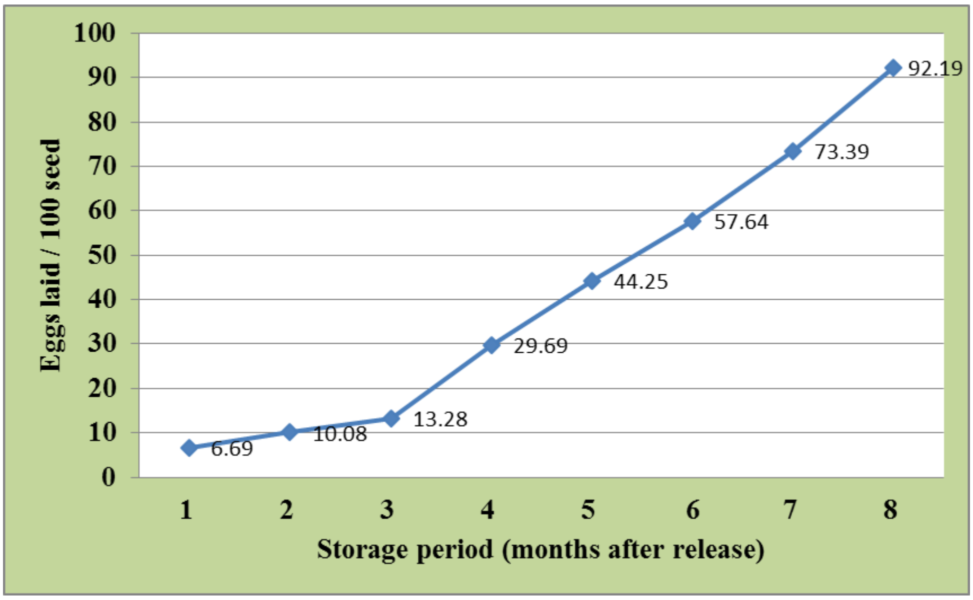

Fig 1: Mean number of eggs laid / 100 seed by *C. chinensis* over 8 months of release in greengram

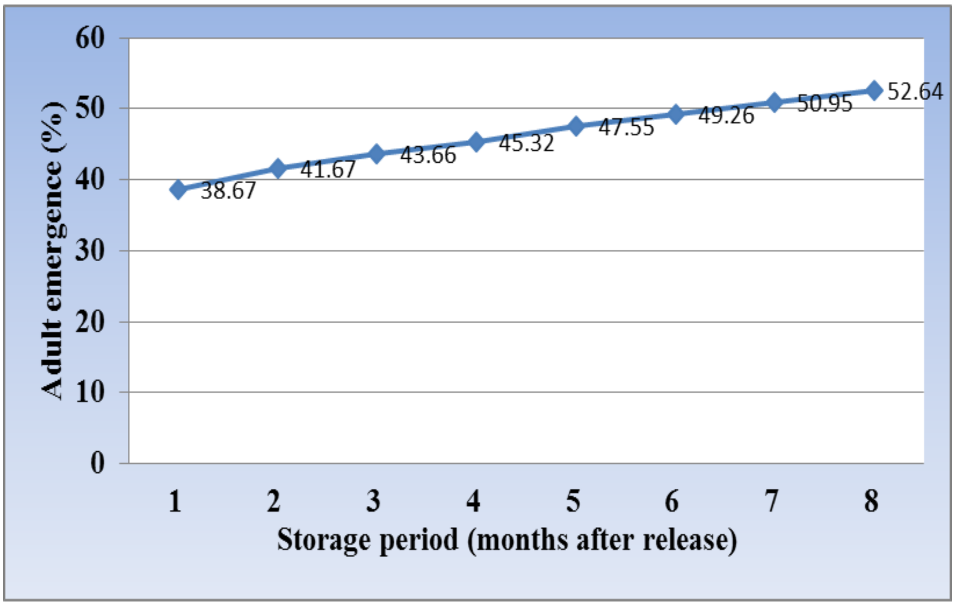

Fig 2: Mean adult emergence of *C. chinensis* over 8 months of release in greengram

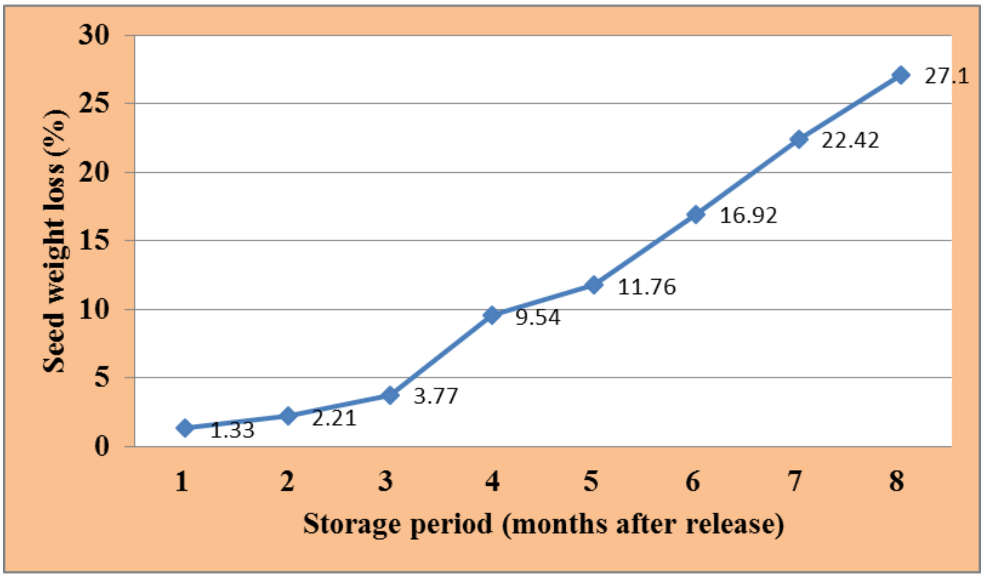

Fig 3: Effect of pulse bruchid infestation on seed weight loss in greengram under storage

5. Conclusion

Screening of 12 greengram genotypes for resistance to pulse bruchid, *Callosobruchus chinensis* revealed that five genotypes *viz.*, PM-5, LGG-610, LGG-607, GGG-1 and LGG-595 were resistant which could be used in breeding programme for the development of resistant cultivars. Highest number of eggs laid (73.17 / 100 seed), adult emergence (63.20%), seed weight loss (29.21%), growth index (2.83) and shorter mean development period (25.81 days) were recorded in WGG-42, which was found to be highly susceptible to pulse bruchid.

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