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Diversity and losses caused by pulse beetle in North-Western Himalayan region of India

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Abstract

Pulses constitute a significant category of crops in India, contributing substantially to its export revenue. India holds the distinction of being the largest producer of pulses globally. Pulses typically contain 20 to 25 percent protein by weight, which is twice the protein content found in wheat and three times that in rice. Pulses are infested by many insect-pests during storage, however, pulse beetle is one of the major pest causing substantial losses in different pulses. Out of the eight locations surveyed in Himachal Pradesh, *C. chinensis* was predominant and was found at all locations, while *C. analis* and *C. maculatus* was found at fewer places. The predominant specie of pulse beetle, *C. chinensis* caused damage and weight loss of different pulses at different locations. As far as orientation studies, out of eight pulses tested, the predominant specie i.e. *C. chinensis* oriented maximum towards green gram and least towards horse gram and kidney bean.

Keywords: Pulse beetle, *Callosobruchus chinensis*, green gram, chickpea, damage, weight loss, weight loss, kidney bean

Introduction

Bruchids, also known as pulse beetles, pea beetles, or bean beetles, primarily feed on legumes of the tribe Phaseoleae. The genus *Callosobruchus* is widely distributed in tropical and subtropical regions and infests seeds of mung bean, green gram, chickpea, and other pulses both before and after harvest. In India, 117 species of bruchids from 11 genera have been identified infesting various pulses, with *C. maculatus*, *C. chinensis* and *C. analis*, being the predominant species (Dias, 1986) [8]. While these beetles cause minor damage in the field, infested seeds stored after harvest allow adults to emerge and lay eggs on nearby seeds, leading to more severe secondary infestations. Females typically lay eggs singly on the surface of grain sheaths in the field or on dried seeds during storage. Upon hatching, larvae bore into the seed beneath the egg, leaving the eggshell attached to the seed. Larvae remain inside seed, and the presence of a sealed exit hole indicates the pupal stage (Singh and Pandey, 2001) [21]. Adults require neither food nor water and can reproduce immediately after emergence. This pest spends its entire immature life cycle within individual legume seeds, causing weight loss, reducing germination potential, and diminishing both market and nutritional values of the affected commodity.

Pulses are crucial field crops stored by farmers in modern bins or traditional structures for consumption and seeding purposes. In Himachal Pradesh, pulses are extensively consumed, and several insect pests, including pulse beetles, have been documented causing significant damage to stored pulses. Effective management of this pest requires a comprehensive understanding of its diversity across different districts of the state and the potential losses it can inflict on various pulse crops. Hence, this study was conducted to address these issues.

Material and Methods

Survey studies of pulse beetle in Himachal Pradesh

To know the presence of a particular specie of pulse beetle, samples of different pulses infested with pulse beetle were collected from different parts of Himachal Pradesh. Different species of pulse beetles were brought to laboratory and identified by running proper taxonomic key using a proper magnifying glass and microscope.

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Maintenance of culture of pulse beetle

A stock culture of pulse beetles was established using adult beetles collected from infested grains found in farmers' storage containers across various locations. To ensure the health of the pulse grains, they were meticulously cleaned and sifted to remove any grain fragments or insects. Additionally, the grains underwent disinfection to eradicate any visible or hidden infestations. The culture was then propagated and maintained in a laboratory at ambient room temperature. Fresh grains were regularly provided to sustain the beetle's development. After allowing the beetles to lay eggs for more than one week, they were removed from the jars using a Two-in-one pitfall trap and probe tap, and subsequently discarded. Grains containing eggs from the stock culture were segregated and transferred to new large plastic jars to establish a pure culture of pulse beetles. This culture was carefully maintained, and newly emerged beetles were used to perpetuate the pure culture of *C. chinensis* which maintained on green gram.

Quantitative losses caused by pulse beetle

Grains infested by pulse beetles from various locations were collected and brought to the laboratory to assess the quantitative losses caused specifically by *C. chinensis*. The evaluation was conducted on a host-by-host and location-specific basis at room temperature. To achieve this, 10 pairs of pulse beetles and one hundred randomly chosen seeds from different pulses (gathered from diverse locations) were placed in a 100g capacity plastic container. Observations focused on determining the number and weight of damaged versus undamaged seeds.

Subsequently, calculations were performed to quantify weight loss and grain damage in different pulses using established formula (Adams and Schulten, 1978)^[1].

a) Grain damage (%) = no. of grains damaged/total no. of grains in a vial x100

$$b) \text{ Weight loss (\%)} = \frac{(UND) - (DNU)}{U(ND+NU)} \times 100$$

U: wt. of un-damaged grains (g)

NU: no. of un-damaged grains

D: wt. of damaged grains

ND: no. of damaged grains

Orientation of *C. chinensis* towards different pulses

To conduct orientation studies, twenty grams of each type of pulse were placed in small circular containers, arranged in a circular pattern within a larger plastic box (see Figure 1). In the center of this setup, one hundred adults of *C. chinensis* were released. To ensure proper aeration, small holes were made in the lid of the box using a sharp pin. The experiment was replicated three times, and the number of adults oriented towards each type of pulse was recorded at 24, 48, and 72 hours after the initial release.

Results and Discussion

Species diversity in different parts of Himachal Pradesh

Surveys were conducted at eight distinct locations in Himachal Pradesh, namely Chamba, Kullu, Sirmaur, Sarkaghat, Solan, Una, Palampur, and Barot, to assess the species diversity of *Callosobruchus* spp. The collected pulse beetles were identified at CSIR-IHBT, Palampur, Himachal Pradesh. Morphological studies using taxonomic keys were performed on characteristics such as antennae, hind legs,

sternites, and elytra, revealing variations among the species within the genus *Callosobruchus*. Three predominant species of pulse beetle were identified: *C. chinensis*, *C. maculatus*, and *C. analis*. *C. chinensis* was found infesting pulses across all eight surveyed locations. *C. maculatus* was observed infesting pulses in Palampur, Barot, Chamba, and Una, while *C. analis* was found in Kullu and Solan (see Table 2).

Harish *et al.* (2018)^[9] conducted a survey across six districts of the Hyderabad-Karnataka region, identifying *C. analis*, *C. maculatus*, *C. chinensis*, and *Tribolium castaneum* as infesting major stored pulses, ranking *C. analis* as the dominant species among stored pulses. Kumar *et al.* (2005)^[12] reported *C. chinensis* as the predominant pest during their survey in Dharwad district, Karnataka. Similar findings were recorded by Hampanna *et al.* (2006)^[10] in Raichur district, Karnataka, where *C. chinensis* was identified as the most economically significant pest of chickpeas.

In Jaipur district, Sharma *et al.* (2017)^[3] conducted a survey across five randomly selected tehsils (Chaksu, Amer, Phagi, Phulera, and Jhotwara), noting increased moisture content and damage caused by *C. chinensis*. Similarly, Bajiya (2010)^[3] surveyed stored mung beans in these tehsils and observed infestations and losses attributable to *C. chinensis* across all tehsils.

Raina (1970)^[19] reported three species of *Callosobruchus* in India: *C. chinensis*, *C. maculatus*, and *C. analis*, with extensive studies focusing particularly on the first two species. *C. chinensis*, originally described in China in 1758, is known as the Adzuki bean weevil in Japan (Ishii, 1952)^[11] and as the pulse beetle in India (Srivastava and Bhatia, 1959)^[22]. *C. chinensis* spread from Asian countries to East America and subsequently to West Africa and the Mediterranean basin (Calderon, 1958).

Conducted a survey across three districts of West Bengal (Coochbehar, Alipurduar, and Jalpaiguri) to study the diversity and abundance of pulse beetle species infesting stored chickpeas from 2018 to 2020. They identified four species: *C. chinensis*, *C. maculatus*, *C. analis*, and an unidentified *Callosobruchus* sp. Coochbehar district exhibited the highest diversity, as indicated by species evenness (0.49), Brillouin index (1.17), Hill index (0.49), Simpson index (0.68), Shannon index (1.23), and effective diversity (3.43), followed by Jalpaiguri with Brillouin index (1.04), species evenness (0.46), Simpson index (0.64), Shannon index (1.09), Hill index (0.48), and effective diversity (2.99). Chakraborty also noted *C. chinensis*, *C. maculatus*, and *C. analis* as the most abundant pulse beetle species in the north-eastern regions of India, feeding on various hosts such as Bengal gram, green gram, red gram, and cowpea.

Black gram, cultivated in states including Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, Haryana, West Bengal, Andhra Pradesh, Karnataka, and Himachal Pradesh, suffers severe damage from pulse beetles. Green gram grown in states like Madhya Pradesh, Rajasthan, Uttar Pradesh, Punjab, Maharashtra, Haryana, West Bengal, Andhra Pradesh, Karnataka, Orissa, and Bihar also faces significant storage damage, primarily due to *C. chinensis*.

Quantitative losses caused by *C. chinensis* at different locations

C. chinensis infested various pulses across eight surveyed locations. The grains were brought to the laboratory to

assess infestation levels by this pulse beetle. In Palampur, it primarily affected horse gram, chickpea, green gram, kidney bean, and soybean. Green gram showed the highest damage and weight loss, followed by chickpea, with kidney bean exhibiting the least infestation. In Sarkaghat, *C. chinensis* was found infesting green gram and chickpea, causing more damage and weight loss in chickpea compared to green gram. At Chamba, black gram and horse gram were infested, with horse gram showing greater damage and weight loss. In Solan district, green gram, chickpea, and kabuli chickpea were affected, with green gram and chickpea experiencing higher damage compared to other districts. In Sirmaur district, infestation mainly occurred in green gram and black gram, with black gram showing more damage and weight loss than green gram. Kullu district saw *C. chinensis* infesting green gram, black gram, horse gram, and kabuli chickpea, with green gram showing the highest damage followed by kabuli chickpea, horse gram, and black gram. In Una district, infestation was found in green gram, black gram, and chickpea, with green gram showing the maximum damage and weight loss, possibly due to higher temperatures. Lastly, at Barot district, kidney bean and horse gram were infested, with kidney bean showing higher damage and weight loss compared to horse gram during laboratory evaluation.

The larvae of these beetles live inside the grains, filling the burrows with their excrement and dead bodies (Atwal, 1976)^[2], rendering the kernels unsuitable for milling if they contain more than 0.5% insect-infested material (Cotton, 1941)^[5].

Pathania *et al.* (2011)^[18] screened black gram varieties across six districts of Himachal Pradesh against *C. chinensis*, highlighting maximum seed damage, weight loss, and foreign matter in Kullu district, with Kangra district showing the least damage. Shafique and Ahmad (2002)^[20] conducted laboratory tests on various cultivars of lentil, mung bean, chickpea, black gram, and cowpea, revealing significant differences in adult development, oviposition, and weight loss among cultivars, indicating varying levels of resistance to *C. analis*.

Manohar and Yadav (1990)^[14] studied the damage caused by *C. maculatus* on cowpea cultivars, noting varied losses in apparent weight and seed viability among different varieties. Das *et al.* (2002)^[6] investigated the impact of *C. chinensis* on chickpea, arhar, green gram, peas, and kidney bean under controlled conditions, reporting significant emergence of adults in Kabuli chickpea and minimal or no infestation in kidney bean. Singh and Pandey (2001)^[21] studied soybean varieties against *C. chinensis*, finding substantial infestation levels and reductions in grain weight and seed germination in damaged seeds compared to healthy ones.

Orientation studies

An independent experiment was undertaken to investigate the orientation behavior of *C. chinensis* adults towards various pulses at 24, 48, and 72 hours post-release (Table 3). The results showed that, after 24 hours, the highest number of adults oriented towards green gram (22.33), followed by chickpea and kabuli chickpea (both 17.00), while the lowest orientation was observed towards kidney bean (7.33), followed by horse gram and black gram (both 7.67). This

trend of maximum orientation towards green gram persisted at 48 and 72 hours, with kidney bean and horse gram consistently receiving the least orientation. The differences in orientation numbers between pulses at different time intervals (24, 48, and 72 hours) were statistically significant. Therefore, the preference for orientation after 72 hours followed this descending order: green gram > chickpea > cowpea > kabuli chickpea > soybean > black gram > kidney bean > horse gram (Table 3).

Previously, Patel (2019)^[17] assessed 30 chickpea genotypes for *C. chinensis* orientation over similar time intervals, while Mehta and Kumar (2021)^[15] conducted a similar study on *Sitophilus oryzae* with wheat varieties. Mahor and Shrivastava (2018)^[7] explored the influence of chickpea genotypes on *C. maculatus* orientation, finding significant differences in adult preferences among various genotypes. Genotypes JGG-1, JG-16, JG-317, JKG-3, and JG-92-1 exhibited minimal orientation, whereas JG-130 and JSC-43 were most preferred by the beetles for orientation.

Table 1: Different pulses to be screened against *C. chinensis*

S. no	Pulses	Scientific name	Weight of 100 seeds (g)
1.	Cowpea	<i>Vigna unguiculata</i>	21.024
2.	Chickpea	<i>Cicer arietinum</i>	22.643
3.	Horse gram	<i>Macrotyloma uniflorum</i>	3.090
4.	Kabuli chickpea	<i>Cicerkabulium</i>	54.701
5.	Kidney bean	<i>Phaseolus vulgaris</i>	35.562
6.	Black gram	<i>Vigna mungo</i>	4.709
7.	Green gram	<i>Vigna radiate</i>	3.439
8.	Soybean	<i>Glycine max</i>	26.228

Table 2: Different species of *Callosobruchus* reported from different locations

S. no	Location	<i>Callosobruchus</i> Species		
		<i>C. chinensis</i>	<i>C. maculatus</i>	<i>C. analis</i>
1.	Palampur	+	+	--
2.	Sarkaghat	+	--	--
3.	Chamba	+	+	--
4.	Solan	+	--	+
5.	Sirmaur	+	--	--
6.	Kullu	+	--	+
7.	Una	+	+	--
8.	Barot	+	+	--

Table 3: Orientation of *Callosobruchus chinensis* towards different pulses

S. No.	Pulses	Number of adults oriented after		
		24hr	48hr	72 hr
1.	Cowpea	5.67 (2.37)	7.00 (2.60)	13.00 (3.59)
2.	Chickpea	17.00 (4.12)	18.33 (4.28)	22.67 (4.76)
3.	Horse gram	7.67 (2.75)	7.00 (2.63)	3.67 (1.91)
4.	Kabuli chickpea	17.00 (4.12)	18.00 (4.24)	11.00 (3.28)
5.	Kidney bean	7.33 (2.70)	4.00 (1.97)	4.33 (2.06)
6.	Black gram	7.67 (2.76)	5.00 (2.21)	5.33 (2.29)
7.	Green gram	22.33 (4.72)	32.00 (5.65)	34.33 (5.86)
8.	Soybean	15.33 (3.90)	7.67 (2.74)	5.67 (2.36)
	CD (0.05)	(0.49)	(0.69)	(0.59)

*Figures in parentheses are square root transformed values

Table 4: Damage caused by *C. chinensis* at different locations of Himachal Pradesh

Host	Location							
	Palampur	Sarkaghat	Chamba	Solan	Sirmaur	Kullu	Una	Barot
	Damage (%)							
Horse gram	22.67 (28.39)	0.00 (0.29)	21.00 (27.24)	0.00 (0.29)	0.00 (0.29)	30.00 (33.17)	0.00 (0.29)	25.33 (30.16)
Chickpea	36.67 (37.26)	40.00 (39.21)	0.00 (0.29)	38.67 (38.41)	31.00 (33.78)	33.00 (34.99)	42.67 (40.76)	0.00 (0.29)
Green gram	40.33 (39.41)	31.00 (33.81)	33.67 (35.39)	46.67 (43.08)	25.33 (30.16)	39.33 (38.83)	63.33 (52.77)	41.33 (40.00)
Black gram	29.67 (32.98)	25.33 (30.09)	19.33 (25.90)	24.67 (29.72)	38.00 (38.04)	19.67 (26.23)	37.67 (37.83)	0.00 (0.29)
Kidney bean	10.67 (18.95)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	35.33 (36.39)
C.D. (P=0.05)	(4.00)	(4.79)	(5.36)	(4.53)	(4.02)	(5.06)	(4.89)	(4.78)

*Figure in parentheses are angular transformed values

Table 5: Weight loss caused by *C. chinensis* at different locations of Himachal Pradesh

Host	Location							
	Palampur	Sarkaghat	Chamba	Solan	Sirmaur	Kullu	Una	Barot
	Weight loss (%)							
Horse gram	3.59 (10.91)	0.00 (0.29)	5.63 (13.69)	0.00 (0.29)	0.00 (0.29)	6.05 (14.23)	0.00 (0.29)	4.29 (11.87)
Chickpea	12.99 (21.12)	13.65 (21.68)	0.00 (0.29)	13.49 (21.55)	10.40 (18.74)	8.29 (16.74)	10.11 (18.49)	0.00 (0.29)
Green gram	14.36 (22.27)	10.11 (18.52)	13.02 (21.09)	15.93 (23.52)	7.54 (15.78)	11.14 (19.49)	16.71 (24.12)	10.65 (19.03)
Black gram	9.26 (17.71)	8.63 (17.06)	3.85 (10.87)	6.52 (14.43)	11.97 (20.24)	4.09 (11.64)	8.48 (16.93)	0.00 (0.29)
Kidney bean	4.43 (12.15)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	7.52 (15.74)
C.D. (P=0.05)	(0.83)	(1.55)	(3.69)	(3.48)	(2.99)	(1.03)	(1.52)	(2.98)

*Figure in parentheses are angular transformed values



Fig 1: Male and female of *C. chinensis*



Fig 3: Male and female of *C. analis*



Fig 2: Male and female of *C. maculatus*

Conclusion

From our present study, it can be concluded that, *C. chinensis* was predominant specie in NW Himalayan region of India and was found at all eight locations surveyed. *C. maculatus* was found at four locations out of eight while, *C. analis* was found only at two locations out of all surveyed. Pulse beetle caused damage and weight loss in different grains at different places. As far as orientation of adults of pulse beetle is concerned, maximum adults oriented towards green gram while, lowest towards horse gram and kidney bean. From our study, farmers and researchers can easily come to the conclusion about the specie of pulse beetle in that area and the extent of quantitative losses it is causing.

Author Contribution

Vasu Mehta carried out the experimental trials, designed the study and drafted the manuscript. RS Chandel designed the study, critically analysed and revised the manuscript

Declarations.**Conflict of Interest**

The authors declare that they have no conflict of interest. Also, no research grant from any type of funding agency was received for this whole research work.

Ethics approval

Not applicable.

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