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Host Preference Studies on Stored Pulses to Pulse bruchid, *Callosobruchus phaseoli* (Gyllenhal) (Chrysomelidae: Coleoptera)

G. Haripriya[1], R. Arulprakash[2]*, P.S. Shanmugam[2] and D. Amirtham[3]
[1]Research Scholar, Department of Agricultural Entomology,
Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[2]Assistant Professor (Agricultural Entomology), Department of Agricultural Entomology,
Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[3]Assistant Professor (Biochemistry), Department of Biochemistry,
Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.

(Corresponding author: R. Arulprakash)*
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ABSTRACT: Pulses, being an essential source of proteins, serves as the indispensable food crop throughout the world. In storage, pulses witness heavy loss and damage due to various biotic and abiotic constraints. Among them, the bruchid infestation was very crucial. The present study was focussed on the host preference of bruchid, *Callosobruchus phaseoli* to various pulses seeds *viz*., green peas, chickpea, kidney beans, black beans, greengram, blackgram, horsegram, cowpea, and lablab. Results revealed that *C. phaseoli* preferred larger pulses seeds for oviposition than the smaller ones and maximum adult emergence was observed in lablab and cowpea. In blackgram, black beans and kidney beans (both speckled and brown types), despite oviposition, none of the grubs turned in to adults and there was no sign of damage to the seeds. Correlation studies showed that except seed dimension (Length, breadth and surface area) other biophysical characters (Seed colour, lustre, texture and sphericity) did not have any influence on *C. phaseoli* biology. Biochemical profiling of blackgram and black beans would reveal the exact cause of resistance.

Keywords: *Callosobruchus phaseoli*, pulses, host preference, biology, biophysical parameters.

INTRODUCTION

Pulses are indeed an important source of dietary fibre and high-quality protein. They play a vital role in food and nutritional security, with a lot of potential in meeting future global food security, nutrition, and environmental sustainability needs (Singh *et al*., 2016). In India, pulses have been cultivated over an area of 28.8 million hectares, with total production of 25.72 million metric tons, yielding about 892 Kg/ha annually (Anonymous, 2022), and thus being the world's largest producer, consumer and importer of pulses (Vishwakarma *et al*., 2019). Nevertheless, there are some constraints exists in the production as well as the post-production of pulses. Being highly proteinaceous, pulses are more prone to storage pests and that alone accounts for 5-10 % losses in pulses (Lal and Verma 2007).

Pulse beetle, *Callosobruchus* spp. (Chrysomelidae: Coleoptera), also called bruchids, are quite well regarded as the most devastating pulse storage pest, particularly in the tropics and subtropics, causing notable losses to the stored pulse commodities (Mishra *et al*., 2017). The extent of damage and lossincurred by bruchids is variable depending on the crop and species. Among the species, *Callosobruchus maculatus* (Fabricius) and *C. chinensis* (Linnaeus) are the most notorious infesting several pulses such as cowpea, mung bean, black gram, chickpea, horse gram etc., during storage. In recent times, the bruchid species *C. phaseoli* has known to infest stored lablab seeds. During the recent survey, occurrence of *Callosobruchus phaseoli* (Gyllenhal) was recorded in stored lablab seeds and until now this species has not been reported from Tamil Nadu. *C. phaseoli* measures about 2.7 – 3.5 mm length and 1.5 – 1.7 mm width, having golden yellow brown body colouration. Like any other bruchid, it also glues its egg to the seed surface, the emerging grub entirely damages the cotyledon, and the adults make their way out by making circular exit holes. In view of *C. phaseoli* may expand its host range in future, the present study was focussed on identifying the host preference of bruchid by studying its biology in various pulses and to determine the range of susceptibility in pulses genotypes.

MATERIALS AND METHODS

Experiment details. The study was conducted at the Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu from November 2021 to May 2022 by utilising Completely Randomized Design with three replications.

Seed materials. Different types of uninfested pulses *viz*., green peas (*Pisum sativum*), chickpea (*Cicer arietinum*) (Desi and kabuli type), kidney beans (*Phaseolus vulgaris*) (Speckled and brown), blackgram (*Vigna mungo*), greengram (*Vigna radiata*), cowpea (*Vigna unguiculata*) and horsegram (*Macrotyloma uniflorum*) were used for the host preference study and were procured from the local markets. Black kidney beans, a local landrace collected from Kolli hills, Tamil Nadu and dolichos bean (*Lablab purpureus*) (Variety CO(GB)14), obtained from the Department of Vegetable Sciences, TNAU, Coimbatore, were also included in this study. All the seed materials were pre-conditioned in the deep freezer at -23˚C for two weeks to eliminate the hidden infestation by any other storage pests and then the seeds were thawed at room temperature for five days before experimentation.

Bruchid culture. *Callosobruchus phaseoli* adults used in this experiment were obtained from the pure culture maintained at Seed Health Laboratory, Directorate of Seed Centre, TNAU, Coimbatore. The male and female beetles were differentiated using the key characters (Kingsolver, 2004). Ten pairs of freshly emerged adults were released in uninfested white lablab seeds (100 g) kept in a plastic container (10 cm diameter, 20 cm height). Seeds were exposed for oviposition for two days and adults were removed. Freshly emerged adults from the culture were used for the experiments. The culture was retained throughout the study by periodical sub-culturing.

'No-choice' study. The host preference of *C. phaseoli* to different pulses was determined using no-choice method of screening (Aidbhavi *et al.,* 2021). The number of seeds taken for study varied according to their size *viz*., kidney beans - 20 nos.; green peas, chickpea, lablab, kolli hills bean - 30 nos. each); cowpea - 40 nos. and greengram, blackgram, horsegram - 50 nos. each. The pre weighed seeds were taken in plastic vials (7.5 cm height, 5 cm diameter) three pairs of mated beetles were released in each vial and allowed to oviposit for four days. The biological parameters *viz*., oviposition (no. of eggs laid per ten seeds), egg hatching success (Giga and Smith 1987), adult emergence (%) and Mean Developmental Period (MDP) were observed. Finally, seed damage (%) and weight loss (%) were computed (Seram *et al.,* 2016). Based on the above parameters, Susceptibilty Index (SI) (Howe, 1971) was calculated, and pulses seeds were categorized into immune (0.000), resistant (0.001-0.050), moderately resistant (0.051-0.055), moderately susceptible (0.056-0.060), susceptible (0.060-0.065) and highly susceptible (>0.065).

Seed biophysical characteristics. The qualitative (Seed colour, lustre and texture) and quantitative (Seed dimensions, surface area and sphericity) parameters of different pulses were analysed to understand whether it has any relationship with the host susceptibility to *C. phaseoli*. The qualitative aspects like seed colour and lustre were visually observed. Seed texture was analysed under LEICA stereo zoom microscope (Model: S8APO). Seed length, breadth, width and seed coat thickness were measured using digital vernier calliper (Model: Kency). From the above parameters, seed surface area and seed sphericity were worked out as per Sewsaran *et al.* (2019).

Statistical analysis. The data were statistically analysed by one way ANOVA using SPSS software version 22.0 and the means were compared by Duncan's Multiple Range test (DMRT) at 5.0 % significance level. To stabilize the variance, data in percentage were transformed using arc-sine transformation, while others were transformed by square root transformation. To understand the mechanism of resistance, seed biophysical parameters were correlated with the biological parameters of *C. phaseoli*.

RESULTS AND DISCUSSION

A. Screening by no-choice test

Biological parameters of *C. Phaseoli* obtained from the host preference study were given in Table 1. Eggs of *C. phaseoli* were observed on all the pulses seeds but there was significant variation in the rate of ovipositon. Maximum oviposition was observed in kidney beans (speckled) (70.17 eggs/10 seeds) and minimum in horsegram seeds (2.60 eggs/10seeds). Maximum eggs on kidney beans (speckled) might be due to the larger size, because seed size influence the ovipositon pattern of bruchid (Lambrides and Imrie 2000).

Table 1: Categorization of pulses seeds based on Susceptibility Index.

Susceptibility Index	Category	Pulses seeds
0.000	Immune	Blackgram, blackbeans (Land race), Kidney beans (Speckled & brown)
0.001-0.050	Resistant	Green peas, horsegram, chickpea (Desi type), and greengram
0.051-0.055	Moderately resistant	Chickpea (Kabuli type)
0.056-0.060	Moderately susceptible	-
0.061-0.065	Susceptible	-
>0.065	Highly susceptible	Cowpea and lablab

The hatching success of bruchid eggs had a significant variation between the pulses. Hatched eggs were differentiated from the unhatched ones by their dirty white opaque colour (Unhatched eggs are shiny and translucent). Maximum percentage of egg hatching was noticed in chickpea (Desi type) (94.72%) and in green peas (94.43%), whereas only 33.70% eggs were hatched in kidney beans (Brown).

Maximum adult emergence (%) was observed in lablab seeds (78.11%). In blackgram, black beans (local land race) and kidney beans (speckled and brown) no adult emergence was observed. Despite higher percentage of hatching success, the seeds of blackgram, black beans and kidney bean has not supported the larval development. This might be due to the presence of antinutritional factors in the seed cotyledon that has hindered the larval development. Under laboratory condition in lablab seeds, *C. phaseoli* adult emergence occurs in 25-28 days after oviposition. However, in this study, Mean Developmental Period (MDP) of *C. phaseoli* varied significantly in different pulses. MDP ranged from 0.0 to 32.78 days, with green peas registering the longest MDP. *C. chinensis* had a prolonged developmental period in the resistant chickpea varieties (Ahmad *et al.,* 2017).

Per cent seed damage was higher in lablab (93.33%) followed by cowpea (85.83%), which also recorded maximum weight loss (29.95%). The seed damage and weight loss had a prominent association with the adult emergence rates. Tripathi *et al*., (2020) also found a significant positive relationship between adult emergence and percent seed weight loss. Based on SI, different pulses seeds were categorized and mentioned in Table 2.

B. Biophysical seed characters

The results of qualitative and quantitative seed characters are presented in Table 3. There is a variation in seed coat colour among the different pulses seeds, however most of them had smooth texture and shiny lustre. The current findings revealed that seed coat colour did not have any impact on *C. phaseoli* oviposition, because there was no variation in the number of eggs laid on the light as well as dark coloured pulses seeds. In contrast, Esen *et al.* (2019) observed that seed colour of peas influenced the oviposition by *C. chinensis*, wherein black and brown coloured varieties had significantly lesser number of eggs per seed than yellow and green varieties.

Seed lustre also had no effect on the oviposition. This finding contrasted with Duraimurugan *et al*., (2014), they reported that small and shiny green gram seeds had fewer eggs compared to the large and dull seeds.

The seed texture of pulses seeds used in this study did not show much variation as most of them had smooth texture, except chickpea (Desi and kabuli type) and blackgram. The roughness of black gram seed could be a factor for non-preference by *C. phaseoli*. A similar attribute was noticed by Shaheen *et al*. (2006) wherein the resistant chickpea cultivars had rough and wrinkled seed coats.

The quantitative seed parameters *viz.,* seed dimensions, seed coat thickness, seed surface area and seed sphericity, all exhibited significant variations between the pulse seeds. The seed coat thickness was minimum in the small sized pulses seeds like blackgram, greengram, horsegram and cowpea. Chickpea (Desi type) had the maximum seed coat thickness of 0.18 mm. As far as the Seed Surface Area (SSA) is concerned, Chickpea (Kabuli type) had the maximum SSA of 307.02 mm^2 and horse gram had the minimum of 31.37 mm^2. Seed sphericity denotes the roundness of the seed, that is, higher the value of seed sphericity, the more spherical is the seed (Wood *et al*., 2012). The seed sphericity of pulses seeds ranged from 53.71% (Kidney beans brown) to 90.29% (Green peas).

Table 2: Biological parameters of *Callosobruchus phaseoli* in different pulses.

Sr. No.	Pulse crop	Oviposition/10 seeds*	Hatching success (%)**	No. of adults emerged*	MDP (days)*	Adult emergence (%)**	Seed damage (%)**	Weight loss (%)**	SI*
1.	**Green peas**	55.67 (7.46)^{g}	94.43 (76.36)^{h}	3.00 (1.86)^{b}	32.78 (5.77)^{e}	1.78 (8.65)^{b}	5.56 (13.48)^{b}	4.42 (12.14)^{b}	0.007 (0.71)^{a}
2.	**Chickpea (Kabuli type)**	26.33 (5.13)^{c}	90.07 (71.78)^{g}	15.00 (3.91)^{c}	27.73 (5.31)^{c}	28.21 (32.33)^{d}	21.67 (27.71)^{c}	5.01 (12.92)^{b}	0.052 (0.74)^{c}
3.	**Chickpea (Desi type)**	33.67 (5.80)^{d}	94.72 (76.73)^{h}	29.33 (5.45)^{d}	30.67 (5.58)^{d}	29.12 (32.92)^{d}	24.44 (29.55)^{c}	8.85 (17.30)^{c}	0.048 (0.74)^{bc}
4.	**Kidney beans (Speckled)**	70.17 (8.38)^{i}	59.01 (50.21)^{c}	0.00 (0.71)^{a}	0.00 (0.71)^{a}	0.00 (4.05)^{a}	0.00 (0.64)^{a}	0.68 (4.74)^{a}	0.000 (0.71)^{a}
5.	**Kidney beans (Brown)**	59.33 (7.70)^{h}	33.70 (35.48)^{a}	0.00 (0.71)^{a}	0.00 (0.71)^{a}	0.00 (4.05)^{a}	0.00 (0.64)^{a}	0.78 (5.07)^{a}	0.000 (0.71)^{a}
6.	**Blackgram**	2.87 (1.69)^{a}	46.81 (43.17)^{b}	0.00 (0.71)^{a}	0.00 (0.71)^{a}	0.00 (4.05)^{a}	0.00 (0.41)^{a}	0.94 (5.53)^{a}	0.000 (0.71)^{a}
7.	**Greengram**	6.00 (2.45)^{b}	74.46 (59.72)^{e}	12.00 (3.53)^{c}	31.92 (5.69)^{e}	39.96 (39.47)^{e}	19.33 (26.01)^{c}	12.46 (20.62)^{d}	0.050 (0.74)^{bc}
8.	**Cowpea**	25.92 (5.09)^{c}	88.47 (70.17)^{g}	65.67 (8.13)^{e}	24.73 (5.02)^{b}	63.50 (53.17)^{f}	85.83 (68.02)^{d}	29.95 (33.15)^{e}	0.073 (0.76)^{e}
9.	**Horsegram**	2.60 (1.61)^{a}	66.98 (54.97)^{d}	2.33 (1.68)^{b}	30.44 (5.56)^{d}	18.20 (25.50)^{c}	4.67 (12.42)^{b}	3.85 (11.31)^{b}	0.041 (0.74)^{b}
10.	**Black kidney beans (Land race)**	39.00 (6.24)^{e}	80.58 (63.87)^{f}	0.00 (0.71)^{a}	0.00 (0.71)^{a}	0.00 (4.05)^{a}	0.00 (0.52)^{a}	0.95 (5.49)^{a}	0.000 (0.71)a
11.	**Lablab**	50.89 (7.13)^{f}	87.30 (69.15)^{g}	119.33 (10.94)^{f}	27.65 (5.31)^{c}	78.11 (62.48)^{g}	93.33 (75.36)^{e}	13.62 (21.63)^{d}	0.068 (0.75)^{d}
	F value	940.16	126.99	475.56	5727.34	239.04	392.97	182.83	183.80
	p value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	SE	0.11	1.72	0.22	0.04	1.95	1.90	0.94	0.002

Data represents mean of three replications. *Values in parenthesis are square root transformed values. **Values in parenthesis are arcsine transformed values. Means in a column followed by same letter do not differ significantly at p=0.05 based on Duncan's Multiple Range test.

C. Correlation study

Correlation analysis was performed to understand the influence of biophysical parameters of pulses seeds on the biology of *C. phaseoli* (Table 4). Surprisingly, only the ovipositional preference of bruchid had significant positive association with the seed length (r=0.803), breadth (r=0.763) and seed surface area (r=0.721). This elucidates that *C. phaseoli* had a high preference to larger seeds for oviposition, and it is evident that greater the seed size and area, higher is the rate of oviposition by bruchid.

Table 3: Qualitative and quantitative parameters of different pulses seeds use in the experiment.

Sr. No.	Pulse crop	Seed colour	Seed texture & lustre	Seed length mm	Breadth mm	Width mm	Seed coat thickness mm	Seed surface area mm^2	Seed sphericity %
1	**Green peas**	Green	Smooth, dull	7.06 ± 0.13^{c}	6.24 ± 0.32^{c}	5.93 ± 0.23^{e}	0.12 ± 0.01^{c}	127.85 ± 6.92^{d}	90.29 ± 0.85^{h}
2	**Chickpea (Kabuli type)**	White	Wrinkled, dull	12.25 ± 0.40^{e}	8.94 ± 0.17^{f}	8.89 ± 0.06^{g}	0.14 ± 0.01^{c}	307.02 ± 9.35^{i}	80.79 ± 1.37^{fg}
3	**Chickpea (Desi type)**	Dark brown	Rough, dull	10.35 ± 0.10^{d}	7.06 ± 0.14^{d}	7.21 ± 0.21^{f}	0.18 ± 0.01^{d}	203.73 ± 3.85^{f}	77.82 ± 0.11^{ef}
4	**Kidney beans (Speckled)**	Light brown, speckled	Smooth, shiny	15.65 ± 0.21^{f}	7.80 ± 0.11^{e}	6.17 ± 0.21^{e}	0.12 ± 0.01^{bc}	258.54 ± 3.87^{g}	58.01 ± 0.73^{b}
5	**Kidney beans (Brown)**	Purplish brown	Smooth, shiny	17.34 ± 0.34^{g}	8.03 ± 0.08^{e}	5.83 ± 0.19^{e}	0.11 ± 0.02^{bc}	271.82 ± 1.32^{h}	53.71 ± 1.17^{a}
6	**Blackgram**	Black	Rough, dull	4.93 ± 0.07^{ab}	3.58 ± 0.02^{a}	4.12 ± 0.04^{c}	0.07 ± 0.01^{a}	54.60 ± 0.47^{b}	84.61 ± 0.81^{g}
7	**Greengram**	Green	Smooth, shiny	4.49 ± 0.21^{a}	3.42 ± 0.03^{a}	3.45 ± 0.06^{b}	0.07 ± 0.01^{a}	44.17 ± 1.49^{b}	83.69 ± 2.69^{g}
8	**Cowpea**	Light brown	Smooth, shiny	6.73 ± 0.06^{c}	5.05 ± 0.27^{b}	3.99 ± 0.20^{c}	0.07 ± 0.01^{a}	82.44 ± 3.46^{c}	76.10 ± 2.24^{de}
9	**Horsegram**	Light brown	Smooth, shiny	5.23 ± 0.05^{b}	3.56 ± 0.15^{a}	1.71 ± 0.07^{a}	0.07 ± 0.00^{a}	31.37 ± 0.24^{a}	60.40 ± 0.40^{b}
10	**Black beans (Land race)**	Black	Smooth, shiny	9.79 ± 0.32^{d}	6.78 ± 0.12^{d}	5.04 ± 0.08^{d}	0.09 ± 0.01^{ab}	150.54 ± 2.96^{e}	70.81 ± 1.75^{c}
11	**Lablab**	Dark brown	Smooth, dull	12.20 ± 0.14^{e}	9.38 ± 0.01^{f}	6.28 ± 0.12^{e}	0.13 ± 0.01^{c}	250.79 ± 3.86^{g}	73.28 ± 0.58^{cd}
	F value			406.84	186.06	167.28	15.402	547.98	73.27
	p value			0.000	0.000	0.000	0.000	0.000	0.000
	SE			0.31	0.23	0.21	0.13	6.11	1.95

Data represents mean of three replications Mean ± SE. Means in a column followed by same letter do not differ significantly at p=0.05 based on Duncan's Multiple Range test.

Table 4: Correlation between seed biophysical and biology parameters of *C. phaseoli.*

	Seed length (mm)	Breadth (mm)	Width (mm)	Seed coat thickness (mm)	Seed surface area (mm2)	Seed sphericity (%)	Oviposition /10 seeds	Hatching success (%)	No. of adults emerged	MDP (days)	Adult emergence (%)	Seed damage (%)	Weight loss (%)	Susceptibility index
Seed length (mm)	1.000													
Breadth (mm)	0.852**	1.000												
Width (mm)	0.647**	0.845**	1.000											
Seed coat thickness (mm)	0.556	0.744**	0.845**	1.000										
Seed surface area (mm2)	0.917**	0.960**	0.873**	0.748**	1.000									
Seed sphericity (%)	-0.621*	-0.252	0.143	0.036	-0.325	1.000								
Oviposition/10 seeds	0.803**	0.763**	0.555	0.544	0.721*	-0.362	1.000							
Hatching success (%)	-0.275	0.185	0.310	0.404	0.018	0.576	-0.011	1.000						
No. of adults emerged	0.057	0.358	0.142	0.221	0.182	0.112	0.118	0.438	1.000					
MDP (days)	-0.440	-0.113	-0.003	0.209	-0.203	0.480	-0.300	0.728*	0.384	1.000				
Adult emergence (%)	-0.149	0.133	0.009	0.066	0.001	0.201	-0.159	0.511	0.911**	0.591	1.000			
Seed damage (%)	-0.047	0.235	0.070	0.091	0.073	0.166	0.022	0.483	0.958**	0.434	0.948**	1.000		
Weight loss (%)	-0.309	-0.136	-0.162	-0.149	-0.244	0.264	-0.190	0.483	0.683*	0.501	0.833**	0.852**	1.000	
Susceptibility Index	-0.249	0.031	0.008	0.091	-0.057	0.236	-0.334	0.595	0.735**	0.751**	0.931**	0.818**	0.805**	1.000

* Significant at 5% level of significance; ** Significant at 1% level of significance

This is in consistent with Holay *et al.,* (2017) who reported that the seed surface area of cowpea seeds was positively correlated with the number of eggs laid. Furthermore, the seed biophysical characters had no effect on the susceptibility of pulses. The bruchid biological parameters such as number of adults emerged, percent adult emergence, seed damage, weight loss was highly associated with one another. The SI had a significant positive correlation with number of adults emerged (r=0.735), MDP (r=0.751), percent adult emergence (r=0.931), seed damage (r=0.818), and weight loss (r=0.805). Thus, the present study infers that the major reason behind bruchid resistance in some pulses may be attributed to the seed biochemical factors or the presence of any antinutritional substances and this was found consistent with several reports (Srinivasan and Durairaj 2007; Saruchi and Thakur 2014; Swamy *et al*., 2020).

CONCLUSION

The degree of susceptibility to *C. phaseoli* varied between the different types of pulses. *C. phaseoli* prefers to breed in lablab and cowpea. Whereas seeds like blackgram, black beans and kidney beans did not support the larval development of *C. phaseoli*. Correlation studies revealed that biophysical parameters of pulses did not influence the growth and development of bruchid. Resistance in blackgram and beans could be attributed to the antinutritional substances (Secondary metabolites) in them. Therefore, to understand the mechanism of resistance, biochemical profiling of pulses is a prerequisite.

FUTURE SCOPE

Biochemical profiling of pulse genotypes can be accomplished to have a deeper understanding on the mechanism of bruchid infestation. Host preference studies for *C. phaseoli* can be further explored on wild pulses for the identification of elite resistant sources.

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