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Oviposition Behaviour of Pulse Beetle (*Callosobruchus chinensis* L.) on Different Stored Pulses

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ABSTRACT

Background: Adult female Pulse beetles, *Callosobruchus chinensis* L. use sensory receptors to detect host seed properties like texture, chemical composition and size for oviposition. Seed characteristics *viz*. size, texture and chemical cues significantly affect oviposition success and larval development. Perceiving these factors is crucial for managing infestations and reducing damage to stored pulses.

Methods: Oviposition behaviour was studied over two consecutive generations during 2022-23, using five stored pulses (chickpea, cowpea, lentil, pea and kidney bean) based on seed size, texture and shape.

Result: The longest oviposition duration was observed on cowpea, followed by kidney bean, while the shortest period on lentil. The highest number of eggs were laid on the larger-sized kidney bean seeds, followed by cowpea. The pulse beetles showed a preference for larger seeds with a smooth seed coat, over rough-textured seeds and kidney-shaped pulses for oviposition. Number of eggs laid increased with increase in seed size. Thus, the higher egg counts on kidney bean and cowpea seeds can be attributed to their larger size, smoother texture and kidney-shape. Oviposition duration and fecundity were higher in second generation in all the types of pulse seeds except kidney beans where larval development couldn't take place in first generation.

Key words: *Callosobruchus chinensis,* Oviposition behaviour, Stored pulses.

INTRODUCTION

Among the storage pests, the pulse beetles (*Callosobruchus* spp.), cause significant losses to pulses both in field and storage (Kalpana *et al*., 2022). *Callosobruchus chinensis* L. is a cosmopolitan and one of the most destructive pests in the seed stock. They are the most important bruchid pest in India (Mishra *et al*., 2013). They infest a variety of economically important legumes *viz*., cowpea, lentil, green gram and black gram (Srinivasan and Durairaj, 2008) and affect the seeds' germinability and nutritive value. They are prolific breeder and complete several generations in a year. Population development depends on the reproductive potential of the insect and oviposition behaviour is a key factor in the beetle's reproductive success and damage to stored pulses (Cury *et al*., 2019). Once mated, the female pulse beetles choose pulse whole seeds that provide optimal conditions for laying their eggs, which are glued to seed surface. Upon hatching, larvae burrow through the seed coat, reaching the endosperm and consume the inner content of seeds rendering them unsuitable for consumption, replanting, or sprouting (Guedes and Yack, 2016).

Oviposition behaviour in *C. chinensis* involves physiological and behavioural events, starting with egg movement through the oviduct and ending with egg placement on suitable substrates (Blumer and Beck, 2008). Egg laying behaviour ensures the fitness of a species that helps preserve the genetic continuity from one generation to the next and ensures a stable population. Before oviposition, females use sensory receptors on antennae, tarsi and ovipositor to detect environmental cues on the host surface, assessing texture, chemical composition and other properties (Yack, 2016). Eco-physiological and chemical factors, such as nutritional and allelochemical compounds, impact egg deposition and larval development (Singer, 1987). Seed coat guides oviposition (Kellouche *et al*., 2004) and adult beetle spreads eggs evenly when seed size is uniform (Cope and Fox, 2003). In storage, different pulse seeds vary in their size, mass and surface

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area, affecting egg-laying behaviour of the pulse beetle (Singha and Rajkumari, 2021). Perceiving these factors is crucial for assessing variation in fecundity in different stored pulses for reducing and managing pulse beetle infestations. This study examines how seed physical characteristics influence oviposition.

MATERIALS AND METHODS

Study location

The laboratory experiments were carried out in the Department of Agricultural Entomology at Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India during June-July of 2022 and 2023.

Maintenance of stock culture of *C. chinensis*

A stock culture of *C. chinensis* was established on healthy chickpea seeds and maintained in plastic containers with muslin-covered openings. To obtain freshly emerged and unmated adults, one egg per seed was allowed to hatch in glass vials and excess seeds were removed using a fine needle. The emerged adults were sexed based on identification keys of Khare (1993) and used in the experiments. The rearing setup was maintained at 27±2° C in a BOD incubator to ensure stable conditions.

Methodology

Oviposition duration

A freshly emerged adult pair (0-24 hrs) of pulse beetle was released in a plastic container (3cm diameter and 4 cm height) with 5 g of each pulse seeds (chickpea, cowpea, lentil, pea and kidney bean) and oviposition duration was recorded for two consecutive generations (Gen1 and Gen2). The experiment was repeated.

Oviposition behaviour

Oviposition behaviour, in terms of fecundity, was studied in various seed size, texture and shape. A newly emerged adult pair (0-24 hrs) was released in a plastic container (3 cm diameter and 4 cm height) with 10 g of each tested seed separately. The experiment was laid out according to the following set-up.

Seed size

Seeds within each pulse type were grouped into bigger (10 g), smaller (10 g) and mixed sizes (5 g + 5 g) (*i.e*. combination of bigger and smaller sized seeds). Bigger and smaller sized seeds of individual pulse were determined visually. The number of eggs laid on seed of each size was counted for 7 consecutive days across two generations (Gen1 and Gen2) and the experiment was repeated.

Seed texture

Two seed coat textures (smooth and rough) were observed in five tested pulses. The number of eggs laid on seeds under these two textures was recorded for 7 consecutive days across two generations and the experiment was repeated.

Seed shape

The number of eggs laid by adult insects on seeds of different shapes (round, kidney, flat and triangular) was counted for 7 consecutive days. The study was conducted across two generations and repeated.

RESULTS AND DISCUSSION

Oviposition behaviour of the *C. chinensis* was studied on two aspects such as oviposition duration and fecundity based on size, shape and texture of seeds of five different host pulses.

Effect of different pulses seeds on the oviposition duration of *C. chinensis*

The oviposition duration of the pulse beetle, varied significantly among the pulses (Table 1, individual year and pooled mean data). In Gen1, cowpea had the longest duration (7.50 days), followed by kidney bean (6.83 days), while lentil had the shortest period (4.50 days). In Gen2 also, cowpea and lentil recorded the longest and shortest oviposition duration (8.50 and 5.00 days respectively). No oviposition occurred in kidney bean in Gen2 due to failure of adult emergence in Gen1. Overall, the oviposition duration ranged from 4.50-7.50 days in the Gen1 and 5.00-8.50 days in Gen2, with a longer egg-laying period in Gen2 for most pulses except kidney bean (pooled mean data).

Table 1: Oviposition duration of *C. chinensis* in June-July, 2022 and 2023.

Pulses	Mean oviposition duration (days)					
	1st Trial (June-July 2022)		2nd Trial (June-July 2023)		Pooled mean	
	*Gen1	Gen2	Gen1	Gen2	Gen1	Gen2
Chickpea	5.33^{ab}*	6.33^{b}	5.33^{a}	6.67^{b}	5.33^{b}	6.50^{b}
Cowpea	7.33^{c}	8.33^{c}	7.67^{b}	8.67^{c}	7.50^{d}	8.50^{d}
Lentil	4.33^{a}	4.67^{a}	4.67^{a}	5.33^{a}	4.50^{a}	5.00^{a}
Pea	6.33^{bc}	7.00^{b}	6.67^{b}	7.33b	6.50^{c}	7.17^{c}
Kidney bean	6.67^{c}	-	7.00^{b}	-	6.83^{cd}	-
S.Em±	0.333	0.288	0.394	0.333	0.240	0.220
CD	1.050	0.941	1.24	1.08	0.700	0.650

*Numbers followed by same letters are statistically at par by DMRT; *Gen= Generation.

Oviposition is influenced by the seed size, texture, seed mass and chemical cues of the particular type of pulse seed. The adult pulse beetle is aphagous, entirely relying on nutrients derived during its larval stage. Its reproductive potential depends on the type of host seed it consumed at its larval stage. Hence, its fecundity and duration of egg laying varied according to the host pulse it consumed. Earlier studies also revealed that oviposition period of pulse beetle varied in different pulses. It ranged between 7.88±1.20 and 8.10±1.25 days in cowpea and chickpea respectively (Vyas, 2004; Pokharkar and Mehta, 2011); between 7.1±0.2 and 7.9±0.1 days in chickpea, cowpea and pea respectively (Sharma *et al*., 2016) and among different varieties of red gram, it ranged from 5.60-7.20 days (Harsh *et al*., 2022).

Effect of seed size on oviposition of *C. chinensis*

Oviposition of Gen1

There were substantial differences in the mean number of eggs laid in five different types of pulses (Table 2). Kidney bean seeds harboured the highest number of deposited eggs (86.61), followed by cowpea (79.06), while lentil recorded the significantly lowest number of eggs (41.72). The mean number of eggs laid was higher in big sized seeds (87.13) than mixed sized (67.07) and small sized seeds (59.17) (pooled mean). It was, therefore, confirmed that an increase in seed size was associated with an increase in egg number.

Egg laying varied among pulses due to their difference in seed size. The large- sized kidney bean seeds had the maximum egg deposition (103.67), followed by the mixed seeds (79.84) and the small seeds (76.34). Parallel outcome was observed in cowpea, with the most in big-sized seeds (94.50) and the least in small-sized seeds (67.50). Pea and chickpea also followed same size-depended trend. The large sized lentil seeds received the maximum eggs (56.33) followed by mixed seeds (40.34) while small seeds had the lowest number of eggs (28.50).

Oviposition of Gen2

The mean number of eggs laid in five different types of pulses varied significantly (Table 2). Maximum number of eggs laid in Gen2 was 82.17 on cowpea seeds, followed by pea (78.39) while, lentil (43.22) had the minimum. Since the first generation's egg failed to hatch, there was no egg laying in kidney bean in Gen2. We demonstrated earlier that seeds of kidney bean had thickest seed coat, lower protein and carbohydrate content, higher phenol, tannins, trypsin inhibitor and protease inhibitor activity that might have led to the failure of the larva to hatch and enter into the seed in Gen 1 (Mounika, 2024).

Among the different sizes of seeds, greater number of eggs (85.09) was observed in bigger sized seeds than mixed (66.21) and small sized seeds (57.96). Thus, size of the seed had an impact on the egg laying. The number of eggs increased with seed size.

The interaction effect shows that, big sized cowpea seeds received the highest deposited eggs (97.67), followed by mixed cowpea seeds (78.17) and small cowpea seeds (70.67) while small lentil seeds received the lowest number of eggs (30.50).

The beetles displayed a strong preference towards bigger size seeds irrespective of pulse type. It might be due to the ability of the female to judge accurately the seed mass during the oviposition for ensuring future offspring development. Adult female apparently determines the relative amounts of resources available in the seed by detecting size differences between seeds (Cope and Fox 2003). Mitchell (1990) demonstrated that females distributed eggs in proportion to relative seed mass rather than relative seed surface area. Various scholars cited that pulse beetle preferred the larger-sized seed for oviposition

Table 2: Fecundity of *C. chinensis* based on seed size in June-July, 2022 and 2023.

Pulses	No. of eggs /10 g of seeds							
	Gen1 (Pooled mean of 1st and 2nd Trial) (June-July of 2022 and 2023)				Gen2 (Pooled mean of 1st and 2nd Trial) (June-July of 2022 and 2023)			
	Big	Small	Mixed	Mean	Big	Small	Mixed	Mean
Chickpea	89.50(9.49)*	59.84(7.77)	67.67(8.26)	72.33$^{b\#}$(8.51)	91.84(9.61)	64.00(8.03)	69.84(8.39)	75.22^{b}(8.68)
Cowpea	94.50(9.75)	67.50(8.25)	75.17(8.70)	79.06^{d}(8.90)	97.67(9.91)	70.67(8.44)	78.17(8.87)	82.17^{d}(9.07)
Lentil	56.33(7.54)	28.50(5.39)	40.34(6.39)	41.72^{a}(6.44)	56.84(7.57)	30.50(5.57)	42.34(6.55)	43.22^{a}(6.56)
Pea	91.67(9.60)	63.67(8.01)	72.34(8.54)	75.89^{c}(8.72)	94.00(9.72)	66.67(8.20)	74.50(8.66)	78.39^{c}(8.86)
Kidney bean	103.67(10.21)	76.34(8.77)	79.84(8.97)	86.61^{e}(9.31)	0.00	0.00	0.00	0.00
Mean	87.13^{c}(9.32)	59.17^{a}(7.64)	67.07^{b}(8.17)		85.09^{c}(9.20)	57.96^{a}(7.56)	66.21^{b}(8.12)	
		Gen 1				Gen 2		
	Pulses	Size	Pulses × Size		Pulses	Size	Pulses × Size	
S.Em±	0.023	0.018	0.041		0.024	0.021	0.041	
CD	0.064	0.050	0.116		0.067	0.059	0.116	

Factorial CRD was done; *Figures in parentheses are square root transformed values;
Numbers followed by same letters are statistically at par by DMRT, *Gen= Generation.

(Ofuya, 1998, Kar and Ganguli, 2016, Chakraborty and Mondal, 2016). De Sa *et al.* (2014) reported that shape, size of the seeds, texture and thickness of seed-coat and toxic compounds on the seed coat affect the oviposition of the female *Callosobruchus* spp. However, Prajapathi *et al.* (2018) obtained a little higher number of eggs on medium sized seeds, although the lowest in small seeds. Umadevi *et al.* (2018) observed that chickpea varieties with higher test weight, white -colour and smoother seed surface received a greater number of eggs from pulse beetle.

Oviposition behaviour of *C. chinensis* based on seed texture

Seed textures were categorised as smooth (kidney bean, cowpea, pea and lentil) and rough (chick pea). Results revealed that the number of eggs were always higher on smooth seeds than rough textured seeds except lentil (Table 3, pooled mean data). In Gen1, the highest number of eggs were recorded on kidney bean (100.17) while it was cowpea (96.50) in Gen2 as no adult was produced in kidney bean in Gen1. Eggs laid on pea were 88.83 (smooth surface) and 93.00 while on chickpea (rough surface) 86.83 and 90.16 in Gen1 and Gen2 respectively. Minimum number of eggs were recorded on the lentil in two generations (54.34 and 57.16 respectively). The oviposition number in descending order followed the trend: kidney bean > cowpea > pea >chickpea>lentil in Gen1 while it was: cowpea> pea >chickpea>lentil in Gen2.

The female beetle laid more eggs on smooth -textured seeds than on rough ones, indicating that smooth coated seeds facilitated farm attachment of the eggs. Conversely, rough seed coat received lower number of eggs, likely due to their uneven, thicker seed coat and testa, retarding egg deposition or chemical cues that dissuaded oviposition. Texture of the seed coats affected oviposition and development and the females avoided rough seed coats for egg laying in *C. maculatus* (Ajayi and Lale, 2001; Bansod *et al.,* 2006). Though a smooth- textured, lentil seeds received the least number of eggs, likely due to its smaller surface area limits oviposition sites; if lentil seeds had the same surface area as chickpea, they could accommodate more eggs. Jones and Vamosi (2010) found the discrimination behaviour of adult female towards the size of the host seed to increase the fitness through increased fecundity of the offspring generations. Chakraborty and Mondal (2016) observed that kidney beans with a broad, polished seed coat received the maximum egg loads while Senthilraja and Patel (2024) demonstrated a clear preference of the pulse beetle for smooth and brown testa over wrinkled and white coated cowpea seeds. Both studies

Table 3: Fecundity of *C. chinensis* based on seed texture in June-July, 2022 and 2023.

Pulses	Texture	Mean no. of eggs/ 10 g of seeds					
		1st Trial (June-July 2022)		2nd Trial (June-July 2023)		Pooled mean	
		Gen1	Gen2	Gen1	Gen2	Gen1	Gen2
Chickpea	Rough	87.33$^{b\#}$(9.37)*	90.33^{b}(9.53)	86.33^{b}(9.32)	90.00^{b}(9.51)	86.83^{b}(9.34)	90.16^{b}(9.52)
Cowpea	Smooth	92.33^{c}(9.63)	96.00^{c}(9.82)	93.33^{c}(9.69)	97.00^{d}(9.87)	92.83^{c}(9.66)	96.50^{d}(9.84)
Lentil	Smooth	54.67^{a}(7.43)	57.33^{a}(7.60)	54.00^{a}(7.38)	57.00^{a}(7.58)	54.34^{a}(7.43)	57.16^{a}(7.60)
Pea	Smooth	89.00^{bc}(9.46)	92.67^{bc}(9.65)	88.67^{bc}(9.44)	93.33^{c}(9.69)	88.83^{b}(9.45)	93.00^{c}(9.67)
Kidney bean	Smooth	100.67^{d}(10.06)	0.00	99.67^{d}(10.01)	0.00	100.17^{d}(10.03)	0.00
S.Em±		0.076	0.068	0.090	0.047	0.053	0.038
CD		0.239	0.223	0.283	0.155	0.155	0.112

*Figures in parentheses are square root transformed values; #Numbers followed by same letters are statistically at par by DMRT; *Gen= Generation.

Table 4: Fecundity of *C. chinensis* based on seed shape in June- July, 2022 and 2023.

Pulses	Shape	Mean number of eggs/10 g of seeds					
		1st Trial (June- July 2022)		2nd Trial (June- July 2023)		Pooled mean	
		Gen1	Gen2	Gen1	Gen2	Gen1	Gen2
Chickpea	Triangular	84.33$^{b\#}$(9.21)*	87.67^{b}(9.39)	86.67^{b}(9.34)	89.00^{b}(9.46)	85.50^{b}(9.27)	88.33^{b}(9.42)
Cowpea	Kidney	94.00^{c}(9.72)	95.67^{c}(9.81)	94.67^{c}(9.76)	96.67^{c}(9.86)	94.33^{d}(9.73)	96.17^{d}(9.83)
Lentil	Flat	54.00^{a}(7.38)	56.33^{a}(7.54)	53.67^{a}(7.36)	56.00^{a}(7.52)	55.16^{a}(7.36)	56.16^{a}(7.52)
Pea	Round	88.33^{b}(9.43)	91.67^{bc}(9.60)	91.00^{c}(9.57)	93.33^{c}(9.69)	89.66^{c}(9.49)	92.44^{c}(9.64)
Kidney bean	Kidney	100.00^{d}(10.02)	0.00	100.67^{d}(10.06)	0.00	100.33^{e}(10.04)	0.00
S.Em ±		0.089	0.092	0.072	0.068	0.055	0.053
CD		0.282	0.301	0.228	0.220	0.160	0.156

*Figures in parentheses are square root transformed values; #Numbers followed by same letters are statistically at par by DMRT, *Gen= Generation.

showed that pulse beetle laid more eggs on seeds with larger areas having polished surfaces.

Effect of seed shape on oviposition of *C. chinensis*

Results showed significant variation in egg laying among different pulses due to variation in shapes (Table 4). In Gen1, the maximum number of eggs were laid on kidney bean seeds (100.33) followed by cowpea (94.33) and the minimum on flat-shaped lentil (55.16) seeds. In Gen2, maximum number of eggs were deposited on cowpea seeds (96.17) but minimum on the lentil (56.16) seeds. Thus, the oviposition number in descending order followed the trend: kidney shaped > round > triangular >flat.

Female *C. chinensis* preferred kidney-shaped seeds for their larger surface area and curvature, while round, triangular and flat seeds received fewer eggs due to narrower or uneven surfaces. The beetle favors smooth surfaces, with lentil seeds having the least egg laying due to their small size and flat shape. Gupta and Apte (2016) stated that pulse beetle preferred larger and heavier seeds with smooth surface as they offer greater oviposition area. Aarthi and Selvanarayanan (2023) found that green gram and broad bean seeds harboured the highest and lowest egg counts, indicating these two host seeds as the most and least preferred hosts for *C. chinensis* respectively. Mounika (2024) described that the female beetles identify the seed size according to surface curvature of the pulse. She also noted that pulse beetles preferred larger, smooth-coated seeds for oviposition, which aligns with the current findings, where kidney bean and cowpea, with larger sizes and smooth surfaces, were preferred.

CONCLUSION

Oviposition preference by pulse beetles is influenced by host features such as seed size, coat texture and shape. The longest oviposition duration occurred on cowpea, followed by kidney bean, while lentil had the shortest duration. The highest number of eggs was laid on larger kidney bean seeds, followed by cowpea. The beetles preferred larger, smooth-textured seeds as kidney-shape for oviposition. Thus, the highest egg count on kidney bean and cowpea seeds is attributed to their larger size, smooth texture and kidney shape.

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Conflict of interest

Authors declare that there is no conflict of interest regarding publication of this article.

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