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Screening of chickpea varieties for resistance against the pulse beetle, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

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Abstract

The experiment was conducted during September 2020 - July 2021 at Junagadh Agricultural University, Junagadh, Gujarat, India. The pulse beetle *Callosobruchus maculatus* (F.) is an economically important and most destructive pest of pulses including chickpea. The pest being an internal feeder is known to cause considerable losses to chickpea seeds during storage. Establishment of management strategies against *C. maculatus* for safe storage of chickpea seeds is important. So, attempts were made to study the various aspects pertaining to safe storage of chickpea seeds against *C. maculatus*. Ten varieties (GG 1, GG 2, GJG 3, GG 4, GG 5, GJG 6, JG 16, Dahod yellow, KAK 2 and AKG 1303) were tested for their susceptibility to *C. maculatus* based on the population growth, weight loss and germination loss during storage and categorized as highly resistant, resistant, susceptible and highly susceptible. In a nutshell, varieties GG 4 and GG 5 which fall under the highly resistant (HR) category whereas Dahod yellow, JG 16, GJG 6 and GJG 3 were resistant (R). GG 2 and GG 1 were susceptible (S) and the varieties KAK 2 and AKG 1303 were found highly susceptible (HS) to *C. maculatus*. None of the varieties tested was found to be immune against *C. maculatus*. Wrinkled or reticulated and dark seed varieties, these features seemed to be important in the preference of the bruchid for host selection. These results suggests that resistant varieties can be used against pulse beetle.

Keywords: Pulse beetle, *Callosobruchus maculatus*, chickpea, resistant varieties

1. Introduction

Chickpea (*Cicer arietinum* L.), originating from Southeastern Turkey, holds a pivotal position among global leguminous crops (Ladizinsky, 1975; Toker, 2009) [9, 22]. It dominates as the foremost *rabi* season food legume worldwide, cultivated across 15 m ha and yielding 15.9 mmt (Anonymous, 2023) [2]. Known for its nutritional richness, chickpea provides essential energy, protein, minerals, vitamins, fiber, and beneficial phytochemicals. Its nutrient-dense profile, often likened to "poor man's meat," makes it a popular choice for both human consumption and animal feed (Jukanti *et al.*, 2012; Kaur and Prasad, 2021) [7, 8].

The cultivation of chickpea spans approximately 57 countries, showcasing its adaptability to diverse environmental conditions (Mallikarjuna *et al.*, 2000) [10]. India, as the largest producer, contributes significantly with 65% of the global production (9.07 mt) (Merga and Haji, 2019) [11]. Despite advances in breeding for increased yields and resistance to diseases, chickpea suffers notable losses post-harvest, estimated at 8.41%, with insect pests alone accounting for 1.18% in storage. Among these pests, *Callosobruchus* spp. (Chrysomelidae: Bruchinae: Coleoptera) are particularly destructive, causing extensive economic losses by rendering stored chickpeas unfit for consumption or seeding within 4-6 months. Their rapid multiplication in favorable conditions of high humidity and optimal temperature exacerbates these losses (Appleby and Credland, 2004) [3].

It is known to spend its entire immature life in individual legume seeds, feeds internally and hence responsible for huge weight loss decrease in germination potential thereby diminishing the market as well as the nutritional value of the commodity. The grub feeds and develops exclusively on the seed, while adults do not require food or water and spend their limited life

span in mating and oviposition on seeds. Female beetle attaches its eggs individually on the testa of the seeds, hatched grubs borrow into the seed, feed on endosperm and embryo, undergoes a series of molts and pupates just underneath the seed coat. Although, the seed is still intact, around 1-2 mm hole is apparent at the location where the beetle is pupating. The adult that results from pupation chews through the seed coat and emerges from the seed, thus responsible for the formation of characteristic bored holes on the seed coat. The infestation of *C. maculatus* may commence from field or threshing floors, where matured seeds of chickpea may get infested. It causes (5-10%) damage in temperate regions and (20-30%) damage in tropical regions.

Pest control in stored grains, including legumes, heavily relies on synthetic pesticides such as deltamethrin and malathion, alongside botanicals and physical methods like sun drying, to protect crops (Shaheen and Khaliq, 2005; Sharma *et al.*, 2007)^[17, 19]. However, concerns over pesticide resistance, residues, and environmental impacts on treated seeds often make them unsuitable. In response, there has been a growing emphasis on reducing chemical reliance and minimizing seed loss to bruchid attacks through enhanced host plant resistance in leguminous crops (Brewer and Horber, 1984; Shaheen *et al.*, 2006)^[4, 18]. Cultivating tolerant or resistant chickpea cultivars offers a sustainable and cost-effective alternative that complements cultural and biological pest control methods without requiring additional farmer knowledge or costs. Studies assessing resistance in leguminous crops against bruchid species (Sarwar, 2012; Raghuwanshi *et al.*, 2016)^[1, 16, 14] are crucial, with our study focusing on evaluating ten chickpea varieties for resistance against *C. maculatus*. By advocating for host plant resistance over synthetic pesticides, our research aims to promote the integration of resistant chickpea cultivars into comprehensive pest management strategies.

2. Materials and Methods

This experiment was conducted during September 2020 - July 2021 at Department of entomology Junagadh Agricultural University, Junagadh, Gujarat for six months.

2.1. Chickpea varieties

Ten desi chickpea varieties (GG 1, GG 2, GJG 3, GG 4, GG 5, GJG 6, JG 16, Dahod yellow, KAK 2, and AKG 1303) (Table 1) sourced from Pulse Research Station Junagadh, Agricultural University Gujarat, were assessed for their susceptibility to *C. maculatus*. Prior to the experiment, it was ensured that the chickpeas were free from any insect infestation.

Table 1: List of desi chickpea varieties

Chickpea varieties	Seed shape and surface	Seed color	100-seed weight (g)
GG 1	Smooth and round	Reddish brown	18.0
GG 2	Smooth and round	Reddish brown	30.5
GJG 3	Smooth and round	Brown	17.4
GG 4	Owl's head	Yellow	22.7
GG 5	Angular	Brown	21.37
GJG 6	Angular	Brown	22.45
JG 16	Owl's head	Brown	25
Dahod yellow	Rough	Yellowish brown	15
KAK 2	Smooth	White	38
AKG 1303	Smooth	Brown	21.7

2.2. Insect material and maintenance

The *C. maculatus* used in this study obtained from a laboratory

colony maintained at the Department of Entomology, J.A.U. Junagadh. They were reared on chickpea seeds under controlled conditions of 30±2 °C and 65±5% relative humidity in darkness. Chickpea seeds were cleaned and treated at 50 °C for four hours to eliminate any potential infestations before being used for rearing (Patil, 2007)^[13]. Eggs laid on these seeds were monitored daily in fresh jars until adult emergence, ensuring a consistent supply of beetles for the experimental investigations.

2.3. Resistance tests

The evaluation of chickpea varieties for their resistance or susceptibility to *C. maculatus* was conducted under a Completely Randomized Design (CRD). A no-choice method was employed, where each chickpea variety (GG 1, GG 2, GJG 3, GG 4, GG 5, GJG 6, JG 16, Dahod yellow, KAK 2, and AKG 1303) was individually screened against *C. maculatus*. In this method, 50g of seeds from each variety were placed in separate glass jars (7×8 cm²), with each jar serving as one replication for the variety. The experiment included three replications for each of the ten chickpea varieties.

For each replication, ten pairs of one day old adults of *C. maculatus* were introduced into the jars and allowed access to the seeds for oviposition. The jars were covered with double-layered muslin cloth and secured with rubber bands to prevent insect entry and escape while ensuring adequate air circulation. After one week of exposure for oviposition, the adult insects were removed, and the jars were kept under controlled conditions of 30±2 °C and 65±5% relative humidity. Emerging adult *C. maculatus* beetles from each jar were counted after 3 and 6 months of storage. Both live and dead adults were recorded, and seed weight loss was calculated as a % to assess the extent of damage caused by the beetles. This methodology aimed to evaluate the relative resistance or susceptibility of the chickpea varieties to *C. maculatus* infestation under controlled storage conditions.

2.4. Data collection and analysis

In the evaluation of chickpea varieties for resistance or susceptibility to *C. maculatus* using the no-choice test, several parameters were assessed: population growth (adult emergence), seed weight loss, and germination loss caused by the bruchid. Population growth was determined by counting the number of adult beetles that emerged from each variety after 6 months of storage. Seed weight loss was quantified by randomly selecting 100 grains from each replication of the variety, separating undamaged grains from those damaged by the bruchid larvae, and weighing them using an electronic balance. The % weight loss was calculated based on the formula:

Furthermore, germination loss was assessed by comparing initial germination rates with those after 6 months of storage for each replication of every variety. Chickpea varieties were categorized into four groups of susceptibility to *C. maculatus*: highly resistant, resistant, susceptible, and highly susceptible. This categorization was based on the mean values of population growth, % weight loss, and % germination loss, using a scale modified from Ghelani (2016)^[6] specifically for chickpea as given below.

Statistical analysis employed Duncan's Multiple Range Test (DMRT) to identify significant differences among the chickpea varieties for each parameter, with a significance level set at **p*<0.05. This rigorous evaluation aimed to provide insights into the relative resistance or susceptibility of the tested chickpea varieties to *C. maculatus* infestation under controlled experimental conditions, aiding in the selection of more resilient

cultivars for effective pest management strategies in chickpea cultivation.

3. Results and Discussion

Ten chickpea varieties (GG 1, GG 2, GJG 3, GG 4, GG 5, GJG 6, JG 16, Dahod yellow, KAK 2, and AKG 1303) were subjected to laboratory testing to assess their resistance to *C. maculatus* using seed weight loss, germination loss, and population growth as key parameters. Based on their responses to *C. maculatus* infestation, these cultivars were categorized into four groups: highly resistant, resistant, susceptible, and highly susceptible.

3.1. Population growth (adult emergence)

The emergence of adult *C. maculatus* serves as a crucial indicator of the susceptibility or resistance of chickpea varieties to infestation. Over a period of three months, significant variation was observed in the number of adults that emerged among the ten tested chickpea varieties, reflecting their varying levels of resistance. Among the varieties tested, KAK 2 exhibited the highest susceptibility, with 17.68 adults emerging, indicating a lack of effective resistance mechanisms against *C. maculatus*. Similarly, AKG 1303 showed high susceptibility with 17.12 adult emergences. These findings suggest that both KAK 2 and AKG 1303 are likely to experience considerable post-harvest losses due to their inability to control pest development effectively. Varieties GG 1 and GG 2 showed intermediate susceptibility, with fewer adults developed compared to KAK 2 and AKG 1303, and were statistically similar to each other in their resistance levels. GG 5 and GG 4 demonstrated the highest resistance, with the least number of adults emerging, and were also statistically equivalent to each other in terms of their resistance. Following a 6-month storage period, KAK 2 and AKG 1303 remained the most susceptible varieties, with 21.12 and 20.35 adult emergences, respectively. Both varieties continued to exhibit statistically similar levels of susceptibility. Conversely, GG 5 maintained the lowest adult emergence, followed closely by GG 4, with statistically comparable levels of resistance.

3.2. Seed weight loss of chickpea varieties

Weight loss in chickpea seeds due to *C. maculatus* infestation after both three and six months of storage provides critical insights into the resistance or susceptibility of each variety. After three months of storage, KAK 2 and AKG 1303 showed

the highest weight losses at 36.83% and 34.26%, respectively, indicating their high susceptibility to pest infestation. GG 1 and GG 2 followed with weight losses of 31.89% and 32.05%, respectively, demonstrating moderate susceptibility. In contrast, GG 4 and GG 5 exhibited the least weight loss at 15.19% and 13.54%, respectively, with GG 5 showing the highest resistance. After six months of storage, GG 4 and GG 5 maintained their resistance, with weight losses of 23.42% and 19.04%, respectively. These varieties continued to exhibit significantly lower weight losses compared to others, indicating their strong and durable resistance against *C. maculatus*.

3.3. Germination loss

Assessing germination loss due to *Callosobruchus maculatus* infestation after six months of storage provides critical insights into the resilience of chickpea varieties. Among the tested varieties, KAK 2 and AKG 1303 exhibited the lowest germination %, indicating their high susceptibility to pest damage. GG 4 and GG 5, on the other hand, demonstrated the least germination loss. GG 4 showed a decline from 77.39% to 54.14%, indicating a loss of 23.25%, while GG 5 decreased from 79.5% to 55.97%, with a loss of 23.53% (Table 2). These results underscore the strong resistance of GG 4 and GG 5 to *C. maculatus*, maintaining higher germination rates compared to other varieties. GG 5 particularly stood out with the least germination loss, highlighting its superior resistance and suitability as a resilient variety against *C. maculatus* infestation. GG 4 also showed notable resistance, making it a viable option for cultivation in regions prone to such pests.

The study assessed ten desi chickpea varieties (GG 1, GG 2, GJG 3, GG 4, GG 5, GJG 6, JG 16, Dahod yellow, KAK 2, and AKG 1303) against *C. maculatus* using a no-choice test, focusing on population growth (adult emergence), seed weight loss, and germination loss. The study demonstrated varying susceptibilities among the tested chickpea varieties to *C. maculatus* infestation. KAK 2 emerged as the most susceptible variety based on population growth (adult emergence), seed weight loss, and reduction in germination % after three and six months of storage. Following KAK 2, AKG 1303 also showed high susceptibility. Notably, KAK 2, characterized by its smooth, cream-colored seed coat and large seed size, exhibited particularly high susceptibility to *C. maculatus* (Table 1). These findings align with previous research by Eker (Eker *et al.*, 2018) [5] who similarly found that pulse beetles preferentially laid eggs on chickpea cultivars with smooth seed coats in antibiosis tests.

Table 2: Susceptibility of chickpea varieties to *C. maculatus* based on population growth, weight loss and loss of germination per cent

Varieties	No. of adults developed after months of storage		Weight loss (%) months after storage		Germination (%)	
	3 Months	6 Months	3 Months	6 Months	Before infestation	After 6 months of storage
GG1	16.4 ^c	20.32 ^{ab}	31.89 ^c	50.62 ^b	76.66 ^d	41.95 ^d
GG2	15.47 ^d	19.66 ^{bc}	32.05 ^c	51.35 ^b	76.23 ^e	42.53 ^d
GJG3	14.65 ^e	18.64 ^{cd}	28.12 ^e	48.24 ^c	77.09 ^c	46.16 ^c
GG4	12.47 ^h	16.47 ^f	15.19 ^f	23.42 ^e	77.39 ^b	54.14 ^a
GG5	11.7 ⁱ	16.06 ^f	13.54 ^g	19.04 ^d	79.5 ^a	55.97 ^a
GJG 6	13.15 ^g	16.92 ^{ef}	28.78 ^{de}	47.49 ^c	75.04 ^f	47.3 ^c
JG16	13.61 ^{fg}	17.96 ^{de}	29.37 ^{de}	47.94 ^c	77.09 ^c	49.42 ^b
Dahod yellow	13.96 ^f	18.66 ^{cd}	29.82 ^d	48.24 ^c	79.5 ^a	49.39 ^b
KAK 2	17.68 ^a	21.12 ^a	36.83 ^a	54.18 ^a	75.04 ^f	33.01 ^e
AKG1303	17.12 ^b	20.35 ^{ab}	34.26 ^b	53.93 ^a	75.07 ^f	34.05 ^e
F cal.	177.729	23.93	313.86	486.34	309.48	115.06

4. Conclusion

The study assessed chickpea varieties' resistance to *Callosobruchus maculatus* based on adult emergence, weight loss, and germination loss after three and six months of storage. GG4 and GG5 showed the highest resistance with minimum adult emergence, low weight loss, and reduced germination loss. In contrast, KAK 2 and AKG1303 were highly susceptible, showing significant adult emergence, weight loss, and reduced germination rates. Moderately resistant varieties, including Dahod Yellow, JG16, and GJG 6, demonstrated intermediate resistance levels.

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