Effect of temperature and relative humidity on oviposition, survival and seed damage by *Callosobruchus maculatus* on different hosts and evaluation of pigeon pea (*Cajanus cajan*) cultivars for resistance

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ABSTRACT

The present study was carried out during 2021-2023 at Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, to investigate the influence of temperature and relative humidity on oviposition, adult survival, and seed damage caused by the pulse beetle, Callosobruchus maculatus (F.), in various pulse crops, and evaluated the resistance of selected pigeon pea [Cajanus cajan (L.) Millsp] cultivars. Experiments were conducted under three environmental conditions, viz. 20°C/80% RH, 30°C/70% RH, and 40°C/60% RH. The experiment was laid out in a completely randomized design (CRD) with four replications. Soybean (Glycine max) was identified as the most preferred host for oviposition, with egg deposition per seed reaching 4.17, 5.44, and 2.64 under the respective conditions. Pigeon pea supported the highest adult survival (22.34, 64.65 and 13.31%), whereas lentil exhibited the lowest oviposition and survival, indicating strong resistance. Seed damage was highest in pigeon pea (27.04–36.59%), followed by green gram and chickpea, while lentil remained largely unaffected. Further evaluation of 15 pigeon pea cultivars commonly grown in western Uttar Pradesh (India) revealed significant variation in susceptibility. Cultivar BWR-23 exhibited the lowest adult emergence (19.23%), indicating resistance, whereas BSMR-146 and BSMR-198 showed the highest oviposition rates (6.61 and 6.46 eggs per female, respectively). BSMR-198 recorded the highest seed damage, followed by WSA-1, BSMR-146, T-21, and Bahar. These results highlight the significant influence of environmental conditions on pest behaviour and demonstrate variability in resistance among pigeon pea varieties. The findings provide critical insights for breeding programs and support the development of integrated pest management strategies targeting C. maculatus.

Keywords: *Callosobruchus maculatus*, Oviposition, Pigeon pea, Seed damage, Temperature, Varietal resistance

Grain legumes, or pulses, are essential components of vegetarian diets due to their rich nutritional profile, providing proteins, fiber, vitamins, minerals, and high-quality carbohydrates. India plays a significant role in global pulse production, contributing approximately 25% of the total output and accounting for 27% of global consumption (Bharathi *et al.* 2017). Major tropical and subtropical pulse crops such as chickpea, black gram, pigeonpea, green gram, and lentil serve as vital sources of protein for both human consumption and livestock feed. Despite their importance, India faces stagnation in pulse production, resulting in a widening demand-supply gap and increased dependence on imports. Post-harvest storage losses, mainly caused by

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bacterial, fungal, insect, and rodent infestations, further aggravate the issue, with insect pests alone responsible for 10–50% of the losses.

Among these storage pests, pulse beetles including Callosobruchus chinensis, C. maculatus, and C. analis are major culprits, infesting a wide range of pulses such as green gram, cowpea, lentil, black gram, and pigeon pea (Singh and Boopathi 2022a, 2022b). Callosobruchus maculatus is particularly damaging, capable of completing multiple generations within stored seeds. Females oviposit on seed surfaces, with larvae burrowing into the seed and developing within the endosperm, thereby reducing seed weight, viability, and market value if not effectively controlled (Mofunanya and Namgbe et al. 2016). Resistance in legumes is influenced by seed characteristics such as colour, texture, size, and hardness (Appleby and Credland 2003), while environmental factors particularly temperature and humidity profoundly affect pest biology. Elevated temperatures accelerate development, and high humidity enhances larval feeding and infestation levels (Mphosi and Modise 2012). These factors also modulate host preference and seed vulnerability.

Pigeon pea [Cajanus cajan (L.) Millsp], a key tropical pulse crop, is notably susceptible to C. maculatus; however, variations in susceptibility among cultivars offer promising avenues for identifying resistant genotypes. This study investigates the effects of temperature and relative humidity on C. maculatus oviposition, survival, and seed damage across multiple legumes, with a particular focus on assessing resistance among pigeon pea cultivars. The findings aim to support integrated pest management (IPM) strategies and promote sustainable pulse storage and production systems.

MATERIALS AND METHODS

Culture of Callosobruchus maculatus: The present study was carried out during 2021-2023 at Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh. The culture of *C. maculatus* was maintained on cowpea under controlled laboratory conditions (27 ± 1 °C temperature and $70 \pm 1\%$ relative humidity). Initially, adult beetles were collected from infested stored grains and introduced into open-mouthed plastic containers (10 cm × 5 cm) containing 100 g of disinfested cowpea seeds. These containers were kept inside wire mesh and glass cages to prevent escape. After 3–5 days, once oviposition was complete, the beetles were removed. Newly emerged adults were transferred to fresh cowpea seeds for mating and subsequent egg laying. This process was repeated at 10-day intervals to maintain a continuous supply of all developmental stages for experimental purposes.

Host preference of C. maculatus: To assess host preference, an experiment was conducted using eight pulse crops: pigeonpea, pea, lathyrus, green gram, black gram, soybean, chickpea, and lentil. Equal-sized compartments were created within a single container, each filled with 100 g of a test pulse. One hundred uniformly aged adult beetles (mixed sexes) were released at the center of the container, allowing them free movement to choose a preferred host. The study was conducted under three environmental conditions, $20 \pm 1^{\circ}\text{C/80} \pm 1\%$ RH, $30 \pm 1^{\circ}\text{C/70} \pm 1\%$ RH, and $40 \pm 1^{\circ}\text{C/60} \pm 1\%$ RH. The experiment was laid out in a completely randomized design (CRD) with four replications. Observations were recorded after five days, noting the number of eggs laid per seed.

Evaluation of pigeon pea cultivars for resistance: 15 pigeon pea cultivars varying in seed colour, size, and weight (Supplementary Table 1) were evaluated for resistance to *C. maculatus* using a forced-choice method. For each cultivar, 100 g of seeds were placed in four vials (6.5 cm height × 1.5 cm diameter), covered with muslin cloth to allow ventilation. One male and one female adult beetle (1-2 days old) were introduced into each vial. After 24 hours of incubation, the beetles were removed. The mean oviposition rate was calculated by counting the number of eggs on 50 randomly selected seeds using a 10 X magnifying lens. Survival from egg to adult was determined as the ratio of the total number of emerged adults to the number of eggs laid per seed (Howe 1971). Seed damage was assessed by calculating the percentage of damaged seeds among 50 randomly selected seeds from each treatment.

Table 1 Host preference of C. maculatus cultured on different pulses at different temperature and humidity conditions during 2021-22

Hosts	20°C temperature and 80% RH			30°C temperature and 70% RH			40°C temperature and 60% RH			
	No. of eggs laid*	% survival**	% seed damage**	No. of eggs laid*	% survival**	% seed damage**	No. of eggs laid*	% survival**	% seed damage**	
Pigeonpea	15.00	14.50	20.75	21.25	81.00	36.50	6.00	5.00	4.00	
	(3.93)	(22.37)	(27.08)	(4.66)	(64.24)	(37.15)	(2.54)	(12.91)	(11.53)	
Chickpea	9.00	12.75	11.50	16.50	73.25	22.75	4.25	4.00	2.25	
	(3.08)	(20.90)	(19.81)	(4.12)	(58.85)	(28.47)	(2.17)	(11.53)	(8.58)	
Pea	10.50 (3.31)	5.50 (13.54)	5.00 (12.91)	16.25 (4.09)	13.75 (21.75)	6.00 (14.14)	5.00 (2.34)	0.00 (0.00)	0.00 (0.00)	
Lathyrus	8.75	10.50	10.50	15.25	63.00	21.00	3.50	3.50	1.75	
	(3.04)	(18.89)	(18.89)	(3.96)	(52.51)	(27.26)	(2.00)	(10.75)	(7.52)	
Green gram	9.00	13.75	12.25	10.25	76.50	29.25	3.25	4.50	4.00	
	(3.08)	(21.74)	(20.47)	(3.27)	(60.97)	(32.72)	(1.93)	(12.22)	(11.53)	
Black gram	7.25	3.25	6.25	7.50	4.75	11.25	2.25	2.00	3.00	
	(2.78)	(10.25)	(14.64)	(2.82)	(12.57)	(19.58)	(1.65)	(7.85)	(9.97)	
Soybean	16.50	2.50	4.25	27.50	2.75	4.00	6.00	0.00	0.00	
	(4.12)	(8.83)	(11.87)	(5.29)	(9.50)	(11.22)	(2.54)	(0.00)	(0.00)	
Lentil	5.75 (2.50)	0.00 (0.00)	0.00 (0.00)	6.00 (2.54)	0.00 (0.00)	0.00 (0.00)	2.25 (1.65)	0.00 (0.00)	0.00 (0.00)	
SEM	0.327	0.301	0.228	0.77	0.645	0.275	0.323	0.250	0.125	
CD (P=0.05)	0.959	0.903	0.670	2.28	1.895	0.807	0.948	0.734	0.367	

^{*}Transformed values in to √n+0.5 are given in parenthesis; **Transformed values into angular are given in parenthesis. SEM, Standard error of mean; Cd, Critical difference.

Table 2 Host preference of C. maculatus cultured on different pulses at different temperature and humidity conditions during 2022-23

Hosts	20°C temperature and 80% RH			30°C temperature and 70% RH			40°C temperature and 60% RH		
	No. of eggs laid*	% survival**	% seed damage**	No. of eggs laid*	% survival**	% seed damage**	No. of eggs laid*	% survival**	% seed damage**
Pigeonpea	14.50	14.00	20.25	22.75	81.00	36.00	5.50	5.50	4.50
	(3.87)	(21.95)	(26.73)	(4.82)	(64.24)	(36.85)	(2.44)	(13.54)	(12.22)
Chickpea	9.50	13.25	10.75	15.25	70.25	23.75	3.75	4.00	2.75
	(3.16)	(21.33)	(19.11)	(3.96)	(56.92)	(29.15)	(2.06)	(11.14)	(9.50)
Pea	11.00	5.00	4.50	17.50	13.50	6.50	4.50	0.00	0.00
	(3.39)	(12.91)	(12.22)	(4.24)	(21.54)	(14.73)	(2.23)	(0.00)	(0.00)
Lathyrus	9.25	10.00	10.00	14.75	62.50	20.00	4.00	3.00	2.25
	(3.18)	(18.42)	(18.42)	(3.90)	(52.22)	(26.55)	(2.12)	(9.83)	(8.58)
Green gram	8.00	13.50	11.75	9.75	77.00	29.00	3.25	4.75	3.00
	(2.91)	(21.54)	(20.03)	(3.20)	(61.32)	(32.56)	(1.93)	(12.57)	(9.97)
Black gram	7.75	3.25	6.25	7.25	4.50	11.00	2.75	3.00	3.50
	(2.87)	(10.25)	(14.32)	(2.78)	(12.22)	(19.35)	(1.80)	(9.97)	(10.75)
Soybean	16.50 (4.12)	2.50 (8.83)	3.75 (11.14)	29.25 (5.45)	2.50 (9.04)	4.00 (11.22)	6.00 (2.54)	0.00 (0.00)	0.00 (0.00)
Lentil	5.75 (2.48)	0.00 (0.00)	0.00 (0.00)	4.25 (2.17)	0.00 (0.00)	0.00 (0.00)	1.750 (1.50)	0.00 (0.00)	0.00 (0.00)
SEM	0.275	0.241	0.265	0.631	0.508	0.405	0.228	0.319	0.191
CD @5%	0.807	0.713	0.779	1.853	1.491	1.189	0.670	0.936	0.561

^{*}Transformed values in to $\sqrt{n+0.5}$ are given in parenthesis; **Transformed values into angular are given in parenthesis. SEM, Standard error of mean; CD, Critical difference.

Statistical Analysis: Data on host preference and cultivar resistance were subjected to one-way analysis of variance (ANOVA) using SPSS software (Version 26.0). Tukey's Honest Significant Difference (HSD) test was employed for mean separation at a significance level of $P \le 0.01$.

RESULTS AND DISCUSSION

Host preference of C. maculatus: During 2021–2022, soybean recorded the highest mean oviposition by C. maculatus, with 16.50, 27.50, and 6.00 eggs per 50 seeds under 20°C/80% RH, 30°C/70% RH, and 40°C/60% RH conditions, respectively (Table 1). In contrast, lentil consistently exhibited the lowest oviposition across all tested environmental conditions, indicating its low attractiveness to C. maculatus. Among all temperature and humidity combinations, 30°C/70% RH proved most favourable for oviposition across all pulse types. Pigeon pea also exhibited high oviposition rates, suggesting susceptibility. These findings align with Bidar et al. (2021), who reported lentil as the least preferred host for oviposition by C. maculatus.

Regarding survival, pigeon pea showed the highest adult emergence with mean values of 14.50, 81.00, and 5.00 under 20°C/80% RH, 30°C/70% RH, and 40°C/60% RH, respectively (Table 1). Lentil was the most resistant host, with no adult emergence observed under any environmental condition. Green gram also supported high survival, indicating preference. In contrast, soybean exhibited low survivorship, confirming the findings of Falke *et al.* (2021), who identified soybean as a poor host for *C. maculatus* development. Lentil again emerged as the most resistant

pulse, corroborating the reports of Bidar et al. (2021).

Seed damage followed a similar trend. Pigeon pea recorded the highest damage levels, with 20.25%, 36.00%, and 4.60% seed damage under the three respective environmental conditions during 2022–2023 (Table 2). No seed damage was recorded on lentil, reaffirming its resistance. Green gram and chickpea also showed significant seed damage, whereas soybean had comparatively lower damage levels. Across all pulses, 30°C/70% RH was the most conducive condition for oviposition, survival, and seed damage, except in lentil. This is consistent with Longanathan *et al.* (2011), who reported similar environmental preferences by pulse beetles.

Evaluation of pigeon pea cultivars under forced-choice test: Among the 15 pigeon pea cultivars evaluated, BSMR-146 (42.90 \pm 0.062) and BSMR-198 (41.18 \pm 0.087) recorded the highest oviposition rates per female (Table 3). In contrast, the local cultivar showed substantially lower oviposition (16.03 \pm 0.176). Both BSMR-146 and BSMR-198 are characterized by large, dark brown seeds, a trait previously linked to increased beetle attraction (Sewsaran et al. 2019, Bashir et al. 2014). Chen et al. (2019) also associated seed coat colour with oviposition behaviour in bruchids.

Across both years of study, cultivar BWR-23 consistently exhibited the lowest survival (10.86 \pm 0.068%), while BSMR-198 (60.11 \pm 0.037%) and BSMR-146 (44.17 \pm 0.027%) recorded the highest. Genotypes BWR-23, GAVT-82-104, and PA-8508 demonstrated notable resistance with minimal survival. Similarly, BSMR-198 sustained the highest seed damage, followed by WSA-1, BSMR-146, T-21,

Table 3 Effect of different pigeon pea cultivar on the fecundity, per cent survival and per cent seed damage of *C. maculatus* on the basis of forced-choice test during 2021–2022 and 2022–2023

Cultivar	No. of eggs laid*			(% survival**		% seed damage**		
	2021–2022	2022–2023	Mean	2021–2022	2022–2023	Mean	2021–2022	2022–2023	Mean
T-21	12.75	12.75	12.75	40.37	43.82	42.10	35.50	36.00	35.75
	(3.64)	(3.64)	(3.64)	(39.74)	(41.44)	(40.59)	(36.57)	(36.87)	(36.71)
BWR-23	7.00	7.25	7.13	10.85	10.87	10.86	10.00	11.50	11.25
	(2.73)	(2.75)	(2.75)	(19.22)	(19.23)	(19.23)	(18.40)	(19.68)	(19.04)
GAVT 82-104	11.25	11.75	11.50	20.47	20.62	20.55	11.50	12.50	12.00
	(3.40)	(3.49)	(2.95)	(26.89)	(26.96)	(26.93)	(19.78)	(20.56)	(20.17)
UPAS- 120	7.50	8.50	8.00	43.33	43.30	43.30	34.00	16.00	25.00
	(2.76)	(2.96)	(2.87)	(41.14)	(41.15)	(41.15)	(35.58)	(23.36)	(29.47)
ICPL-267 A	8.75	8.75	8.75	39.30	47.62	43.46	22.00	22.00	22.00
	(3.00)	(3.01)	(3.00)	(43.16)	(43.63)	(43.40)	(27.63)	(27.81)	(27.72)
PA-104	6.00	6.00	6.00	31.73	32.45	32.09	20.50	22.50	21.50
	(2.52)	(2.54)	(2.53)	(34.27)	(34.700	(34.49)	(26.86)	(28.19)	(27.51)
PA-8507	6.25	7.00	6.63	32.57	32.42	35.00	20.50	22.51	21.50
	(2.57)	(2.71)	(2.64)	(34.79)	(35.28)	(35.04)	(26.86)	(28.17)	(27.52)
PA-8508	6.50	8.00	7.25	21.57	21.87	21.72	12.00	13.50	12.75
	(2.63)	(2.91)	(2.77)	(27.66)	(27.86)	(27.76)	(20.24)	(21.47)	(20.86)
Bahar	9.75	10.50	10.13	41.35	42.67	42.01	34.00	35.00	34.50
	(3.17)	(3.30)	(3.24)	(40.01)	(40.76)	(40.39)	(25.64)	(36.24)	(30.92)
WSA-1	5.00	6.00	5.50	36.20	41.68	38.94	38.50	39.50	39.00
	(2.34)	(2.54)	(2.44)	(26.96)	(40.20)	(38.58)	(38.33)	(38.93)	(38.63)
WSA-2	5.00	5.50	5.25	30.22	32.87	31.60	15.00	21.00	19.00
	(2.32)	(2.39)	(2.76)	(33.38)	(39.94)	(36.66)	(22.59)	(27.15)	(24.87)
BSMR-146	42.75	43.00	42.88	39.00	49.27	39.14	35.50	39.50	37.50
	(6.57)	(6.59)	(6.58)	(44.44)	(44.58)	(44.51)	(36.52)	(38.92)	(37.72)
BSMR-198	40.75	41.60	41.17	58.37	61.87	60.12	78.50	79.50	79.00
	(6.00)	(6.25)	(6.12)	(49.83)	(51.88)	(50.86)	(62.45)	(63.40)	(62.92)
MTH-15	9.25	9.75	9.50	41.47	42.02	41.75	23.00	24.50	23.75
	(3.10)	(3.18)	(3.14)	(39.97)	(40.41)	(40.19)	(28.63)	(29.62)	(29.13)
Local	15.50	16.50	16.00	36.77	27.20	36.99	21.50	23.50	22.50
	(3.96)	(4.12)	(4.04)	(37.29)	(37.57)	(37.43)	(27.59)	(28.95)	(28.63)
SEM	0.22	0.21		0.94	1.08		1.55	1.81	
CD	0.53	0.52		2.28	2.68		3.75	4.39	

^{*}Transformed values in to $\sqrt{n+0.5}$ are given in parenthesis; **Transformed values into angular are given in parenthesis. SEM, Standard error of mean; CD, Critical difference.

and Bahar. These results further highlight the susceptibility of large, dark brown seeds to beetle infestation. The findings are supported by Loko *et al.* (2022), who emphasized seed physical characteristics in host resistance. WSA-1, a white, medium-sized cultivar with a tough seed coat, recorded the lowest oviposition, while BWR-23 exhibited minimal survival and damage. This is in agreement with results from Naseri *et al.* (2022) and Samyukhta *et al.* (2020), who found similar resistance traits in cowpea and green gram genotypes.

ANOVA revealed significant differences in fecundity, survival, and seed damage across temperature, humidity, and host plant species (P<0.05). Significant interaction effects (Temperature \times RH \times Host) were also observed. Tukey's HSD analysis confirmed that soybean, pigeon pea, and lentil significantly differed in their susceptibility

to *C. maculatus*. The 30°C/70% RH condition was optimal for pest development, while 40°C/60% RH supported the least activity, with beetle development nearly absent on pea, soybean, and lentil. Among pulses, soybean recorded the highest oviposition; black gram and lentil showed the least. Pigeonpea supported the highest survival and seed damage, whereas black gram, pea, and soybean recorded negligible damage. Among pigeonpea cultivars, BSMR-146 attracted the highest oviposition, while BSMR-198 showed maximum survival and seed damage. BWR-23 and WSA-1, with white-coloured, tough, medium-sized seeds, consistently demonstrated resistance.

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