

Research article

Reproductive potential and development of *Callosobruchus chinensis* on legumes and cereals commodities

Muhammad Amjad Bashir*, Abid Mahmood Alvi, Rehana Bibi, Tasleem Ahmad, Hina Naz

Department of Plant Protection, Faculty of Agricultural Sciences, Ghazi University, DG Khan, Punjab, Pakistan

Abstract

The fecundity and development of *Callosobruchus chinensis* on legumes and cereals were studied *in vitro* from April to September. The temperature during the investigation was 30.2-34°C and relative humidity was 46-61%. The data was taken at the interval of 24 hours. The highest fecundity of *C. chinensis* (54.40) eggs was recorded on whole black gram and the lowest fecundity (6.8) was recorded on wheat. The adult female life was non-significance here and the longest of 8.6 days on red cowpea and the shortest of 6.2 days was determined in white cowpea. The longest adult life of male (6.2 days) was recorded on mung bean and the shortest was recorded on white whole gram. The longest egg to adult period of 18 days was recorded on lentil. The highest emergence rate (85%) was recorded on lentil and the lowest (61.54%) was recorded on white crushed gram. The highest male to female ratio (5.34:1) was calculated on whole black gram, the heaviest individual of 5.0 mg was recorded on white cowpea and the lightest (3.13 mg) was recorded on whole black gram.

Key words: *Callosobruchus chinensis*, edible seed, legumes, storage pest.

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*Corresponding author Muhammad Amjad Bashir Email amjadhajbani@hotmail.com



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Introduction

Cereals are crops of the family *Gramineae* planted primarily for their edible seeds for human consumption and as a fodder for animals feeding. They provide about 60% of the calories and 50% of the protein to human race [1]. The economy of Pakistan and many other agricultural countries depends directly upon cereals and indirectly the prosperity of all nations depends upon increased production of cereals. Besides a major source of food for human beings, the cereals are used as a feed for livestock and as an industrial raw material in many food and non-food products. Cereal crops are cultivated upon an area of 13099500 million hectares with an average production of 34302500 million tons in Pakistan, while areas under cultivation and production in Punjab remained 9605300 million hectares and 2578500 million tons respectively (Agricultural statistics of Pakistan 2010-2011).

Similar to cereal, the pulses are also a good source of food nutrients (proteins, carbohydrates, lipids, vitamins and minerals) and energy [2] and comprise the major portion of Pakistani food (diet). They are cultivated upon an area of 1328800 million hectares in Pakistan with an average production of 656000 tones while the area under pulses cultivation and production in Punjab is 1127600 hectares with an average production of 522000 million tons (Agricultural statistics of Pakistan 2010-2011). Both the cereal and pulses are infested by a large number of insect species in

fields, but due to some management and control measures, the pests are controlled, but the damage is not recovered. After harvest, the crop product is stored as grains for future consumption; even at this stage the commodities are not safe. In stores, the grains and pulses are attacked by a number of storage pests. The storage pests cause colossal damage to the commodities which reduces not only the quantity, but also the quality of seeds.

Among those, the cow-pea beetle, *Callosobruchus chinensis* is the most notorious storage pests of the common legumes (Mung bean, mash, gram, lentil, garden pea, cow-pea, black gram and soybean), which is widely spread in Asia, Africa, Central and South America. The body length of the adult *C. chinensis* ranges from 2.5 mm to 3 mm. The color of the elytra in this specie varies from light yellow to brownish red with black and white spots forming bands near the tip. Antennae are pectin in males and serrate in females. Cowpea adults live for an average of 12 days and adult females lays 60 eggs in her life span on dry seeds or pods of the mature beans. Just after hatching the larvae entered the seed for their entire larval feed. The whole development requires 18- 60 days depending upon temperature, humidity and food [3].

Keeping in view the importance of crops and their storage, a research project was designed to screen the storage pest non-supporting legumes and cereals by evaluating their destruction due to the cowpea beetle.

Materials and methods

Experiment conditions and materials

The experiment was conducted in the laboratory of the College of Agriculture, Dera Ghazi Khan, Punjab, Pakistan from April to September, 2012. The culture of the test insect was raised under controlled laboratory conditions on various kinds of legumes stored at the research laboratory of Entomology. The other materials like food commodities (pulse and cereals), containers, mason jars, plastic bags, chemicals and muslin cloth etc. were purchased from the local market. Before starting the experiment, the seven types of legumes, including mung bean (*Vigna radiate*), black gram (*Cicer orientinum*), white cowpea (*Vigna unguiculata*), red cowpea (*Cigna unculata*) and lentil (*Lens culinaris*) and three types of cereal grains include wheat grains (*Triticum aestivum*), maize grain (*Zea mays*) and rice (*Oryza sativa*) were conditioned at $4\pm 1^\circ\text{C}$ for 10 days to make sure that the commodities are free of any infestation.

Reproductive potential of *C. chinensis*

For the study of reproductive potential of *C. chinensis*, one pair of one day old adult was released on 10 grams of the legume and cereal grains contained in the Mason jar. There were 10 treatments consist of seven legumes and three cereals. Each treatment was replicated five times. In each replicate, the food commodity was changed daily to facilitate the data recording and rectify any error in eggs counting that may happen otherwise. Eggs counting were done in each replicate at the interval of 24 hours until the female insect dies away. Daily record of laboratory temperature and humidity was maintained throughout the experimental period.

Development and life cycle estimation

Another experiment was conducted to determine the development and effect of food commodities on the life cycle of *C. chinensis* on legumes and cereals. For this experiment, one pair of adult beetle was released on each commodity. Observations of egg laying were made every day. The grains containing eggs were separated and preserved for future studies. Keeping the record of egg laying and onward observations were helpful in the determination of number of egg laying by the female and viability of eggs i.e. total number of eggs hatched out. Similarly, larval and pupal periods were calculated through the recorded information. The data was converted into percentage in order to facilitate the description of results. After the completion of the experiment, the

data was statistically analyzed using SPSS version 11.5 statistical software (SPSS, Chicago, USA) and results were evaluated after proper tabulation of the observations.

Results

Fecundity of *C. chinensis*

The fecundity of *C. chinensis* on three cereals and seven legumes is depicted in Table 1. The data shows that on the first day, the highest fecundity was recorded on red cowpea, which was 15.8 eggs, followed by maize, mung bean, white cowpea, black gram, white whole gram and lentil where the numbers of eggs were recorded as 14.0, 11.6, 9.8, 8.6 and 8.4, respectively, while the lowest fecundity of 7.4 eggs was recorded on white crushed gram. Moreover, in wheat and rice grains, no egg laying was recorded on the first day. The statistical analysis of the data reveals that although the red cowpea and maize were statistically similar to each other, but were significantly different from the other treatment. Similarly, maize and mung bean gave a similar response, but found significantly different from the rest of the treatments which were statistically similar among themselves. The lowest fecundity found in crushed gram, was significantly different from all other treatments.

On the second day, red cowpea and mung bean showed the highest fecundity where the count of eggs was 18.89 and 18.6, respectively. The lowest fecundity of 2.2 eggs per female was recorded on wheat grains. There was no egg laying on rice grains throughout the life period of females. The statistical analysis of the data showed that red cowpea, mung bean, white cowpea and whole black gram were similar to each other, but significantly different from the rest of the treatments. White whole gram and white cowpea were statistically similar to each other while significantly different from the remaining treatments. Moreover, response of whole gram, white crushed gram and lentil were almost similar to each other, but significantly different from the rest of the treatments. Similar results were obtained from maize and wheat.

On the third day, the highest fecundity was shown by whole black gram which was 18.2 followed by white crushed gram, mung bean, whole gram, white cowpea, red cowpea, lentil and maize in descending order where the fecundity was 13, 11.6, 11.0, 10.6, 10.2, 7.2 and 2.0, respectively. The lowest fecundity was 1.2 recorded on wheat grains. No egg laying was recorded on rice. It is revealed from the statistical analysis that whole black gram and maize were significantly different from all the tested treatments

Table 1 Fecundity of *Callosobruchus chinensis* on different commodities.

Treatments	Mean number of eggs laid after (days)					Total
	1	2	3	4	5	
Wheat	0.0e	2.2de	1.2e	0.0e	0.0e	5.8d
Rice	0.0e	0.0e	0.0e	0.0d	0.0e	0.0d
Maize	12.0ab	2.8d	2.0e	7.2c	1.0dc	21.60d
Whole-white gram	8.6	11.0bc	11.0bc	12.4a	2.8cd	34.80b
Crushed white	7.4d	9.2c	7.2d	7.4bc	1.6cde	27.20b
Lentil	8.4cd	9.2c	7.2cd	7.4bc	1.6cde	27.20c
Red cowpea	15.8a	18.8a	1.2bc	11.60abc	6.0a	52.40a
Mung bean	11.6bc	18.6a	11.6bc	11.20abc	7.2a	52.80a
White cowpea	9.8cd	14.6ab	10.6bc	11.80abc	3.6bc	40.60b
Whole black gram	6.8c	16.8a	18.2a	11.80abc	7.0a	54.40a

but were statistically similar to each other. Moreover, the response of five treatments (whole gram, white crushed gram, red cowpea, mung bean and white cowpea) was similar to one another while significantly different from the rest of the treatments. Similar results were obtained by lentil, whole gram, red cowpea, mung bean and white cowpea. Analysis of the data further reveals that maize and lentil were significantly different from all other treatments but were similar to each other. The lowest fecundity was recorded in wheat, which was statistically similar to rice and maize, but different from the remaining tested treatments.

On the fourth day, the highest fecundity was recorded on white crushed gram which was 14.4 followed by white cowpea, black gram, red cowpea, whole gram, mung bean and lentil where eggs were counted as 11.80, 11.80, 11.60, 11.20 and 7.4, respectively, and the lowest fecundity was 7.2 on maize. Moreover, on wheat and rice no egg laying was recorded in this observation. The statistical analysis shows that white crushed gram was similar to white whole gram, red cowpea, mung bean, white cowpea and whole black gram, but different to other treatments. Similarly, lentil was non-significant to maize, whole gram, red cowpea, white cowpea and black gram.

On the fifth day, the highest fecundity was recorded on mung bean and whole black gram where the eggs were counted as 7.2 and 7.0, respectively, followed by red cowpea, white whole gram, white cowpea, white crushed gram and lentil giving 6.0, 5.6, 3.6, 2.8 and 1.6 eggs, respectively. The lowest fecundity was recorded on maize, which was only 1.0 egg while no egg laying was recorded on wheat and rice. The analysis of the data showed that fecundity response by red cowpea mung bean, whole black gram and white whole gram was non-significant among them, but significantly different from the rest of the treatments. White cowpea and white whole gram were statistically similar to each other, but

different from the other treatments. Similar results were obtained with white crushed gram, lentil and white cowpea. Lentil and maize showed significantly different response from the other treatments, but were similar to each other. The lowest fecundity on the fifth day was recorded on wheat, which was significantly different from other treatments, but similar to rice, maize and lentil.

On the sixth day, the females were died and no egg laying was recorded in any one of the treatments. The total number of eggs laid by a female of *C. chinensis* during her lifespan was the highest in black gram where (54.4) eggs were deposited followed in descending order by red cowpea (52.4), mung bean (52.8), white cowpea, whole gram, white crushed gram, lentil and maize. The lowest fecundity was recorded on wheat, which showed 5.8 eggs while no eggs were recorded on rice. It was clear from analysis of the data that the fecundity of the pest on black gram, mung bean and red cowpea were non-significant to each other but different from the other treatments. Similarly, white cowpea, white whole gram and white crushed gram were statistically similar in response of egg laying with each other but significantly different from the rest of the treatments.

Adult life

The data regarding the life cycle of adult males and females of *C. chinensis* is shown in Table 2. The observations showed that the longest female life was recorded on wheat and maize, which was 7.0 days and the lowest on rice which was 5.6 days. Statistically, all the treatments had similar effects on life span of the female *C. chinensis*. The average life of the female was obtained as 5.6-7.0 days.

The longest male life of the *C. chinensis* was recorded on mung bean which was 6.2 days followed by red cowpea, wheat, white cowpea, white crushed gram; whole black gram maize, lentil and white whole gram and the smallest male life was recorded on rice which was 4.8 days. The statistical analysis

of the data indicates that mung bean caused significantly different effects on the life span of the insect compared to rice, maize, white whole gram and whole black gram only. The life of insect on rice, white whole gram and lentil were also significantly different from other treatments.

Table 2 Mean longevity of *Callosobruchus chinensis* on different commodities.

Treatments	Longevity	
	Female	Male
Wheat	7.0	6.0ab
Rice	5.60	4.80b
Maize	7.0	5.60cd
White whole gram	6.40	5.0ab
White crushed gram	6.40	6.0bcd
Lentil	6.40	5.40ab
Red cowpea	6.80	6.0a
Mung bean	6.40	6.20ab
White cowpea	6.20	6.0ab
Whole black gram	6.40	5.80 b

Life cycle

The life cycle of *c. chinensis* was studied on aforesaid commodities. The results revealed that the longest egg period of 10 days was recorded on wheat (Table 3). Similarly, 6 days egg period was recorded in white crushed gram, red cowpea and mung bean and 5 day egg period was obtained in lentil. It was further observed that the larvae hatched from the eggs without breaking the eggshells and tried to penetrate down and started feeding inside the grains. The larvae failed to further develop into the pupal stage in wheat, maize and red cowpea. It was noticed that after initial feeding the larvae on these treatments were died.

For larval development, the results further revealed that the longest larval period was recorded in white crushed gram and white cowpea, which was 9 days followed by whole gram, mung bean and black gram, where the larval period was 8 days in all the treatments. The shortest larval period was recorded in lentil which was 7 days. It was observed that the larvae got developed and transformed into pupal form on these treatments. The grains were damaged by making holes where the population took place.

The observation about the developmental period of the pupal stage revealed that the largest pupal period was 3 days in white crushed gram and white cowpea and 2 days in whole gram, lentil, mung bean and black gram. The life cycle from egg through pupa (3 stages) was calculated from the available information reported above. Hence the longest egg to adult period was recorded as 18 days in white crushed gram followed mung bean and white cowpea showing 16 days on both treatments. The shortest life

cycle (egg, larvae and pupa) was recorded in lentil and whole black gram which was 14 days.

Table 3 Life cycle of *Callosobruchus chinensis* on different commodities.

Treatments	Duration of different life stages(days)			
	Egg	Larval	Pupal	Egg-Adult
Wheat	10	-	-	-
Rice	-	-	-	-
Maize	9	-	-	-
White whole gram	4	8	2	14
White crushed gram	6	9	3	18
Lentil	5	7	2	14
Red cowpea	6	-	-	-
Mung bean	6	8	2	16
White cowpea	4	9	3	16
Whole black gram	4	8	2	14

Emergence rate

To determine the emergence rate of the adults on cereals and legumes, some grains with known number of eggs were separated and kept under observation for further development. It was clear from the data (Table 4) that all the eggs were hatched in to larvae. This study could not have been possible in wheat, rice, maize and red cowpea. The reason was that no eggs were deposited on rice, while on the later; the transformation of pupae from the larvae was not occurred throughout the study period.

In the remaining six treatments (whole gram, white crushed gram, lentil, mung bean, white cowpea and whole black gram), the development through the adult stage was observed, but showing varying rates of emergence. The maximum emergence was recorded on lentil which was 85%, followed by in descending order white cowpea, black gram, white whole gram and mung bean where adult emergence rate from the pupal stage was obtained as 84.0, 82.61, 76.47 and 64.7%, respectively.

Table 4 Percent emergence of pulse beetle *Callosobruchus chinensis* on different commodities.

Treatments	Mean numbers				
	Egg	Larvae	Pupae	Adult	Emergence
Wheat	12	12	-	-	-
Rice	-	-	-	-	-
Maize	19	19	-	-	-
White whole gram	17	17	13	13	76.47
White crushed gram	13	13	8	8	61.54
Lentil	20	20	17	17	85.00
Red cowpea	23	23	-	-	-
Mung bean	17	17	11	11	64.71
White cowpea	25	25	21	21	84.00
Whole black gram	23	23	19	19	82.61

Sex ratio and body weight

Numbers of male and female adults were recorded separately in each treatment to find the sex ratio. Additionally, the body weights of newly emerged

adults were also recorded to estimate the amount of food, preferably consumed by the individual on the tested commodities so the highest male to female ratio was recorded in mung bean which was 8:1. In whole black gram, the number of male and female adults was found equal which made the sex ratio as 1:1 while in white cowpea the number of females was greater than the male adults so it gave male to female ratio as 1:2.67 (Table 5).

Regarding the record of body weight, the weights for both the sexes were recorded separately, and then average was calculated. The highest body weight per individual adult was found in white cowpea, which was 5.0mg where the weight of the male was 4.0mg and of the female 6.0mg followed by white whole gram whole gram white crushed gram, lentil and mung bean where the average weight per insect was recorded as 3.63, 3.50, 3.38 and 3.34 mg, respectively. The separately recorded body weight of the adult was the lowest in whole black gram, where the weight of male adult was obtained as 2.0mg and female adult as 4.25mg and the average weight per insect was 3.13mg.

Discussion

Regarding the fecundity of *C. chinensis* on cereals, the obtained results reveal that the eggs were noticed on cereals as well as leguminous grains, but larval through adult development did not take place on cereals. Moreover, no eggs were found on rice grains among the tasted cereals which may be due to its polished, slippery and hard surface. Among the pulses, the highest fecundity was recorded on whole black gram showing 54.40 eggs. The results of the present investigation are partially in conformity with the findings earlier [3]. They reported *C. maculatus* as a major pest of the legumes, but eggs were rarely and occasionally recorded on cereals. It was further reported that *C. chinensis* laid on an average 70 eggs at $28\pm 2^{\circ}\text{C}$ and $70\pm 5\%$ relative humidity. The variations between the results of the present study and the previous researchers are maybe due to environment (temperature and humidity), laboratory conditions, varieties of the commodities utilized and methods of observation and storage. These results are partially supported by those obtained by that females of *C. chinensis* preferred to ovipositioning in varieties with larger seeds. Similarly, Chevan et al. [4] reported that rough seed surface were less preferred and moreover brown, black, gray and red colored seeds were more preferred than white colored seeds. The highest egg period was recorded on cereals which was 9-10 days, which may be because of non-

preference for these grains. While among legumes, the highest egg period was recorded on white crushed gram, red cowpea and mung bean, which was 6 days followed by lentil which was 5 days. So the egg period varied from 4-10 days. These results are in agreement with the result of earlier research [3] on the biology of *C. chinensis* which reported that the incubation period lasted for 4-5 days.

In case of cereals, it was observed that eggs were laid occasionally. After hatching, the initial feedings were done by larval but soon after initial feeding larvae died away. Similar results were reported who stated that eggs were occasionally laid on stored cereals, but larvae did not survive [5]. On legumes, the red cowpea reflected a discouraging effect for the beetles to feed on and to get developed in this commodity. It was observed that after hatching the larvae could feed for a short period and then larvae did not survive on red cowpea and also only died away which may be because of hard and slippery seed coat of the red cowpea grains. The result is supported by earlier [6] that cluster bean is unsuitable for development of the *Callosobruchus* spp. While in remaining treatments, the highest larval period was recorded on white crushed gram and white cowpea, intermediate was recorded on white whole gram, mung bean and whole black gram while the lowest was recorded on lentil. The larval period varied from 7-9 days on different food commodities. The present findings were in accordance with Shivanna et al. [7] that *C. chinensis* preferred red gram followed by lentil, black gram and cowpea for growth and development.

Since the larvae could not survive on tested cereals and on one of the legumes i.e. red cowpea so further development of bruchid could not be studied while in case of remaining treatments the pupal period was in the range of 2-3 days. Moreover, no significant difference occurred in pupal periods on the tested leguminous grains. The combined larval and pupal period of *C. chinensis* in this investigation varied from 9-12 days. These results are partially in agreement with the results reported earlier [3] in which the combine larval and pupal period of *C. chinensis* was recorded 10-13 days. Life period of adult for both the genders (males and females) was recorded separately. For males of *C. chinensis*, significant differences were found among the tested treatments. Maximum was 6.2 days recorded on mung bean, which was significantly different from other treatments. However, age of male adults on wheat, white crushed gram, red cowpea, mung bean, white cowpea and whole black gram was statistically

Table 5 Comparison of sex ratios and body weights of the adults produced by a pair of *Callosobruchus chinensis* on different commodities.

Treatments	Eggs	Means adults emergence			Sex ratio	Body weight/individual (mg)		
		Male	Female	Total		Male	Female	Avg.
Wheat	12	-	-	-	-	-	-	-
Rice	-	-	-	-	-	-	-	-
Maize	19	-	-	-	-	-	-	-
Whitewhole gram	17	10	3	13	3.3:1	2.25	5.0	3.63
White crushed gram	13	6	2	8	3.0:1	2.25	4.75	3.50
Lentil	20	9	8	17	1.1:1	2.50	4.25	3.38
Red cowpea	23	-	-	-	-	-	-	-
Mung bean	17	8	3	11	2.67:1	2.67	4.0	3.34
White cowpea	25	8	13	21	1.1:63	4.0	6.0	5.0
Whole black gram	23	16	3	19	5.34:1	2.0	4.25	3.13

similar to each other. On the other hand, the differences of the age of female were statistically nonsignificant. These results are somewhat in the line with already reported results [7] that the range of adult ages was 5-20 days and the highest adult's life was in May and October and the minimum in April, June, July and December.

The life cycle (egg to adult period) was not studied in one of the tested leguminous grains and all of the cereal grain, because the larval development did not take place on these commodities. Among the remaining commodities, the longest egg to adult period was observed on white crushed gram followed mung bean and on white cowpea shortest life cycle was recorded on white whole gram lentil and black gram. In this study, the life cycle ranged from 14-18 days. These results are in conformity with earlier [3] who reported that the incubation period lasted from 4-5 days and combine larval and pupal period ranged from 10-13 days. The present results are also supported by Singh et al. [9] who studied the effect of different leguminous grains on feeding and development of *C. chinensis* and reported that red gram followed by lentil, bengal gram, whole black gram and cowpea were preferred by Bruchid.

The highest emergence rate was recorded in lentil, white cowpea and whole black gram which was 85, 84 and 82% and the lowest was 61.54% recorded on white crushed gram. The intermediate population was emerged on white whole gram and mung bean which was 76 and 64%. Singh et al. [9] conducted similar studies and reported that preferred red gram, lentil, whole black gram and cowpea for growth and development. The highest male to female ratio was recorded in whole black gram followed by white whole gram, white crushed gram mung bean and lentil and in white cowpea number of females was greater than male. The body weights of newly emerged adults were also recorded to estimate the amount of food preferably

consumed by the individual on the tested commodities. The highest average weight was calculated as 5.0mg in white cowpea where the weight of a male individual was 4.0mg and that of females was 6.0 mg followed by whole gram, white crushed gram, lentil and mung bean. The lowest average weight was recorded on black gram. The reason for differences in weight of adults on different commodities may be of quality of food and performance of the pest. Some variations in egg and adult production in strains of *C. maculatus* and *C. chinensis* were found and females emerging from adzuki beans were smaller than cowpea and green gram suggesting that azuki bean was inferior as a food substrate [10].

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