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Susceptibility of four Indian grain legumes to three species of stored pest, bruchid (*Callosobruchus*) and effect of temperature on bruchids

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Abstract
Beetles belonging to the *Callosobruchus* genus are of economic importance due to their ability to feed on various legume crops in the field and during storage. The ovipositional preference and adult longevity of the three bruchid species, *i.e. Callosobruchus maculatus* F., *Callosobruchus chinensis* L. and *Callosobruchus analis* F. were evaluated on the four grain legume crops, *Vigna radiata* (mung bean), *Vigna mungo* (black gram), *Vigna unguiculata* (cowpea) and *Cicer arietinum* (green gram). The legume grains differed in their susceptibility to infestation with bruchids. The longevity of the beetles was not affected and nearly remained the same, *i.e*. 10-18 days for all the three species of *Callosobruchus* on all the pulses. The longevity of the bruchid beetles decreased with increase in temperature. Maximum longevity occurred at 30°C, while at and above 42°C the development was seized. The longest developmental period of bruchid beetles was nearly 80-85 days at the lowest temperature, *i.e.* 18°C and the shortest was 23 days for *Callosobruchus chinensis* at 30°C on mung bean. The bruchid infestation diminishes the seed germination ability and seedling growth potential of all the species of legumes.

Keywords: oviposition, longevity, bruchids, legume crops, temperature

1. Introduction

Grain legumes are rich in proteins (20-40%), carbohydrates (50-60%), vitamin B1 and B3, calcium and iron. Worldwide, 840 million people are under nourished mainly due to insufficient proteins, vitamins and minerals contents in their diets. Legumes are combined with cereal grains to get a balanced profile of the amino acids. These crops are infested by over 200 species of insects in India (Anonymous, 2007) [3]. Among them, coleopteron and lepidopteron insects are the major cause of stored food grain damage (Girma 2006; Negamo *et al*., 2007) [13, 25]. Bruchid beetles belong to order Coleoptera of family *Bruchidae*, are the insect pests of stored grains of several legumes (Southgate, 1979) [33]. In India 8.5 per cent of pulses are wasted due to the inadequate storage facilities and their vulnerability to stored grain pests (Shivanna *et al*., 2011) [31]. The degree of damage depends on the storage conditions, containers and grain legume varieties (Nchimbi-Msolla and Misangu 2002; Mebeasilassie, 2004) [24, 21]. The primary infestation occurs in the field, where bruchid adults lay eggs on pods after which larvae hatch, penetrate into the seed and feed on cotyledonary and/or embryonic tissues (Mohan and Subbarao, 2000) [22]. Seeds damaged by bruchids are not fit for consumption (Deshpande *et al*., 2011; Bae *et al*., 2014) [10] or planting and also reduce their aesthetic and market value (Singh, 2011; Sarwar, 2015) [32, 30].

Several options including chemical and physical methods have been employed to control damage caused by insects. Usually, chemicals are used to control the bruchids but the chemical insecticides are expensive and have adverse effects on human health and environment (Bindhu *et al*., 2015). Moreover, the insects are developing resistance to insecticides and are not effective in controlling them. Because of economic, health and environmental considerations emphasis has also been given to, increasing temperatures and imparting resistance in food legumes through crop breeding programs worldwide to prevent bruchid infestation (Kashiwaba *et al*., 2007; Srinives *et al*., 2007) [16, 34]. The development of bruchids is enormously affected by the temperature (Osman *et al*., 2015; Maharjan *et al*., 2017) [26, 20] and the species of grain legumes that in turn affects extent of the damage and weight loss of infested grains (Borikar and Puri, 1985; Lale and Vidal, 2003; Adhikary and Anandamay, 2012; Sujeetha and Srikanth, 2013) [7, 18, 1, 2].

The present study was undertaken to evaluate the susceptibility of four important Indian grain legumes to the infestation of three species of *Callosobruchus* and effect of different temperature regimes on growth, development and longevity of *Callosobruchus* species. The seed germination potential of four grain legumes after bruchid infestation was investigated. The outcomes of the study may be useful for the future management of the most devastating storage pest, *Callosobruchus*.

2. Materials and Methods

2.1 Bruchid beetles maintenance and their infestation

All experiments were conducted in a laboratory at the Department of Zoology, M. D. University, Rohtak, India. Three bruchid beetles including *Callosobuchus maculatus*, *C. chinensis and C. analis* were evaluated and the population of these bruchids were maintained under laboratory conditions on mung bean seeds (*Vigna radiata*) inside a BOD cabinet (Calton-NUAO250485 NSW, New Delhi, India) at 27 ± 2°C, 12:12 Light: Dark conditions and with 70% relative humidity (RH). The bruchids were allowed for mating and oviposition on mung bean seed under laboratory condition. Then parental insects were removed and mung bean seeds containing eggs were transferred to a fresh lot of mung bean seeds in the glass bottles that were covered with pieces of muslin cloth fastened with rubber band to prevent the contamination and escape of bruchids. The emerged adults were used for the bioassay within 48 h of emergence. The left over insects were used to start a new culture so that the cultures with emerging adults were available continuously.

2.2 Procurement of insects and seeds

Three kilograms seeds of each legume species i.e. cowpea (*Vigna unguiculata*), mung bean (*V. radiata*), black gram (*V. mungo*) and green gram (*Cicer arietinum*) were procured from the local market, Rohtak, India. The seeds were firstly cleaned and disinfected by keeping them in a freezer at 5˚C for 7 days to kill all hidden infestations. The disinfected seeds were then placed inside a Hot air oven (Hicon, Delhi, India) at 40˚C for 4 hours before they were stored in plastic containers with tight lids (Ileke *et al*., 2013).

2.3 Susceptibility of different legume grains to Callosobruchus infestation based on weight loss:

To assess the weight loss of different legume grains two experiments (free choice test and no choice test) were conducted separately using the same experimental procedures. The experiments were performed in triplicates. Percent weight loss was calculated as described by Deshpande *et al*., 2011 [10] and Turaki, 2012 by using the following formula:

$$\text{Per cent weight loss of grains} = \frac{\text{UND - DNU}}{\text{U (ND + NU)}} \text{ X } 100$$

NU ND = Number of damaged grains, D = Weight of damaged grains

NU=Number of undamaged grains, U = Weight of undamaged grains

2.3.1 Free choice test

Ten pairs of newly emerged bruchids of each species of the same generation were placed into glass bottles containing 50g seeds of each legume species. The glass bottles were covered with pieces of muslin cloth fastened with rubber band to allow good aeration and to prevent escape of bruchids from bottles. These were kept in the BOD (27 ± 2 ºC and RH 70 ± 5%) without disturbance, to ensure that the beetles mate and oviposit naturally. Each culture was regularly observed and seeds were weighed weekly up to 12 weeks, throughout three successive generations to calculate the weight loss of different legume seeds.

2.3.2 No choice test

Newly emerged 15 male and 15 female adults of each bruchid species were introduced to glass bottles containing 100 g legume seeds of each legume species for 24 h for egg laying on the surface of seeds. After 24 h all the adults were removed and the seeds were examined for egg deposition. The seeds without any eggs were removed. 50 g seeds of each legume grain species bearing approximately 5 eggs per grain were placed in transparent glass bottles (300 ml) and closed by muslin cloth. Regular observations were made for each culture of three bruchid species and seeds were weighed weekly up to 12 weeks to calculate the weight loss.

2.4 Effect of legume species on the reproductive biology and longevity of bruchids

Five pairs of newly emerged bruchid adults (≤ 6 h old) of each species were collected separately from stock cultures reared on mung bean. Each couple was placed in a Petri dish (90x10 mm) containing ten seeds. The seeds were daily examined by magnifying lens to count the number of deposited eggs. The infested seeds were replaced by other non-infested seeds and so forth till the death of bruchid adults. Adults were also checked daily and the alive and dead adults were recorded until the death of all tested female or male bruchids. The experiment was performed in five replicates of each bruchid couple and legume species.

2.5 Effect of temperature on the development and longevity of bruchid beetles

The effect of temperature on the development of bruchid beetles raised on different legume grains was studied at five constant temperature regimes (18, 23, 30, 35 and 42 ± 1°C) and 70 ± 5% RH, uses five different BOD cabinets. The grains were kept at 30 ± 1°C and 70 ± 5% RH for 24 hours before being used for the experiment. Twenty seeds of each legume species bearing fresh eggs of three bruchid species (one egg/grain) of homogenous age (≤ 6 hours old) were placed in separate clean Petri dishes (90x10 mm). Three replicates were performed for each tested temperature. Grains were daily monitored for the development of bruchid beetles, and data was recorded in terms of egg hatching, adult emergence, survival and longevity of emerged adults throughout the experiment for each tested temperature.

2.6 Effect of insect infestation on seed germination

To study the effect of insect infestation on seed germination, the infested and non-infested seeds were germinated on filter paper moistened with distilled water under controlled conditions (27±2°C, 12 h photoperiod and 70±5 % RH) for 72 h. Water was poured regularly to prevent drying. The number of germinated and non-germinated seeds was counted after 12, 24 and 72h and the mean percent germination loss was calculated as described by Jat *et al*. 2013 [15] using the following formula: Number of non-germinated grains Mean germination loss (x 100 Total no. of grains kept for germination

2.7 Data analysis

All data were subjected to one-way analysis of variance (ANOVA) using Vassar stat system, 2017 and means were separated by LSD test at a 0.05 level of significance using Fisher's LSD calculator, 2017.

3. Results

3.1 Seed weight loss assessment

The seed weight loss percentage of four legume species differed significantly after 12 weeks of infestation by adults in free choice test of three *Callosobruchus* species (Fig. 1). *Callosobrucus maculatus* caused the maximum damage to cowpea seeds and the minimum to the chickpea seeds. The minimum damage to chickpea seeds may be due to the coarse surface of these seeds than the cowpea and mung bean seeds. However, *Callosobruchus chinensis* and *Callosobruchus analis* caused the maximum damage to mung bean seeds and the minimum damage to black gram seeds. The grain characters interfere the normal feeding of the insect and affects the biology of the pest adversely and thus make a different susceptibility pattern of the different species of legumes to three species of *Callosobruchus* attack. Seed weight loss by three species of bruchid adults differed significantly ($p < 0.05$) among the four-legume species after 12 weeks of infestation (Fig. 1). The F and P values for *Callosobruchus maculatus* were 63.92 and 0.0001, for *C. chinensis* 22.06 and 0.0003 and for *C. analis* 44.34 and 0.0001, respectively.

The seed weight loss percentages in no choice test of three *Callosobruchu*s species also differed significantly after 12 weeks of infestation (The F and P values for *Callosobruchus maculatus* were 60.19 and 0.0001, for *C. chinensis* 39.75 and 0.001 and for *C. analis* 6.74 and 0.01; Fig. 2). The pattern of damage caused by *Callosobruchus maculatus* eggs and adults was same (in both the cases, cowpea seeds were severely affected then other legume species Figs. 1 & 2) but in case of *Callosobruchus chinensis* and *Callosobruchus analis* the pattern was different as the mung bean seeds were maximally damaged by both bruchid species (Fig. 2). The *Callosobruchus chinensis* and *Callosobrucus maculatus* caused minimum damage to chickpea seeds compared to *Callosobruchus analis*, whereas the extent of damage to black gram seeds by *C. analis* and *C. chinensis* was nearly same but less than *C. maculatus* (Fig. 2).

3.2. Reproductive biology of bruchids:

The order of preference for oviposition for *Callosobruchus maculatus* was *Vigna unguiculata* > *Vigna radiata* > *Cicer arietinum* > *Vigna mung*o; for *Callosobruchus chinensis*, it was *Vigna radiata* > *Vigna unguiculata* > *Vigna mungo* > *Cicer arietinum* and for *Callosobruchus analis*, it was *Vigna unguiculata* > *Cicer arietinum* > *Vigna radiata* > *Vigna mungo*. The fecundity was highest on cowpea seeds for *Callosobrucus maculatus* (195.67±6.35; F=82.79; P≤0.0001), for *Callosobruchus chinensis* fecundity was high (192.67 ± 3.71; F=324.11; P≤0.001) on mung bean seeds, whereas for *Callosobruchus analis* it was high on chickpea seeds (120.24 ± 0.57; F = 83.26; P≤0.0001). *Vigna mungo* was least preferred legume species by *Callosobruhus maculatus* and *Callosobruchus analis* as the lowest fecundity was observed on it by *Callosobruchus maculatus* (78 ± 4.16) and *C. analis* (90.33 ± 0.88). *Cicer arietinum* was least preferred by *C. chinensis* as the fecundity was the lowest (66 ± 2.08) on this grain legume (Fig. 3). The longevity was varied from 10-18 days for all the three species of *Callosobruchus* on all the four grain legumes. However, males were short lived than the females.

3.3. Effect of temperature on development and adult longevity of bruchids

The temperature influences the development of the bruchid beetles especially at egg hatching and larval period. The results showed that the best temperature for bruchid beetle development was 30°C, while at 42°C and above the development was seized as the bruchid eggs at this temperature either failed to hatch or didn't complete their development. At tested lowest temperature of 18°C, the longest developmental period of 85 days was observed for *C. maculatus* on black gram seeds, 80 days for *C. chinensis* on chickpea seeds and 75 days for *C. analis* on black gram seeds. The shortest developmental period of 23 days was observed at 30°C for *C. chinensis* and of 25 days for *C. maculatus* on mung bean and cowpea seeds respectively but for *Callosobruchus analis* developmental period of 29 days was observed on mung bean seeds (Table 1).

Adult longevity decreased as temperature increased expect at 30°C, where it ranged between 10-18 days for all the three species. In general, female bruchid beetles of all the species lived longer than male bruchids irrespective of different temperature regimes (Table 2).

3.4. Seed germination potential

The infested and non-infested seeds germinated were counted after 24 to 72 h of imbibitions. The black gram seeds, infested by all the three insects, showed maximum germination potential whereas mung bean seeds, infested by all the three insects, showed minimum germination potential than the other tested legume species. The bruchid infestation lowers the seed germination ability of legumes (Table 3). Hence, the insect infestation negatively affected the germinability of legume species.

1. Figures and Tables

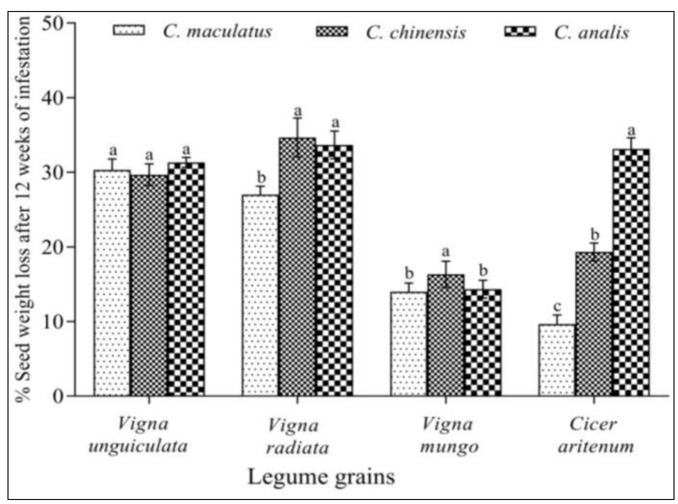

Fig 1: Percent seed weight loss in four legume grains by three *Callosobruchus* species using free choice test. Bars with different letters indicate significant difference (P≤0.05)

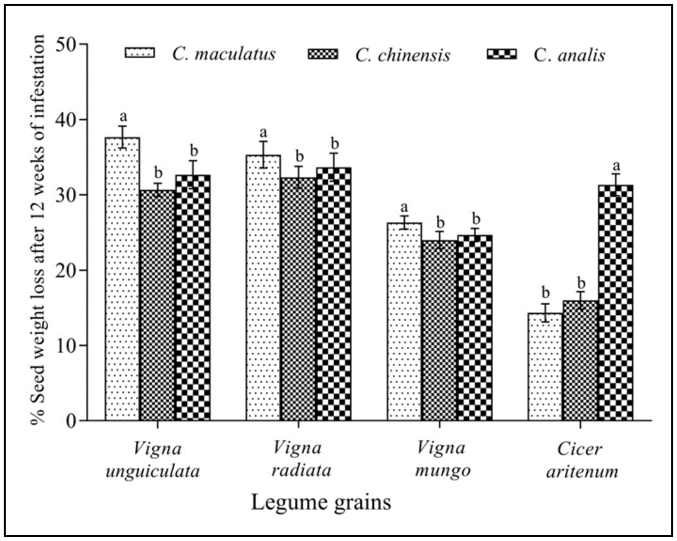

Fig 2: Percent seed weight loss in four legume grains by three *Callosobruchus* species using no choice test. Bars with different letters indicate significant difference (P≤0.05).

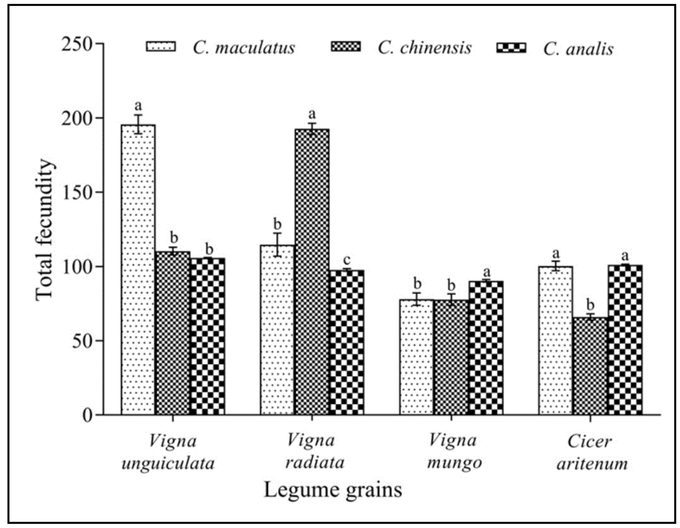

Fig 3: Influence of four legume grains on the fecundity of three species of Callosobruchus. Bars with different letters indicate significant difference (P≤0.05).

Table 1: Effect of different temperatures on the development of three species of Callosobruchus reared on four legume grains (*V. unguiculata*: VU; *V. radiata*: VR; *V. mungo*: VM; *C. arietinum*: CA)

Temperature (°C)	Total developmental period (days)											
	C. maculatus				***C. chinensis***				***C. analis***			
	VU	VR	VM	CA	VU	VR	VM	CA	VU	VR	VM	CA
18	60 ± 2.4^{a}	70 ± 2.7^{a}	85 ± 0.6^{a}	80 ± 1.4^{a}	75 ± 3.1^{a}	60 ± 0.5^{a}	70 ± 1.7^{a}	80 ± 2.3^{a}	70 ± 0.4^{a}	65 ± 1.7^{a}	75 ± 0.9^{a}	70 ± 2.3^{a}
23	45 ± 1.5^{b}	40 ± 3.1^{b}	45 ± 1.8^{b}	47 ± 2.3^{b}	44 ± 0.9^{b}	46 ± 1.6^{b}	48 ± 2.1^{b}	46 ± 3.4^{b}	45 ± 0.9^{b}	45 ± 1.2^{b}	46 ± 0.5^{b}	45 ± 1.5^{b}
30	25 ± 0.9^{c}	28 ± 1.4^{c}	32 ± 2.1^{c}	30 ± 0.5^{c}	31 ± 1.6^{c}	25 ± 1.8^{c}	30 ± 0.9^{c}	28 ± 1.8^{c}	30 ± 1.7^{c}	29 ± 2.1^{c}	31 ± 1.9^{c}	30 ± 0.7^{c}
35	28 ± 1.8^{c}	30 ± 2.1^{c}	30 ± 0.8^{c}	32 ± 1.9^{c}	27 ± 1.9^{c}	28 ± 2.4^{c}	29 ± 1.4^{c}	30 ± 1.9^{c}	31 ± 2.2^{c}	31 ± 0.6^{c}	32 ± 1.3^{c}	31 ± 1.8^{c}
42	No development takes place											

Means in the same column followed by the same letters are not significantly different (LSD P ≤ 0.05)

Table 2: Effect of different temperatures on the longivity of *Callosobruchus*

Temperature (°C)	***C. maculatus***		***C. chinensis***		***C. analis***	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
18	8.5 ± 0.7^{b}	12.3± 1.4^{b}	6.2 ± 1.6^{b}	10.4 ± 1.3^{b}	7.5 ± 2.8^{b}	11.8 ± 1.6^{b}
23	6.4 ± 1.1^{c}	13.7 ± 2.3^{b}	5.9 ± 0.86^{b}	13.9 ± 1.9^{b}	6.8 ± 1.5^{b}	13.1 ± 0.9^{b}
30	11.8 ± 0.8^{a}	17.7 ± 3.1^{a}	11.2 ± 1.7^{a}	18.1 ± 2.1^{a}	10.8 ± 1.3^{a}	17.9 ± 2.1^{a}
35	5.3 ± 0.6^{c}	9.2 ± 1.9^{c}	5.2 ± 1.6^{b}	11.4 ± 1.7^{b}	5.5 ± 0.9^{b}	9.8 ± 0.7^{b}
42	1.05 ± 1.2^{d}	3.19 ± 2.4^{d}	1.5 ± 0.78^{c}	4.82 ± 1.3^{c}	1.58 ±1.7^{c}	3.9 ± 2.3^{c}

Means in the same column followed by the same letters are not significantly different (LSD P ≤ 0.05)

Table 3: Seed germination potential of four legume grains after infestation by three species of *Callosobruchus*

Legume grains	% germination (after 72 hours)		
	C. maculatus	***C. chinensis***	***C. analis***
Vigna unguiculata	75.5 ± 0.31^{b}	80.4 ± 0.11^{a}	81.4 ± 0.11^{a}
Vigna radiate	72.4 ± 0.13^{b}	74.2 ± 0.84^{b}	76.5 ± 0.41^{b}
Vigna mungo	85.2 ± 0.32^{a}	83.2 ± 0.53^{a}	85.1 ± 0.12^{a}
Cicer arietinum	82.1 ± 0.17^{a}	79.3 ± 0.32^{a}	79.3 ± 0.33^{a}

Means in the same column followed by the same letters are not significantly different (LSD P ≤ 0.05)

3.5 Discussion and future prospects

Callosobruchus is a serious oligophagous pest of stored grains (Huignard *et al*., 1996) [14]. In the present study, different legume species showed different degree of susceptibility to infestation of *Callosobruchus* species. Cowpea seeds were found to be more susceptible to the infestation by *C. maculatus* whereas mung bean seeds were more susceptible to the *C. chinensis* and *C. analis*, respectively. This may be due to the smooth seed coats and smaller size of mung bean seeds which were suitable for the insects to hold the seeds while depositing eggs (Girish *et al*., 1974; Mwanze *et al*., 1975; Radha and Susheela, 2014; Osman *et al*., 2015) [12, 23, 28, 26]. However, chickpea was less preferred for oviposition than the other tested legume seeds by all the three species of bruchid. These results are in accordance with the earlier reports (Bhaduria and Jakhmela, 2006) [6]. However, this may be due to the rough surface of seed coat and the large size of chickpea seed which inhibited the egg laying. The grain characters may also interfere the normal feeding of the insect and affects the biology of the pest adversely thus making a legume species resistant to insect attack.

The present study also revealed that the fecundity of *Callosobruchus maculatus* was highest on cowpea seeds whereas those of *C. chinensis* on mung bean seeds. The longevity of all the three species of *Callosobruchus* on all the pulses was also affected by different temperature regimes. The host species has influenced the egg and adult bruchid beetle production. Cowpea and mung bean were the preferred hosts for *Callosobruchus* species to produce higher populations of insect as compared to chickpea and black gram seeds probably due to their smooth surface and nutritional qualities (Wijertane, 1998; Kazemi *et al*., 2009; Shivanna *et al*., 2011; Yunus *et al*., 2015) [36, 17, 31]. The morphology of seeds and their chemical composition played a significant role in the biology of *Callosobruchus*. The cowpea seeds that are rich in protein and sugar proved to be more favorable than other legume seeds for the development of bruchid beetles (Ouali-N'goran *et al*., 2014) [27]. Our studies showed that black gram has also been affected by all the three species of bruchid infestation; however, infestation was low as compared to other *Vigna* species. These results are in contrast to an earlier report (Srinives *et al*., 2007) [34], where *Callosobruchus maculatus* was unable to feed on the black gram seeds. It may be due to different climatic and geographical conditions.

Temperature had an immense effect on developmental stages of all the three species of *Callosobruchus* which failed to develop at 42°C. As the temperature increased, a significant decrease was observed in the total development period of bruchid (Lale and Vidal, 2003; Loganathan *et al*., 2011; Adhikary and Anandamay, 2012) [18, 19 1]. The temperature also affected the longevity of bruchids, the adult longevity decreased as temperature increased (Sujeetha and Srikanth, 2013; Riaz *et al*., 2014; Osman *et al*., 2015; Maharjan *et al*., 2017) [2, 29, 26, 20]. The 27°C to 35°C temperature was found to be the best for bruchid development (23-31 days life cycle), whereas lower temperatures (below 20°C) delayed the life cycle of bruchids (60-85 days) and higher temperature proved to be detrimental as no development takes place at or above 42°C. Thus, the lower and higher temperature ranges can be used to manage *Callosobruchus* species as thermal disinfestations as thermal disinfestations may become the promising tool of physical insect control method for stored grains.

Insect infestation has affected the seed germination (Bewley, 1997; Crawley, 2000) [5, 8]. However, the different insect species caused variable seed damage resulting into diverse seed germination response which varied from a complete inhibition of seed germination to no effect on seed germination (Vallejo-Marin *et al*., 2006) [35]. The insects emerged from holes in seeds (Beck and Blumer, 2007) [4] and these holes interfere with control of water intake during germination. In addition, these holes ease the entry of microorganisms. The consumption of seed reserves by the insects is also a factor that impairs the seed germination (Bae *et al*., 2014; Cruz *et al*., 2015) [11]. In the present study, a direct relationship between higher infestation and lower seed germination was observed. The chickpea seeds that were least affected by insect infestation, showed lower seed mass loss and superior performance during germination (Cruz *et al*., 2015). Future studies are required to determine the lethal low and high temperatures that can be used as thermal disinfectants against pulse beetles without affecting the quality of stored grains.

4. Statement of interest

The authors declare that they do not have conflict of interests.

5. Acknowledgements

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