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Screening of relative susceptibility of different pulses to the pulse beetle, *Callosobruchus maculatus* fab. (Coleoptera: Bruchidae)

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Abstract
A research study entitled "Screening of relative susceptibility of different pulses to *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) and its biology" was undertaken in the laboratory on variety of pulses. The pulses selected for the study were green gram (*Vigna radiata*), Cowpea (*Vigna unguiculata*), Chickpea 'desi' and 'kabuli' varieties (*Cicer arietinum*), Horse gram (*Macrotyloma uniflorum*), Black gram (*Vigna mungo*) and Soya bean (*Glycine max*). The selected pulses were subjected to antibiosis (No-choice confinement test) and antixenosis test (Free-choice test) in the laboratory for their comparative resistance against the pulse beetle. The no-choice bioassay was carried out to assess the influence of each seed type on the egg laying of *C. maculatus* without disturbance by the other seed type. In antixenosis test (Free-choice test), preference and non-preference response of pulse beetle to all test pulses were assessed. The resulting data were subjected to one-way analysis of variance and the mean values obtained were grouped using Least Significant Test (LSD). In no-choice test, maximum number of eggs were found in green gram (61.0±20.74) and minimum in soya bean (30.20±17.17). The adult emergence (63.60±18.06), weight loss (5.39%), frass weight (0.13±0.01), germination (62%) and vigour index (1486.4) and were maximum in green gram. The above said observation confirms through no-choice test that the green gram was highly susceptible to bruchid infestation. Although, the oviposition was observed on black gram (50.60±27.3) and soya bean (30.20±17.17), the adult emergence was nil with lowest percentage of weight loss (black gram 1.07% and soya bean 0.63%). Therefore, the research study confirmed that the black gram and soya bean were highly resistant to *C. maculatus*. Similar observation were also made from free-choice test where the pulse beetle was attracted more towards green gram and cowpea. The pulse beetle preferred soya bean and black gram as a host seed and require less management practices during storage. The present study revealed that the black gram and soya bean were not suitable for the growth of *C. maculatus* and can be recommended for relatively long term storage.

Keywords: relative susceptibility, pulse beetle, *Callosobruchus maculatus, Coleoptera: Bruchidae*

Introduction

Pulses commonly called legumes are the edible seeds of plants from the pea family, cultivated for consumption. The important source of protein, especially for vegetarians or for people who do not get enough protein by eating meat, fish or dairy are pulses. In addition, pulses are a healthy choice for meat-eaters, helping cut off excess fat from diets, and contain zero cholesterol. They are also a good source of dietary fibre, vitamins and minerals, especially iron and zinc. In terms of gram-for-gram nutritional value, pulses are also far cheaper than meats or other sources of protein, offering an economical alternative. (UN News, 2021) [48]

Legumes are an important crop for farmers because they can both sell them and consume them, which helps farming families maintain food security. Legumes also help with economic stability-when dried they can be stored for a long time. They also help to increase the diversity of diets, especially in developing countries (Sakalian, 2020) [37].

India has made remarkable progress in enhancing the pulse production during the past 15 years. The total production of pulses during 2005-06 in India was 13.38 million MT, which increased to 25.58 million MT during 2020-21. This shows an impressive growth of 91% or a compound annual growth rate (CAGR) of 4.42% (Gaur, 2021) [11].

The practice of storing food grains began 4,500 years ago as a safeguard against poor harvests and famine due to adverse weather conditions. Since time immemorial, several species of storage insect pest attacked granaries and other food structures and even today storage losses remain notoriously high. The overall damage caused by stored grain insect pests accounts for 10-40% worldwide annually. In India, 10% of the total production is lost at the farm level during storage. (Chaubey, 2011) [6]

The post-harvest losses and quality deterioration by storage pests are major problem globally. Pulse beetle is the most notorious pest in legumes in majority of tropical countries (Ratnasekar and Rajpakse, 2012) [34]. Among several pests, *Callosobruchus maculatus* (Fabr.) is a major pest of stored legumes. The pest infestation of the pest begins in the field and the population expands rapidly in storage, resulting in reduced seed weight, nutritional quality, and seed viability. Therefore, the grains become unsuitable for human consumption.

During storage, the pulses are attacked by pulse beetle, *Callosobruchus maculatus* (Fabr.) both in the field (before harvest) and storage. The actual damage is caused by the larval stage which spend its entire life within the seed coat. It is usually detectable only during adult emergence, by the time the damage has already been over. (Islam, 2012) [16].

Proper storage of pulses at domestic level is essential to avoid storage losses and enhance food security. Pulses must be stored a safe place until it is needed for consumption and seed purpose. Since pulse production is seasonal and consumption is continuous, safe storage must maintain grain quality and quantity (Gayathri and Manimozhi, 2013) [12].

Various researchers have assessed the genotype susceptibility to *Callosobruchus* species within the legume family (Srinivasan *et al.,* 2008; Kazemi, *et al.,* 2009; Swella and Mushobozy, 2009) [45, 20, 46] within some pulse species, eg. *Vigna* sp. (Asante and Mensah, 2007; Srinivasan and Durairaj, 2008; Badoor *et al.,* 2009) [2, 45, 4] and chickpea (Jha *et al.,* 2002; Aslam *et al.,* 2006; Shaheen *et al.,* 2006) [19, 39, 36]. However, the report on the susceptibility of varietal pulse seeds to *C. maculatus* is meagre. The detection of varietal susceptibility of seed could allow the selection of natural resources for the management of pests by improving genetic resistance in legume seeds. (Tarver *et al.,* 2006; Olakojo *et al.,* 2007) [47, 27]. Screening of resistant variety and use of the same is one of the ecologically sound method and are needed for an integrated approach to pest management. Therefore, the susceptibility of selected, locally available legume seeds were assessed against the pulse beetle, *Callosobruchus maculatus.*

Materials and Methods

An investigation was carried out in the laboratory to find out the relative susceptibility of pulse seeds to the bruchid beetle *C. maculatus*.

Rearing of test insects

Adults of *Callosobruchus* species were collected from household and they were identified under Stereo Binocular Microscope (Karl Zeiss Stemi DV4) using the keys of Southgate and Howe (1958) [43] and Halstead (1963) [14].

A pulse beetle rearing cell was maintained in the laboratory by collecting 10 pairs of *C. maculatus* from the stock and released into separate pre sterilized jars containing green gram seeds. After 7 days, adults were removed and the seeds bearing eggs were placed in a Biological Oxygen Demand (BOD) incubator at 30°± 2°C and 70 ± 5% RH until adult emergence. First generation progeny (F1) was used for bio-assay studies.

Susceptibility test

To evaluate the susceptibility of different legumes procured from the local market were subjected to susceptibility test as prescribed by Shaheen *et al.* (2006) [39]. The selected seeds *viz*., green gram (*vigna radiata*), cowpea (*Vigna unguiculata*), chickpea (*Cicer areitinum*), black gram (*Vigna mungo*), horse gram (*Macrotyloma uniflorum*) and soya bean (*Glycine max*) were subjected to presterilization by keeping the seeds in hot air oven at 160º-180º C for two hours. The sterilized seeds were subjected to antibiosis (No-choice confinement test) and antixenosis (Free-choice test) test in the laboratory for their comparative resistance against the pulse beetle.

Experimental design

No-choice confinement test

In this test, *C. maculatus* was allowed access to only one seed genotype. The no- choice bioassay was carried out in order to assess the influence of each seed type on the egg laying of *C. maculatus* without any hinderance by the other seed type. In antibiosis test, polyethylene bags were used as experimental units with 5 replications. In these bags 25 g of each pulse seed was placed, and 5 pairs of newly emerged adult beetles were released and covered loosely with rubber bands to avoid the escape of beetles. To provide sufficient air, the bags were punched and kept in the laboratory condition for further evaluation.

Observation

The following parameters were recorded to screen the susceptibility of pulse seeds to *C. maculatus***.**

A. Oviposition: The eggs laid by *C. maculatus* was recorded 10 days after artificial introduction of *C. maculatus* in each pulse seeds. The dead insects were removed from the grains.

B. Adult emergence: The number of adults emerged after 25-35 days were recorded depending upon the variety of pulse seeds. The number of days required by pulse beetle from each seed was also observed (Developmental period-Larva to Adult).

C. The percent weight loss: The loss in weight of the grain was observed by removing the frass and the emerged adult. The percent weight loss was calculated at the end of experiment by using the following formula,

$$\text{Percent damage} = \frac{\text{Initial weight-weight of sound grains \& damaged grains}}{\text{Initial weight}}$$

D. Germination and vigour index: To determine the germination and vigour of uninfested and infested seeds by *C. maculatus* 30 days after experiment, seeds were germinated in germination towels following the Wet Towel Method (ISTA, 1996) [18]. Ten seeds from each experiment were taken and soaked in distilled water for 3 hours. The water was decanted, and the seeds were arranged in germination towels and rolled. After one week, the numbers of germinating seeds of varietal pulses were counted and the seedling parameters of root length and shoot length were measured. The vigour index of the seedling was calculated using the following formula.

$$\text{Vigour index} = \text{Germination percentage x (Mean length of root + Mean length of shoot)}$$

Free-choice confinement test

In antixenosis test (free-choice test), preference and non-preference response of pulse beetle to all the test seeds were assessed following the method of Shaheen *et al.,* (2006) [39]. The free-choice bioassay aimed at evaluating the influence of the discriminative attractiveness of the seed type towards the weevil oviposition. Twenty grams of each legume seeds were taken in separate earthen cups and kept in the wooden boxes covered with lid 25x 45 cm^2 and replicated 5 times. Thirty pairs of newly emerged adult beetle were released in the center of every box. The boxes were closed immediately after the release of beetles to avoid their escape and were placed in laboratory condition at a temperature of 30°± 2°C and 70 ± 5% relative humidity. In this test, one box having all the pulse seed in equal weight was considered as one replication. The number of beetles attracted to each cultivar was calculated indirectly by counting the number of eggs laid by *C. maculatus* on ten days after introduction. The dead insects were removed after recording oviposition. Adult emergence, percentage weight loss, germination and vigour index were also observed and recorded as in the case of no-choice confinement test.

Statistical analysis

The data on oviposition, adult emergence and vigour index from free-choice and no-choice tests were subjected to one way analysis of variance. The percentage of weight loss, damage and germination were converted to ARC sine values before subjected to ANOVA.

Results

The observation on Susceptibility of pulses to the pulse beetle, *Callosobruchus maculatus* through no-choice and free-choice method have been tabulated (Table 1 and Table 2). The statistical analysis revealed that significant difference occurred among various pulses infested by pulse beetle, *C. maculatus.*

The observation was made on oviposition followed by adult emergence with number of emergence holes after 25th day of insect infestation, percentage weight loss and frass weight to identify and differentiate the resistant and susceptible varieties of pulses, which will be helpful for management practices.

Susceptiblity test

The Susceptibility of different legumes were evaluated by no-choice and free-choice test.

No-choice test

Oviposition

Oviposition by the beetle on different pulses were recorded after the death of introduce insects (10 days after infestation). The highest number of eggs were laid on green gram (61.00± 20.74). The lowest number of eggs were laid on chickpea 'desi' variety and the value being 26.00±15.07. The egg laying was moderate on cowpea (46.80±19.61), followed by chickpea 'kabuli' (37.20±8.76), horse gram (34.40±6.19) and soya bean (30.20±17.17), which were on par with each other.

Adult emergence

The adult emergence was recorded from 25th day onwards. The data revealed that all the eggs laid by *C. maculatus* did not complete its life cycle and emerged into adults. Therefore, no correlation occurred for oviposition and adult emergence.

The cent percent growth of *C. maculatus* in green gram was possible, which was indicated by the complete hatching of eggs (61.00±20.74) followed by its emergence (63.00±18.06). Here the number of adults exceeded the number of eggs, which may be due to the prior infestation by beetle in the field. The adult emergence was completely nil in black gram and soyabean although the beetle preferred those seeds for egg laying. This might be attributed to the seed characteristics, which is not suitable for its growth. Similar to green gram all the eggs laid on Cowpea, 'desi' and 'kabuli' chickpeas and horse gram become hatched and completed its life cycle and emerged as adults (Cowpea – 46.00±22.62; 'desi' – 29.80±9.93; 'kabuli' – 36.80±10.26; Horse gram 34.60±4.72).

The observation on oviposition and adult emergence revealed that the green gram was considered as highly susceptible variety whereas black gram and soya bean as resistant one to *C. maculatus*. The rest of the pulses such as cowpea, 'desi' and 'kabuli' chickpeas and horse gram were moderately susceptible to bruchid infestation.

Percentage weight loss and frass weight

The quantitative loss caused by *C. maculatus* was expressed as weight loss. The percentage weight loss was recorded after complete emergence of *C. maculatus* on pulses. Similar to egg laying and adult emergence, the percentage weight loss was maximum in green gram (5.39%). This was followed by chickpea 'kabuli' (4.35%), chickpea 'desi' (3.80%), horse gram (3.75%) and cowpea (3.59%). The statistical analysis indicated that the percentage weight loss was on par with each other for the above-mentioned pulses even though slight differences recorded among those varieties. The percentage weight loss was lowest in black gram (1.07%) and soya bean (0.63%) as there was no adult emergence.

The quantity of the frass weight was maximum in green gram (0.13±0.01) as these grains were highly infested by the pulse beetle followed by cowpea and the value of frass weight being 0.09±0.05. As the seeds of black gram and soya bean are more resistant to the beetles and the development of the beetles was retarded, the frass weight in these grains were found to be nil. Rest of the pulses recorded moderate quantity of frass weight (horse gram-0.07±0.03; chickpea 'kabuli'-0.04±0.04; chickpea 'desi'-0.02±0.01).

Germination and vigor index

The present study revealed that the cent percent germination was not recorded in any of the pulse varieties tested. Among the pulses tested, *C. maculatus* infested green gram recorded 62% germination and 1486.4 vigour index. Above 50% germination and higher vigour index was recorded in horse gram (58% and 604.26), black gram (54% and 899.06), and cowpea (54% and 774.2). The germination and vigour index of the seeds of chickpea 'kabuli' was completely arrested in the present study. This might be attributed to insect infestation in chickpea 'kabuli'. Although, the seeds of soya bean were not infested by the beetle, the germination and the vigour of the seeds were nil.. The reason for the significant reduction in percent germination of chickpea varieties and seedling vigour index 30 days after the experiment was due to the insect attack.

Free choice method

Oviposition

In free-choice test, the *C.maculatus* was found to laid maximum number of eggs on cowpea (98.00±54.50) followed by this, the pulse beetle preferred to lay eggs on black gram (69.00±63.68) and soya bean (61.00±35.43). Moderate number of eggs were laid on green gram (48.00±20.19). The *C. maculatus* did not preferred the seeds of 'desi' and 'kabuli' varieties of chickpeas and thereby the number of eggs laid on these grains were minimum (chickpea 'desi'-5.60±2.88; chickpea 'kabuli'-4.20±4.97). The scrutiny of the study showed that the pulse beetle *C.maculatus* selected cowpea as highly preferable seeds and thereby the maximum number of eggs were laid on it.

Adult emergence hole and weight loss

The number of adults emerged from each pulse seed were indirectly recorded by counting the number of adult emergence hole. Since, it may not be possible to identify the beetles from which type of seed it has been emerged. The comparison of egg laying and the number of adult emergence hole showed that not all the eggs were found to hatched and therefore the significant difference occurred between these two parameters. The cowpea seeds were found to be most susceptible when compared to other pulses and the number of adult emergence hole was recorded maximum (64.0±57.7) with the weight loss of 10.1%. The green gram and horse gram were moderately susceptible with 26.0± 15.95 and 10.0±9.0 number of adult emergence hole respectively. Although, the number of adult emergence holes were moderate, the weight loss caused by *C. maculatus* was maximum (11.19%) in green gram. The development of *C. maculatus* on two varieties of chickpea, black gram and soya bean were impossible and thereby the number of adult emergence hole and the percentage weight loss of these grains were minimum (or) nil. Fewer number of adults with minimum weight loss were recorded for 'desi' and 'kabuli' chickpeas, they were statistically on par with black gram and soya bean and the number of holes and weight loss of these grains were nil.

Germination percentage and vigour index

Similar to the observation on no-choice test, none of the pulse seeds recorded cent percent germination and vigour index. The germination and vigour index of green gram was maximum (74.0 and 1779.8) followed by cowpea (54.0 and 1010.4), chickpea 'desi' (50 and 712.3) and horse gram (42.0 and 405.6). The seeds of 'kabuli' chickpea and soyabean were not vigorous and the germination was found to be nil.

Discussion

Insects destroy 5% of the world production of all food grains while they are in storage in elevators, in warehouses or in farms. The losses consist of lowered weight and food value, insect adulteration, heating of grains, mould spoilage and low germination of seed. The use of synthetic insecticides or fumigants against stored product insects is not practiceable due to undesirable residues, alternate methods for beetle control are needed (Deepa *et al.*, 2006). Gene based resistance is one of the most satisfactory and sustainable methods of pest control, particularly as a basic element in integrated pest management approach. (Nalini *et al*., 2012) [25]

In the present investigation, the cultivars variety of mungbean (Green gram) had been used which was more susceptible to pest attack. The study of Venugopal *et al*. (2000) [49] explained the fact for the susceptibility of cultivar variety and the resistance exhibited by the wild variety. The wild seed varieties showed greater amount of resistance to the bruchid attack when compared to that of cultivar varieties. All the cultivar varieties studied showed higher amounts of primary metabolite content with high infestation rate. The wild varieties however showed significantly lower amounts of these primary metabolites and consequently a lower level of infestation. The non–protein anti metabolites (secondary metabolites) such as total phenols, ortho-dihydroxy phenols and tannins were significantly lower in the cultivars. The wild varieties in contrast, revealed higher amounts of these secondary metabolites with lower infestation rate. Deeba *et al*. (2006) [9] have also reported that typically wild mungbean have characteristics of small seed and the presence of a well-formed layer on the seed. These characters act as oviposition deterrents.

The present observations on green gram with maximum susceptibility rate are in conformity with most of the previous workers who reported that no mungbean genotypes was found immune to the pest infestation. (Davis (1959) [7]; Howe and Currie (1964) [15]; Raina (1970) [33] ; Singh. *et al*. (1980) [41]; Hamed *et al*. (1988) [13]; Chakraborty *et al*. (2004) [5]; Deeba *et al*. (2006) [9] and Shivanna *et al*. (2011) [40].

From the present study, it was inferred that the cowpea was susceptible next to green gram. This was supported by several workers. Among the seven *vigna* species studied for its susceptibility by Ofuya, (1987) [26], *Vigna unguiculata* was the most severely affected by *C. maculatus.* Cowpea *V. unguiculata* was significantly more susceptible than soya bean (*Glycine max*) to *C. maculatus* infestation (Pessu and Umeozor, 2004) [31]. Cowpea variety co cp-7 and co-6 was found to be highly susceptible. (Malaikozhundhan and Thiravia, 2011) [22]. Sharah and Ashigar (2012) [42] and Pessu and Umeozor (2004) [31] investigated that the white Cowpea varieties was more susceptible with more population than soya bean.

In the present investigation, the 'Kabuli' chickpeas are affected more by the beetle than the 'desi; chickpea. This was similar to the findings of Reed *et al*. (1987) [36] who expressed thick, rough and tuberculate seed coats of 'desi' was found to be resistant (or) free from damage. The Susceptible nature of 'Kabuli' chickpeas was also reported by Parameshwarappa *et al*. (2007) [29] and Erler *et al*. (2008) [10].

In the present study, all the pulses in no-choice experiment recorded oviposition ranging from 26.0±15.07 to 61.0±20.74. However, the adult emergence was occurred in green gram, chickpea 'desi' and 'kabuli' and horse gram and nil in black gram and soya bean. Therefore, the susceptibility cannot be assessed only based on oviposition. This lends support from previous work by Ahmed *et al*. (1989) [1] who reported that the seed resistance are better indicated by the number of emergence holes than that of the number of eggs present on the seed.

In the present study, the highly infested green gram recorded 62% germination and 1486.4 vigour index while the uninfested soya bean recorded no germination. The reduction in germination percentage of green gram was due to insect infestation. This falls in line with the findings of Megerssa (2010) [24] who reported that germinations were inhibited in treatments with higher number of adult emergence. Parameshwarappa *et al*. (2007) [29] noticed the reason for the reduction in germination of chickpea. According to them, the reduction was due to the increased damage to seed and metabolic wastes such as uric acid produced by bruchids.

Although, the adult emergence registered nil for soya bean, the germination too was not observed in our study. This indicated that the seeds were not viable. Reddy (2012) [35] stated that the soya bean seed is reported to have poor viability and field emergence as compared to other pulse crops due to its inherent seed structure and composition.

In free-choice test of present investigation, the cowpea beetle. *C. maculatus* selected cowpea as a most preferable host. This was in accordance with the findings of Swella and Mushobozy (2009) [46] who reported that the cowpea, garden pea and pigeon pea seeds recorded highest number of eggs and adult emergence, weight loss, shortest developmental period and highest susceptibility indices. Mannan and Bhuiyah (1996) also supported our view that, the hatchability of *C. maculatus* in cowpea was over 85%.

The number of adult emergence holes in 'desi' and 'kabuli' chickpeas, horse gram, black gram and soya bean were minimum or nil in free-choice test of our investigation. This may be due to the pre-adult mortality of *C. maculatus*. The pre-adult mortality of *C.maculatus* in chickpea through free-choice test have also been reported by Panzarino *et al.*, 2011 [2]. Both bruchid development and pre-adult mortality might be due to the presence of multiple antinutritional factors such as enzyme inhibitors; vicilins; lectins contained into the cotyledons (e.g., tannins), which can be added with additive or synergistic action in deterring, poisoning and starving the pulse larvae (Dick and Cleland, 1986; Piergiovanni *et al.*, 1994; Edde and Amatobi., 2000; Lattanzio *et al.*, 2005; Sales *et al.*, 2005; Partida *et al.*, 2007) [8, 32, 21, 38,30].

The beetles were attracted more towards cowpea (98.00±54.50), black gram (69.00±63.68), soya bean (61.00±35.43) and green gram (48.00±20.19). This was evident from the present investigation through fecundity of beetle on the above-mentioned pulses. However, the pulse beetle was not attracted towards rest of the pulses such as, both varieties of chickpeas ('desi'-5.60±2.88; 'kabuli'-4.20±4.97) and horse gram (14.60±9.97). In contrary to this, Islam *et al.* (2007) [17] reported that the *C. maculatus* preferred chickpeas for oviposition and the number of eggs were also more (61%) on this legume. They also reported that chemical cues and/or textures of the seed coat might be the reason for such differential choices.

Conclusion

The overall data revealed that black gram and soya bean were highly resistant against the beetles and thereby they can be stored for longer periods. Desi and kabuli variety of chickpea and horse gram were moderately susceptible to bruchid infestation. The green gram and cowpea were highly susceptible to bruchid infestation and need more management practices to save them from bruchid infestation

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