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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(12): 280-283

www.thepharmajournal.com
Received: 16-09-2023
Accepted: 19-10-2023

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Increased fecundity as a result of multiple mating in *Callosobruchus chinensis* L.

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Abstract
A study was conducted to evaluate the fecundity of *Callosobruchus chinensis* based on variable number of males in the year 2022-23. For this study, Five sets for each type of pulses i.e., pea and chickpea were used in which each having one freshly emerged female of *C. chinensis* with 1, 2, 3, 5 and 7 numbers of newly emerged males (unmated). It was found that maximum number of eggs were obtained on pea (mean= 97.23 eggs) and it was greater than that of chickpea (mean= 91.87 eggs). While, the number of eggs laid by a female varied significantly depending on the number of adult males present. The highest number of eggs (106.00) was observed when one female was exposed to seven males, while the minimum number of eggs (83.92) was recorded when one male was exposed to one female. While considering the interaction effect between the type of pulses and the variable number of males, both types of pulse seeds had the highest fecundity, with single female exposed to seven males *i.e.*, mean fecundity of pea = 111.33 and chickpea = 100.67. The increase in the fecundity from 1: 1 to 1: 7 in respect of female: male, there was an increase in the fecundity by 22.59% and 18.87% in pea and chickpea. The actual number of effective mating for an optimum fecundity and the duration of fecundity in case of multiple mating out of a greater number of males are required to be studied further.

Keywords: Fecundity, *Callosobruchus*, mating, multiple, pulses

1. Introduction
Pulses are typically stored for a year in various types of storage structures until the next crop is harvested. However, during this storage period, a significant loss is often observed due to infestations by various insects. One such insect of common concern is the bruchid, scientifically known as *Callosobruchus chinensis* L. This insect is cosmopolitan in nature and has the capability of attacking a wide range of legumes *viz*., green gram, black gram, chickpea, pigeon pea, and lentils (Sharma, 1984) [15]. The ability of the bruchid to infest and damage multiple types of legumes makes it a serious threat to the stored pulses, leading to potential economic losses and food security concerns. Management strategies are often needed to mitigate these infestations and protect the stored legume crops. The larvae of the bruchid, such as *Callosobruchus chinensis* L., have a destructive impact on stored pulse grains. These larvae bore into the pulse grains, feed and damage the seeds. The damage caused by the larvae makes the affected grains unfit for human consumption, reduces their viability as seeds for planting, and also hinders their potential for use in sprout production.
The life cycle of this species is of particular concern for stored pulses. The larvae of bruchids exclusively feed and develop within the seeds of legumes, causing significant damage. On the other hand, the adult bruchids have a relatively short lifespan, typically lasting one to two weeks. During this time, their primary activities are involved in finding mates, mating and laying eggs on seeds, perpetuating the infestation cycle (Kergoat *et al*., 2007) [8]. Unlike the larvae, the adult bruchids do not require food or water, which further underscores the challenges of managing infestations and preventing damage to stored pulse grains. Effective pest control measures are crucial to safeguard the quality and viability of stored pulses.
Bruchids are widely recognized as the most notorious insect pests affecting pulses. They are responsible for causing extensive damage, with varying reports of up to 50% of the pulse crop being affected during storage over a relatively short period of three to four months (Caswell, 1981) [2]. Importantly, the infestation often commences in the field during the growth of the pulse plants and continues to pose a significant threat during storage.
Over a period of six months, the cumulative loss due to bruchid infestation has been estimated to be around 30-40%. In severe cases of infestation, the damage can be even more catastrophic, reaching up to 100% loss (Lal and Raj, 2012) [10].

These statistics underscore the substantial economic losses and food security risks associated with bruchid infestations in pulses. This calls for an effective pest management strategy to mitigate these losses and safeguard pulse crops throughout their lifecycle, from cultivation in the field to storage.

The reproductive behaviour of *C. chinensis*, such as oviposition (egg-laying), is a critical aspect of their life cycle. The females of this species deposit their eggs on the surface of host seeds, and upon hatching, the larvae burrow their way into the seeds, where they feed and complete their entire developmental process. Importantly, the larvae are unable to move among different seeds, so they are restricted to the seeds that their mother had selected for their survival and development. Therefore, a female's decision regarding where to lay her eggs is influenced by the environment in which her offspring will develop.

Fecundity of the pulse beetle depends on the quality of the seeds from which the adult female derived her nutrition and accumulated resources during her larval life through the pupation as the adult beetle is aphagous. However, oviposition decisions of the adult females are, in part, influenced by the quality of the resource. Females tend to prefer higher quality resources for their offspring to develop (Mitchell, 1975) [13]. Various factors play a role in influencing the ovipositional behaviour of *C. chinensis*, among them the mating process is the one. Fecundity is influenced by the number of mating that may happen during the adult life of the female with the male (Ridley, 1988) [14]. More the number of eggs deposited by the female more the number of future progeny and consequential losses to stored seeds. Therefore, the knowledge on how egg laying is impacted due to a greater number of mating could reflect on the assumption of future population of the insect species. However, there is scanty information on this aspect. To gain a better understanding of how these factors affect the ovipositional behaviour of *C. chinensis*, the studies were conducted on seeds of Chickpea and Pea. The aim of the study was to assess the impact of host on the ovipositional preferences of this insect species and lifetime fecundity due to multiple mating from variable number of males exposed to females. The generated information may be valuable for pest management and crop protection strategies.

2. Materials and Methods

2.1 Study Area

The experiments were carried out in the laboratories of Entomology at Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India during 2022 and 2023.

2.2 Maintenance of Stock culture of *Callosobruchus chinensis*

To conduct this experiments, a stock culture of the pulse beetle, *Callosobruchus chinensis* was used. This stock culture was specifically maintained on healthy pulse seeds of chickpea. The pulse beetles were reared in plastic containers, and the mouth of these containers was covered by muslin cloth, securely fastened in place with rubber bands. For obtaining freshly emerged unmated males and females, only one egg per seed was maintained (destroying the other seeds with the help of a fine needle) in glass vials and the emerged adults were sexed using identifying keys of Khare, (1993) [9] and Hackston, (2014) [6]. Such adult males and females were used as per the plan of the study. The entire rearing system was maintained within a controlled environment at a temperature of 27±2 °C, utilizing a BOD incubator to ensure consistent conditions for the experiments.

2.3 Detailed methodology of the experiment is outlined below

2.3.1 Fecundity based on variable no. of males

For this study, Five sets for each type of pulses i.e., pea and chickpea weighing 5 grms were taken, in which each having one freshly emerged female of *C. chinensis* with 1, 2, 3, 5 and 7 numbers of newly emerged males (unmated) in Plastic container (3 cm dia) and they were kept aside for 7 days and the fecundity were estimated for 7 consecutive days. This experiment was replicated thrice and conducted for 1 generation and the study was repeated.

Basic objective of the study was to see if fecundity is influenced by multiple mating due to presence of a greater number of males *i.e.*, chances of occurrence of multiple mating. Two types of seeds *viz*. chickpea and pea being the most common pulses were used in the study to see if the adult female has ovipositional preference.

2.4. Statistical analysis

The experiments for the research work were formulated and the data were recorded and statistically analysed according to the Factorial Completely Randomized Design (CRD). Duncan Multiple Range Test (DMRT) was also done after transformation of the data to study the difference between the pulses.

3. Results and Discussions

3.1 Fecundity of *Callosobruchus chinensis* based on variable number of males in two different pulses

The egg laying potential of the adult female pulse beetle, *C. chinensis* due to multiple mating by males was studied. In the study one adult female was exposed to different number of males *viz*. 1, 2, 3, 5 and 7 number of males and the fecundity was recorded in one generation and the experiment was repeated. The results are furnished in Table 1 to Table 3

1st Trial

It reveals from the results presented in (Table 1) that in the 1st generation, the number of eggs laid by the adult female differed significantly in the two types of seeds used in the study. Maximum eggs were laid on pea (mean = 97.67 eggs) and it was greater than that of chickpea (mean = 91.33 eggs) and the difference was significant.

It was observed that the fecundity was greatly influenced by multiple mating and egg laying increased gradually due to increased number of males and the difference in mean no. of eggs laid by the female was significant for each level of increase in the number of male adults. Maximum fecundity was noted when one female was exposed to 7 number of males (mean = 107.33 eggs) while the minimum was recorded in case of one female exposed to one male (mean= 83.33 eggs).

When the interaction effect of the type of pulses and the variable no. of males was taken into account, the result was found to be significant. Highest fecundity was noted on both the types of pulse seeds in which one female was exposed to 7 males (mean fecundity - chickpea- =102.00; pea= 112.67).

2nd trial

According to the results shown in (Table 2), there was a significant variation in the number of eggs laid by the adult female between the two types of seeds that were used in the study. Pea received the highest number of eggs (mean = 96.80 eggs), which was significantly greater than chickpeas (mean = 91.86 eggs).

The highest number of eggs (104.66) was seen when a single female mated with 7 no. of males, while the lowest number of eggs (84.50) was noted when a single female mated with one male.

Upon considering the interaction effect between the type of pulses and the variable number of males, a significant result was observed. The two types of pulse seeds recorded the highest fecundity (chickpea = 99.33; pea = 110.00) when one female was exposed to seven males.

3.2 Pooled mean of two experiments

The data in the Table.3 reveal that the number of eggs laid by the adult female differed significantly in the two types of seeds *viz*. chickpea and pea. Maximum number of eggs were obtained on pea (mean= 97.23 eggs) and it was greater than that of chickpea (mean= 91.87 eggs) and the difference was significant.

It was found that there was a significant difference in the mean number of eggs laid by the female for each degree of increase in the number of adult males. One female exposed to seven males resulted in the highest fecundity (mean = 106.00 eggs), whereas one male exposed to one female resulted in the minimum fecundity (mean = 83.92 eggs).

While considering the effect of the interaction between the type of pulses and the variable number of males, the result was found to be significant. Both types of pulse seeds had the highest fecundity, with single female exposed to seven males *i.e.*, mean fecundity of pea = 111.33 and chickpea = 100.67. The increase in the fecundity from 1: 1 to 1: 7 in respect of female: male, there was an increase in the fecundity by 22.59% and 18.87% in pea and chickpea respectively. It was also observed that pea always had greater number of egg deposition than chickpea at each combination of female and males.

Table 1: Fecundity of *Callosobruchus chinensis* based on variable number of males in two different pulses during, June- 2022

Experiment	**Pulses**	**Number of adult males**					**Mean**
		1	**2**	**3**	**5**	**7**	
1st Trial	Chickpea	81.00* (9.03)	89.00 (9.46)	91.33 (9.58)	96.33 (9.84)	102 (10.12)	91.93 (9.607)
	Pea	85.67 (9.28)	92.67 (9.65)	95.33 (9.79)	102 (10.12)	112.67 (10.64)	97.66 (9.897)
	Mean	83.33 (9.15)^a	90.83 (9.56)^b	93.33 (9.69)^c	99.16 (9.98)^d	107.33 (10.38)^e	
		Pulses		No. of males		Pulses x No. of males	
	SEm±	0.024		0.038		0.053	
	CD	0.070		0.111		0.157	

*Mean of three replications; Figures in parenthesis are square root transformed values; Parentheses followed by same letters are statistically at par by DMRT

Table 2: Fecundity of *Callosobruchus chinensis* based on variable number of males in two different pulses during, June- 2023

Experiment	**Pulses**	**Number of adult males**					**Mean**
		1	**2**	**3**	**5**	**7**	
2nd Trial	Chickpea	82.33* (9.10)	89.33 (9.48)	93.00 (9.67)	95.00 (9.77)	99.33 (9.99)	91.86 (9.602)
	Pea	86.67 (9.34)	92.33 (9.64)	95.33 (9.79)	99.67 (10.01)	110.00 (10.51)	96.80 (9.856)
	Mean	84.50 (9.22)^a	90.83 (9.56)^b	94.16 (9.73)^c	97.33 (9.89)^d	104.66 (10.25)^e	
		Pulses		No. of males		Pulses x No. of males	
	SEm±	0.018		0.028		0.040	
	CD	0.053		0.083		0.118	

*Mean of three replications; Figures in parenthesis are square root transformed values; Parentheses followed by same letters are statistically at par by DMRT

Table 3: Pooled mean fecundity of *Callosobruchus chinensis* based on variable number of males in two different pulses 2022-23.

Experiment	**Pulses**	**Number of adult males**					**Mean**
		1	**2**	**3**	**5**	**7**	
1st generation pooled	Chickpea	81.67* (9.06)	89.17 (9.47)	92.17 (9.63)	95.67 (9.81)	100.67 (10.06)	91.87 (9.60)
	Pea	86.17 (9.31)	92.50 (9.64)	95.33 (9.79)	100.83 (10.07)	111.33 (10.57)	97.23 (9.88)
	Mean	83.92 (9.19)^a	90.83 (9.56)^b	93.75 (9.71)^c	98.25 (9.94)^d	106.00 (10.32)^e	
		Pulses		No. of males		Pulses x No. of males	
	SEm±	0.016		0.025		0.035	
	CD	0.044		0.070		0.099	

*Mean of all the three replications; Figures in parenthesis are square root transformed values; Parentheses followed by same letters are statistically at par by DMRT

From all the above tables we found that in between both the pulses more number of eggs were recorded in pea when compared to chickpea it might be due to the presence of smooth seed coat of pea. Relatively less preference of the beetle was seen towards rough seed coat this might be due to their thicker testa or poor nutritive quality of the seed cotyledon or chemical cues presence if any which affect the fecundity. While coming to multiple mating it was revealed that with the increase in number of males, the fecundity of the female rate was also increases.

Enhanced female fecundity in insects associated with multiple mating has been documented in a number of insects (Ridley, 1988) [14]. The results of multiple mating in insects are variable. In *Drosophila hydei* (Diptera: Drosophilidae), fecundity was not affected when females were mated once, twice or thrice in a given morning but it was increased when females were mated one, two, or three times on successive mornings (Markow, 1985) [12]. It was observed by Fox (1993) [5] that multiple-mated females survived longer and had higher fecundity as well than single- mated females. Multiple mating in the female moth *Utetheisa ornatrix* was found to increase the number of egg deposition after the 1st, 2nd 3rd mating but not the 4th and 5th mating (LaMunyon, 1997) [11] while in case of *Papilio xuthus* (Lepidoptera: Papilionidae) fecundity was elevated after each mating (Watanabe, 1988) [16]. Eddy *et al*. (2000) [4] observed that female copulating with the multiple males laid more eggs than those copulating repeatedly with the same male. Fecundity and egg viability of *Coccinella transversalis* females increased with increase in number of mating and increased fecundity due to multiple mating may possibly be owing to the receipt of greater quantities of fecundity stimulants and the nutrients in the male ejaculate which are both stimulatory and nutritional (Xu & Wang 2011, Colares *et al.*, 2015, Xie & Pang, 2016) [17, 3, 18]. In a study, Kar and Ganguli (2016) [7] observed an elevated fecundity when single female was mated to multiple number of males (av. fecundity- 24.5, 34.4, 36.1 and 73.2 when mated with 1, 2, 3 and 4 males, respectively). Bista (2019) [1] also observed that fecundity and egg viability of females of *Coccinella transversalis* enhanced with increase in the number of mating and highest fecundity and egg viability occurred after 10 matings while the lowest values were noted from single mating. In the present study there was an elevated fecundity as the number of males exposed to single female was increased from one to seven and highest fecundity was observed when one female was exposed to seven females. The present findings were in agreement with the above.

4. Conclusion

From the present study it may be concluded that adult female *Callosobruchus chinensis* has a greater ovipositional preference towards pea than chickpea seeds. Egg production by the adult female is greatly increased when there is scope of multiple mating arising out from the presence of a greater number of adult males. Maximum fecundity is achieved from female mated with seven males. The actual number of effective mating for an optimum fecundity and the duration of fecundity in case of multiple mating out of a greater number of males are required to be studied further.

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