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Original article

Varietal susceptibility of certain broad bean seeds to infestation with *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae)



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ABSTRACT

Seeds susceptibility of eight broad bean varieties to *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.) infestation were studied for the first time in free- and no-choice methods in the laboratory of Plant Protection Department, Faculty of Agriculture, Zagazig University. The relation between certain seed physical characteristics and some biological and infestation parameters of both insects in the two studied methods were evaluated. None of these varieties were resistant to both insects, showing various levels of susceptibility. Biological and infestation parameters were significantly different among varieties except the developmental period. In free-choice method, Giza 3 was the most susceptible variety to both insects, since produced the highest progeny of 246.67 and 75.67 adults and susceptibility index (SI) of 10.25 and 7.42, respectively, while the least susceptible variety was Giza 716. In no-choice method, Nubaria 5 and Sakha 1 were the most susceptible varieties to *C. chinensis*, while Nubaria 3 and Giza 3 to *C. maculatus*. Differences between physical characters of varieties were significant. Seed hardness were correlated negatively and seed coat thickness positively with laid eggs, progeny and (SI) of both insects in free-choice method. Also seed coat thickness correlated positively with weight loss and seed damage (%) of *C. chinensis* and negatively of *C. maculatus*. To reduce seed losses the cultivation of the least susceptible variety (Giza 716) is encouraged and considered for breeding purposes to avoid insecticide usage.

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1. Introduction

Legume seeds represent a major source of protein for many worlds population and serve to replace low-protein cereals and root crops (Somta et al., 2008; Rizk, 2011). The most protein consumed by human in developing countries comes from legumes (Hariri, 1981; Huignard et al., 1985; Metwally, 1990; Nagaraja, 2006). Beside human food, their straw serves as animal feed for cattle mainly in rural areas, where the grazing land is limited. Legumes also to improve soil fertility through nitrogen fixation and reduce the external fertilizer input (Okigbo, 1978).

Broad bean (*Vicia faba* L.) is planted in a large scale in Egypt, representing about 10% of the world production of broad bean. Seeds of broad bean, as well as other leguminous seeds are attacked heavily by several bruchid beetles as *Callosobruchus mac-*

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ulatus (F.) and *Callosobruchus chinensis* (L.) (Ofuya & Credland, 1996; Loss, 2006). The infestation with these beetles start in the field on broad bean, pods. After harvest the infested seeds were transmitted to stores where the insects development was continued until adult emergence (Darquenne et al., 1993; Boughdad et al., 1997) causing significant losses of seeds weight and decrease sowing degree and fodder values of seeds (Adamczewski et al., 1992; Boughdad, 1996). Additionally, injured seeds become more easily inhabited by fungi, thus inducing other considerable loss in both seed quantity and quality (Chodulska, 1985; Adamezewski et al., 1992). In addition to, the presence of beetles in broad bean seeds after harvesting decreases the commercial value of the yield (Roubinet, 2018).

The post- harvest seed wastage due to *C. maculatus* and *C. chinensis* can reach 100% during severe infestation (Nchimbi-Mosolla & Misangu, 2002). Random apply of chemical pesticides in controlling stored product pests may lead to residual toxicity, environmental pollution and undesirable effects on non-target organisms. The genetic resistance of host plant could consider an effective and environment friendly management option against stored product pests on various legumes (Stoma et al., 2008). Therefore, breeding plants for resistant against insect pests is considered a main total in insect control inside integrated pest management programs (Amusa et al., 2014; Mohamed et al., 2019). Successful planting requires careful planning at the project levels, including using suitable plant varieties with favorable traits (Chin et al., 2022).

Many authors studied the susceptibility of different legume varieties to infestation with some storage insect pests such as Duan et al. (2014) on *C. chinensis*; Osman et al. (2015); El-Rodeny et al. (2018) on *C. maculatus*; Mahmoud (2020) on *Bruchidius incarnates* (Boh.). The relation between some morphological characters of different cowpea varieties and the resistance to *C. maculatus* was studied by Amusa et al. (2014); Fawki et al. (2012).

There were many different broad bean varieties that have not been studied yet for their susceptibility or resistance to infestation with *C. maculatus* and *C. chinensis* and its relation with some seed physical characters.

Therefore, in this work our objectives were to study the seeds susceptibility of eight broad bean varieties that have not been previously studied to infestation with *C. maculatus* and *C. chinensis* in different bioassay methods and to explain the relation between certain seed physical characters and some biological and infestation parameters of the two tested bruchid beetles.

2. Materials and methods

The research was carried out during 2021 in the laboratory of Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt.

2.1. Source of broad bean seed varieties

Seeds of eight broad bean varieties *Vicia faba* L. viz. Giza 3, Nubaria 5, Australian, Sakha 4, Nubaria 4, Sakha 1, Nubaria 3 and Giza 716 were obtained from Filed Crops Research Institute, Agricultural Research Center (ARC), Dokki, Giza, Egypt. All seeds were sterilized by freezing at -20°C for two weeks. One kilogram seeds of each variety were kept in a plastic jar covered tightly with muslin.

2.2. Rearing insect cultures

The two insect species named the cowpea seed beetle, *C. maculatus* and the pulse beetle, *C. chinensis* were obtained from Stored

Grain Pests Dept., Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Dokki, Giza, Egypt. About 75 pairs of the two insects were put separately in 1 kg glass jar capacity containing 390 g of commercial broad bean seeds and tightly covered with muslin, held in place by rubber bands and kept under laboratory conditions (27.35°C and $53.18\% \text{RH}$) for nearly 5 generations before starting the experiments. The used adults were the newly emerged adults (0- 4hr old).

2.3. Varietal susceptibility of broad bean seeds to infestation with *C. maculatus* and *C. chinensis*

The seeds susceptibility of the tested varieties to infestation with the two aforementioned insect species was determined separately under laboratory conditions at the same afore-mentioned temperature and relative humidity. The biological and infestation parameters were studied by using two techniques named free-choice and no- choice methods.

2.3.1. Free- choice method

Thirty grams of seeds of each variety placed in oval plastic cup (7 cm in length, 4.5 cm in width and 2.5 cm in height). The cups were placed within a big circular plastic plate (29 cm in diameter \times 11.50 cm in height). Twenty seven pairs of newly emerged insects of 0-4hr old were introduced at central and covered with a perforated transparent plastic cover to allow ventilation (Fig. 1). Three replicates of the big circular plastic plate were made for each storage insect bruchid and placed under laboratory conditions 29.35°C and $52.18\% \text{RH}$. After two weeks, all insects were separated and the eggs laid on seeds of each variety were counted.

The infested seeds of each replicate were put in a circular plastic cup (7 cm in diameter and 3.5 cm in height), incubated under laboratory conditions at 28.67°C and $52.88\% \text{RH}$ and examined daily until beginning of adult emergence (Fig. 2). The developmental period was evaluated from infestation start till the appearance of first insect in all replicates. The insects were separated and counted daily to calculate the progeny number. After three days of no adults emergence, each replicate was weighed to calculate weight loss (%). The number of bored and uninfested broad bean seeds was separated and counted to calculate the seed damaged (%) as follows:

$$\text{Seed damaged (\%)} = \frac{\text{Number of bored seeds}}{\text{Total number of seeds}} \times 100$$

The susceptibility index (SI) for each variety was determined according to the equations mentioned by Howe (1971); Dobie (1974); Al-Dosari et al. (2002); Salama & Youssef (2004); Mohamed et al. (2019) as follows:

$$\text{Susceptibility index (SI)} = \frac{\text{Log F1 (Total progeny number)}}{\text{Mean developmental period (day)}} \times 100$$

2.3.2. No- choice method

Thirty grams of each broad bean variety was placed in a quarter- kilo plastic cup (Fig. 3). Three pairs of newly emerged adults (male and female, 0-4hr old) were placed in each cup and tightly covered with muslin, held in place by rubber bands and replicated three times. All replicates were incubated under laboratory conditions at 28.71°C and $51.18\% \text{RH}$. After two weeks, the insects were examined daily and when they were all dead, they were removed. The total number of laid eggs was counted and the number of laid eggs per female was calculated. After 2 weeks of incubation all replicates were checked daily until the beginning



Fig. 1. Oval plastic cups in big circular plastic plate used in free- choice method, **A.** big circular plastic plate without cover, **B.** big circular plastic plate with cover.



Fig. 2. Circular plastic cups which were put in it the infested seeds of each replicate in free- choice method.

of adult emergence to determine the developmental period. The seeds were examined daily until adults emergence ceased. The outgoing adults were separated and counted to calculate the progeny number per female. The weight loss (%), seed damage (%) and (SI) were calculated as mentioned above in free- choice method.

2.4. Seeds physical characters of broad bean varieties

The seeds of each tested variety were picked randomly to measure the following characters:

- Seed length, width, thickness and coat thickness by using a vernier caliper.
- Seed size by water displacement method.
- Seed weight by using an electric balance.
- Number of seeds/30 g was calculated by counting the number of seeds in 30 g of each broad bean variety.
- Seeds hardness was estimated by the aid of a digital force gauge (FGN.S, Japan) at Agricultural Engineering Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

2.5. Relationship between seeds physical characters of broad bean varieties and some biological and infestation parameters of *C. maculatus* and *C. chinensis*

Simple correlation coefficients between certain physical characters and some biological and infestation parameters of *C. maculatus* and *C. chinensis* infesting eight broad bean varieties in free- and no-choice methods were calculated. The biological parameters were the number of laid eggs and progeny number. Also the relation with the larval penetration (%) in no-choice method was calculated. The infestation parameters were SI, weight loss (%) and seed damage (%).

2.6. Statistical analysis

The obtained data were statistically analyzed by using SAS program (SAS, 2004). Simple correlation coefficient was calculated according to Hendy (1969). The means comparison was carried out according to Duncan's (LSD) (Duncan, 1955) at 0.05 probability levels.

3. Results

3.1. Varietal susceptibility of broad bean seeds to infestation with *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.)

The biological and infestation parameters of *C. maculatus* and *C. chinensis* were studied on seeds of eight broad bean varieties to evaluate their seeds susceptibility to insect infestation by using free- and no-choice methods.

3.1.1. Biological parameters of *C. maculatus* and *C. chinensis* on seeds of broad bean varieties in free- and no- choice methods

The biological parameters of *C. maculatus* and *C. chinensis* on eight broad bean seed varieties in free- and no- choice methods were mean number of laid eggs, developmental period (day) and progeny number. As shown in Table 1 the statistical analysis



Fig. 3. Quarter- kilo plastic cups which were used in no- choice method.

Table 1
Ovipositional preference, developmental period and adult emergence of *C. maculatus* and *C. chinensis* on seeds of eight broad bean varieties in free- and no- choice methods.

Broad bean Variety	Mean ± SE of		No- choice method							
	Free- choice method		Developmental period (day)			Progeny no.				
	No. of laid eggs		<i>C. maculatus</i>	<i>C. chinensis</i>	<i>C. maculatus</i>	<i>C. chinensis</i>	<i>C. maculatus</i>	<i>C. chinensis</i>		
Giza 3	194.67 ± 5.36 ^a	316.67 ± 9.84 ^a	25.33 ± 0.33 ^a	23.33 ± 0.33 ^a	246.67 ± 4.33 ^a	72.33 ± 1.54 ^b	29.67 ± 0.67 ^a	25.00 ± 0.00 ^a	31.11 ± 0.11 ^b	54.44 ± 2.38 ^{bc}
Nubaria 5	191.67 ± 1.76 ^a	287.00 ± 9.54 ^b	25.33 ± 0.33 ^a	23.33 ± 0.33 ^a	195.67 ± 11.84 ^b	91.11 ± 5.79 ^a	30.00 ± 0.00 ^a	25.00 ± 0.00 ^a	27.33 ± 2.52 ^{bc}	69.78 ± 6.60 ^a
Australian	190.33 ± 2.03 ^a	235.67 ± 7.84 ^c	25.33 ± 0.33 ^a	23.67 ± 0.33 ^a	141.67 ± 14.19 ^c	67.11 ± 6.31 ^{bc}	30.00 ± 0.58 ^a	25.00 ± 0.00 ^a	23.11 ± 0.78 ^{cd}	51.78 ± 5.40 ^c
Sakha 4	189.00 ± 2.65 ^a	189.33 ± 8.37 ^d	25.67 ± 0.33 ^a	23.67 ± 0.33 ^a	119.33 ± 4.63 ^{cd}	77.78 ± 2.94 ^b	29.67 ± 0.67 ^a	25.00 ± 0.00 ^a	17.67 ± 2.08 ^e	51.67 ± 1.35 ^c
Nubaria 4	151.67 ± 2.60 ^b	136.33 ± 6.74 ^{ef}	25.33 ± 0.33 ^a	23.67 ± 0.33 ^a	101.33 ± 5.36 ^{de}	93.11 ± 0.78 ^b	30.33 ± 0.33 ^a	25.00 ± 0.00 ^a	31.00 ± 2.04 ^b	54.00 ± 2.40 ^{bc}
Sakha 1	105.67 ± 2.60 ^c	117.67 ± 5.78 ^{fg}	26.00 ± 0.58 ^a	23.67 ± 0.67 ^a	87.00 ± 5.20 ^{ef}	78.22 ± 6.02 ^b	29.67 ± 0.33 ^a	25.33 ± 0.33 ^a	24.67 ± 0.39 ^{cd}	66.56 ± 7.22 ^{ab}
Nubaria 3	101.00 ± 3.79 ^c	150.67 ± 8.84 ^e	25.33 ± 0.33 ^a	23.33 ± 0.33 ^a	97.00 ± 4.73 ^{de}	71.11 ± 3.04 ^{bc}	29.33 ± 0.33 ^a	25.33 ± 0.33 ^a	46.22 ± 2.90 ^a	60.22 ± 3.13 ^{abc}
Giza 716	97.00 ± 1.73 ^c	98.00 ± 2.89 ^g	25.33 ± 0.33 ^a	23.00 ± 0.00 ^a	68.33 ± 3.76 ^f	58.67 ± 3.15 ^c	29.33 ± 0.33 ^a	25.33 ± 0.33 ^a	20.67 ± 1.02 ^{de}	47.44 ± 2.11 ^c
Average	152.63	191.42	25.46	23.46	132.13	73.01	29.75	25.12	27.72	56.99
F. test	*	*	NS	NS	*	*	NS	NS	*	*

Means in the same column followed by different letters are significantly different (at $p \leq 0.05$) when analyzed using ANOVA and separated by Duncan test.

* = Significant means.

NS = Non significant means.

cleared that there were significant differences among all tested broad bean varieties and the studied characters in free- and no- choice methods except the mean developmental period which was insignificant. In case of free- choice method the average of developmental period of *C. chinensis* was shorter than *C. maculatus* exhibiting 23.46 and 25.46 days, successively. Moreover, the average number of laid eggs and progeny number were higher (191.42 eggs and 132.13 adults) for *C. chinensis* than those of *C. maculatus* (152.63 eggs and 59.96 adults). Data also showed that the highest mean numbers of laid eggs by *C. chinensis* and *C. maculatus* were recorded on Giza 3 variety (316.67 and 194.67 eggs), while the lowest ones were observed on Giza 716 seeds (98.00 and 97.00 eggs), respectively. The same trend was found in progeny number. The maximum progeny numbers were 246.67 and 75.67 adults on Giza 3 variety and the minimum ones were 68.33 and 36.00 adults on Giza 716 variety for *C. chinensis* and *C. maculatus*, subsequently. As for no- choice method, the average of laid eggs number per female of *C. maculatus* was higher (81.92 eggs) than those of *C. chinensis* (73.01 eggs), but the average of progeny number per female clears opposite trend. It was higher in case of *C. chinensis* (56.99 adults) than *C. maculatus* (27.72 adults). Data also indicated that the highest number of laid eggs per female of *C. maculatus* was 108.56 eggs on Nubaria 3 and those of *C. chinensis* was observed on Nubaria 5 (91.11 eggs). The lowest numbers of laid eggs per female were 48.78 and 58.67 eggs on Giza 716 variety by *C. maculatus* and *C. chinensis*, successively. Concerning the mean progeny number per female of *C. maculatus*, the highest number was recorded on Nubaria 3 seeds (46.22 adults) and the lowest one was emerged from Sakha 4 seeds (17.67 adults). On the other hand, the highest mean progeny number per female of pulse beetle, *C. chinensis* was 69.78 adults on Nubaria 5 and the lowest one was 47.44 adults on Giza 716. It was obvious that the studied broad bean varieties varied in their response to infestation with *C. maculatus* and *C. chinensis*. All tested broad bean seed varieties were found to be higher susceptible to *C. chinensis* than *C. maculatus* whereas all studied parameters recorded higher values in the two tested methods.

3.1.2. Infestation parameters of eight broad bean seed varieties to infestation with *C. maculatus* and *C. chinensis* in free- and no- choice methods

Data presented in Table 2 cleared the effect of infestation with *C. maculatus* and *C. chinensis* on some infestation parameters of broad bean seed varieties i.e., susceptibility index (SI), weight loss (%) and seed damage (%) for both experiments of free- and no- choice methods.

The present data cleared that none of the broad bean varieties used in the study were resistant or tolerant to both *C. chinensis* and *C. maculatus*, indicating different levels of susceptibility. In general the statistical analysis indicated that there were significant differences among infestation parameters of the tested storage bruchids on broad bean seed varieties. The average of susceptibility index was higher for *C. chinensis* which had the same value in free- and no- choice methods (8.88) than for *C. maculatus* which recorded lower values of 6.91 and 6.40, respectively.

As regards free- choice method, the highest values of susceptibility index (SI) and weight loss (%) were observed in Giza 3 variety and the lowest ones were in Giza 716 for both bruchids. It differed in damage traits, in case of *C. maculatus* Nubaria 5 was the most damaged variety (79.81%) and Giza 716 was the least damaged one (50.02%). For *C. chinensis*, the most damaged variety was Australian by 98.53% and the least damaged one was Sakha 1 by 72.55%.

Concerning no- choice method, the highest values of (SI) and weight loss (%) for *C. maculatus* were 7.30 and 10.25% on Nubaria 3 variety, while the lowest (SI) was 5.80 on Sakha 4 and the lowest

Table 2
Varietal susceptibility of broad bean seeds to infestation with *C. maculatus* and *C. chinensis* in free- and no-choice methods.

Broad bean Variety	Mean ± SE of		No-choice method								
	Free-choice method			Susceptibility index (SI)			Seed damage (%)				
	<i>C. maculatus</i>	<i>C. chinensis</i>	Weight loss (%)	<i>C. maculatus</i>	<i>C. chinensis</i>	<i>C. maculatus</i>	<i>C. chinensis</i>	<i>C. maculatus</i>	<i>C. chinensis</i>		
Giza 3	7.42 ± 0.14 ^a	10.25 ± 0.12 ^a	10.99 ± 1.37 ^a	16.37 ± 0.14 ^a	90.79 ± 0.33 ^b	6.65 ± 0.14 ^b	8.85 ± 0.07 ^{bcd}	8.18 ± 0.52 ^b	12.22 ± 1.11 ^{abc}	96.29 ± 2.37 ^a	98.45 ± 0.78 ^a
Nubaria 5	7.29 ± 0.12 ^a	9.82 ± 0.18 ^a	10.07 ± 0.12 ^a	11.90 ± 0.12 ^b	97.88 ± 1.06 ^a	6.37 ± 0.13 ^{bc}	9.27 ± 0.16 ^a	6.10 ± 0.02 ^d	13.00 ± 0.19 ^{ab}	65.50 ± 5.52 ^d	98.85 ± 1.15 ^a
Australian	7.40 ± 0.12 ^a	9.07 ± 0.08 ^b	10.35 ± 0.86 ^a	7.89 ± 0.10 ^d	98.53 ± 0.73 ^a	6.14 ± 0.15 ^{cd}	8.75 ± 0.18 ^{cd}	5.28 ± 0.10 ^e	12.68 ± 0.18 ^{ab}	80.16 ± 1.06 ^{bc}	98.85 ± 1.15 ^a
Sakha 4	7.13 ± 0.14 ^a	8.77 ± 0.07 ^{bc}	6.25 ± 0.28 ^b	9.46 ± 0.19 ^c	98.39 ± 0.81 ^a	5.80 ± 0.28 ^d	8.76 ± 0.05 ^{cd}	5.31 ± 0.07 ^e	10.33 ± 0.03 ^{cd}	65.33 ± 2.86 ^d	91.73 ± 0.86 ^b
Nubaria 4	7.38 ± 0.10 ^a	8.47 ± 0.04 ^{cd}	7.89 ± 0.88 ^b	7.68 ± 0.10 ^d	98.15 ± 0.93 ^a	6.49 ± 0.13 ^{bc}	8.84 ± 0.07 ^{bcd}	6.87 ± 0.27 ^c	11.11 ± 1.11 ^{bcd}	88.80 ± 1.52 ^{ab}	98.01 ± 1.00 ^a
Sakha 1	6.38 ± 0.21 ^b	8.20 ± 0.25 ^{de}	4.05 ± 0.29 ^c	5.10 ± 0.17 ^f	72.55 ± 0.98 ^d	6.30 ± 0.08 ^{bc}	9.18 ± 0.19 ^{ab}	6.49 ± 0.03 ^{cd}	11.61 ± 0.96 ^{abcd}	71.03 ± 4.01 ^{cd}	100 ± 0.00 ^a
Nubaria 3	6.16 ± 0.04 ^b	8.52 ± 0.21 ^{cd}	3.79 ± 0.44 ^c	5.90 ± 0.10 ^e	88.74 ± 0.50 ^b	7.30 ± 0.15 ^a	8.91 ± 0.11 ^{abc}	10.25 ± 0.19 ^a	13.66 ± 0.33 ^a	90.60 ± 2.22 ^a	99.02 ± 0.98 ^a
Giza 716	6.14 ± 0.05 ^b	7.97 ± 0.10 ^e	3.25 ± 0.13 ^c	4.40 ± 0.12 ^g	80.18 ± 0.38 ^c	6.11 ± 0.13 ^{cd}	8.50 ± 0.08 ^d	6.57 ± 0.21 ^{cd}	9.94 ± 0.34 ^d	76.42 ± 2.50 ^c	100.00 ± 0.00 ^a
Average	6.91	8.88	7.08	8.59	90.65	6.40	8.88	6.88	11.82	79.27	98.11
F. test	*	*	*	*	*	*	*	*	*	*	*

Means in the same column followed by different letters are significantly different (at p ≤ 0.05) when analyzed using ANOVA and separated by Duncan test.

* = Significant means.

weight loss recorded 5.28% in Australian variety. The situation was different with *C. chinensis*, where the highest (SI) value (9.27) was in Nubaria 5 and lowest one (8.50) was in Giza 716 variety. Seed damage caused by *C. maculatus*, reached the highest value on Giza 3 seeds by 96.29% and the lowest one on Sakha 4 (65.33%). The most damaged seeds by *C. chinensis* were on Sakha 1 and Giza 716 varieties by same percent of 100.00% and the least damaged one was on Sakha 4 variety by 91.73%.

3.2. Seeds physical characters of eight broad bean varieties

Significant differences were observed among seeds physical characters of eight broad bean varieties with the exception of seed length per width ratio which was insignificant (Fig. 4). It was found that the largest seeds size (0.93 ml³) and the smallest seed hardness (274.10 N) were of Nubaria 4 variety, while the smallest seeds size (0.53 ml³) and the highest hardness (410.43 N) were of Australian variety. In case of seed coat thickness, the highest thickness was of Sakha 4 variety (1.15 mm) and the lowest one was of Giza 716 variety (0.47 mm). For seed thickness, the highest seed thickness (7.38 mm) was of Australian variety and the lowest one (5.25 mm) was of Sakha 4. The lowest seed weight (0.67 mg) and the highest seed number on 30 g (44.00 seeds) was in Australian variety, while opposite trends were recorded in seeds of Nubaria 5 variety, which recorded the highest seed weight (1.01 mg) and the lowest seed number (29.33 seeds).

3.3. Relationship between seeds physical characters of broad bean varieties and some biological and infestation parameters of *C. maculatus* and *C. chinensis*

As evidently given in Table 3, seed physical characters affected differently on the biological and infestation parameters of *C. maculatus* and *C. chinensis* in free- and no-choice methods.

In free-choice method, there was a negative correlation relationship between seed size and each of laid eggs number and progeny number of the two tested insects except the relation with progeny number of *C. chinensis* (r = 0.053) correlated positively. Seed length/ width ratio, seed thickness, seed coat thickness and seed weight was positive with number of laid eggs and progeny number. These relationships were significant only with seed length/ width ratio for *C. chinensis* (r = 0.431* and 0.418*) and highly significant for *C. maculatus* (0.537** and 0.608**), respectively. The laid eggs number and progeny number of the two storage bruchids were correlated negatively with seed hardness and recorded significantly effect in progeny number of *C. maculatus* (r = -0.405*). The relation between seeds number/ 30 g was positive with number of laid eggs and progeny number for *C. maculatus* and negative for *C. chinensis*.

In no-choice method, number of laid eggs per female and progeny number per female of the two storage bruchids were positively correlated with seed size, seed coat thickness, seed hardness and seed weight. There were negative correlation between the two biological parameters and each of seed number/ 30 g and seed thickness except the relation between progeny number per female of *C. chinensis* and seed thickness was positively correlated.

As for the infestation parameters (SI, percent of weight loss and seed damage) of the two tested insects in free-choice method, there was a positive relationship between all infestation parameters and each of seed length/ width ratio, seed thickness and seed weight except the relation between seed weight and seed damage (%) correlated negatively (r = -0.056) for *C. chinensis*. Also, seed coat thickness correlated positively with all tested infestation parameters except in weight loss percent (r = -0.091) and seed damage percent (r = -0.061) caused by *C. macu-*

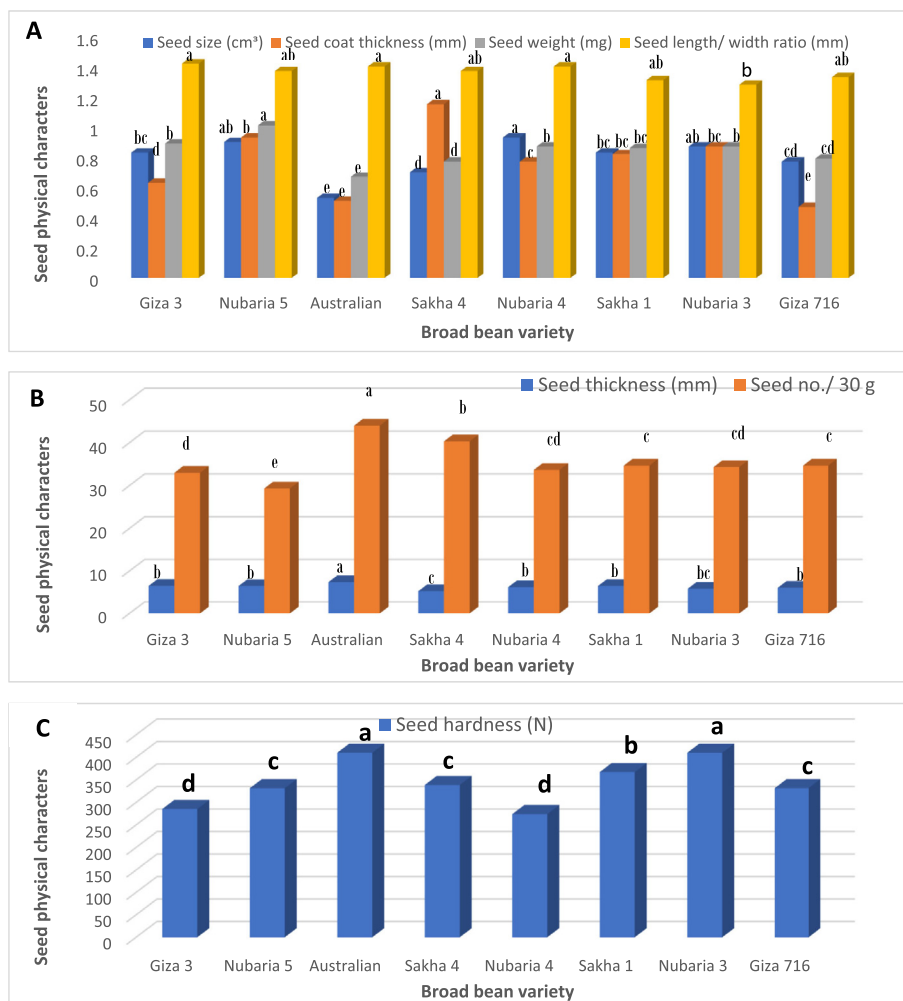


Fig. 4. Seed physical characters of eight broad bean varieties, **A.** Seeds size, seed coat thickness, seed weight and seed length per width ratio, **B.** Seed thickness and seed number on 30 g and **C.** Seed hardness. Means in the same column followed by different letters are significantly different (at $p \leq 0.05$) when analyzed using ANOVA and separated by Duncan test.

latus correlated negatively. Seed hardness correlated negatively with all tested infestation parameters for *C. chinensis* and *C. maculatus* with significant values for weight loss percent ($r = -0.471^*$) caused by *C. chinensis*.

In no-choice method, (SI), weight loss (%) and seed damage (%) correlated positively with each of seed size, seed thickness, seed hardness and seed weight except weight loss (%) caused by *C. maculatus* correlated negatively ($r = -0.190$) with seed thickness. Also seed damage (%) caused by the same insect correlated negatively with both seed hardness ($r = -0.159$) and seed weight ($r = -0.019$). Seed coat thickness and seeds number/30 g correlated negatively with (SI), weight loss (%) and seed damage (%) caused by *C. chinensis* and *C. maculatus* except the relationship between seed coat thickness and (SI) of *C. chinensis*, which correlated positively. Seed length/width ratio correlated negatively with each of (SI) and weight loss (%) caused by *C. maculatus*, whereas $r = -0.060$ and -0.250 , respectively. Opposite trend was observed with seed damage (%). In case of *C. chinensis* seed length/width ratio correlated positively with (SI) and weight loss (%) but correlated negatively with seed damage (%). In general, simple correlation coefficient between biological or infestation parameters of the two storage bruchids in the tested methods and seed physical characters was different from one to other.

4. Discussion

4.1. Varietal susceptibility of broad bean seeds to infestation with, *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.)

The biological and infestation parameters of the two storage bruchids, *C. maculatus* and *C. chinensis* were studied on eight broad bean varieties to evaluate their seeds susceptibility to insect infestation by using free- and no-choice methods.

4.1.1. Biological parameters of *C. maculatus* and *C. chinensis* on seeds of broad bean varieties in free- and no-choice methods

The differences in the studied biological parameters among the eight broad bean varieties were significant except the developmental period which was insignificant. The obtained data are in accordance with Mohamed et al. (2019) who investigated the same tested biological parameters in this study on cowpea varieties infested by *C. maculatus* and *C. chinensis* in free- and no-choice methods and reported that there were significant differences among the cowpea varieties in respect to all tested biological parameters of the two storage bruchids in all experiment methods with the exception of the mean developmental period. The present results clear that Giza 3 was the most susceptible variety to infes-

Table 3Simple correlation coefficients (r) between seeds physical characters of broad bean varieties and some biological and infestation parameters of *C. maculatus* and *C. chinensis* in free- and no- choice methods.

Bioassay method	Parameter	Bruchid	Simple correlation coefficient (r)							
			Seed size (ml ³)	Seed length/width (mm)	Seed thickness (mm)	Seed coat thickness (mm)	Seed hardness (N)	Seed weight (mg)	Seeds no./30 g	
Free-choice	Biological	Laid eggs no.	<i>C. m.</i>	-0.275	0.537**	0.201	0.169	-0.261	0.010	0.203
			<i>C. ch.</i>	-0.098	0.431*	0.324	0.056	-0.165	0.255	-0.098
	Progeny no.	<i>C. m.</i>	-0.155	0.608**	0.273	0.069	-0.405*	0.011	0.158	
		<i>C. ch.</i>	0.053	0.418*	0.308	0.022	-0.323	0.347	-0.242	
No-choice	Laid eggs no./♀	<i>C. m.</i>	0.378	-0.046	-0.257	0.579**	0.090	0.295	-0.151	
		<i>C. ch.</i>	0.238	0.0430	-0.077	0.502*	0.030	0.543**	-0.260	
	Progeny no./♀	<i>C. m.</i>	0.489*	-0.191	-0.025	0.048	0.145	0.342	-0.379	
		<i>C. ch.</i>	0.366	-0.155	0.070	0.258	0.180	0.568**	-0.365	
Free-choice	Infestation	Susceptibility index	<i>C. m.</i>	-0.110	0.628**	0.237	0.030	-0.379	0.054	0.141
			<i>C. ch.</i>	0.066	0.450*	0.294	0.075	-0.268	0.343	-0.230
	Weight loss %	<i>C. m.</i>	-0.122	0.496*	0.481*	-0.091	-0.288	0.120	0.040	
		<i>C. ch.</i>	0.106	0.462*	0.114	0.118	-0.471*	0.359	-0.237	
No-choice	Seed damage %	<i>C. m.</i>	-0.052	0.528**	0.528**	-0.061	-0.255	0.226	-0.046	
		<i>C. ch.</i>	-0.163	0.421*	0.046	0.252	-0.156	-0.056	0.217	
	Susceptibility Index	<i>C. m.</i>	0.438*	-0.060	0.011	-0.050	0.149	0.326	-0.362	
		<i>C. ch.</i>	0.356	0.068	0.103	0.317	0.100	0.515*	-0.342	
Weight loss %	<i>C. m.</i>	0.474*	-0.250	-0.190	-0.029	0.111	0.306	-0.417*		
	<i>C. ch.</i>	0.052	0.035	0.237	-0.024	0.398	0.233	-0.106		
Seed damage %	<i>C. m.</i>	0.180	0.169	0.154	-0.436*	-0.159	-0.019	-0.097		
	<i>C. ch.</i>	0.144	-0.120	0.407*	-0.624**	0.168	0.248	-0.315		

Means in the same column followed by different letters are significantly different (at $p \leq 0.05$) when analyzed using ANOVA and separated by Duncan test.

Simple correlation coefficient is significant (*) at the 0.05 level and highly significant (**) at the 0.01 level of probability.

C. m. = *C. maculatus* and *C. ch.* = *C. chinensis*.

tation with *C. chinensis* and *C. maculatus*, while the least susceptible variety was Giza 716 in free- choice method. These results are in agreement with the findings of [El- Rodeny et al. \(2018\)](#) on other broad bean genotype who indicated that Sakha 3 genotype of broad bean was tolerant to the infestation with *C. maculatus* in storage since it had low number of progeny and short developmental period.

4.1.2. Infestation parameters of eight broad bean seed varieties to infestation with *C. chinensis* and *C. maculatus* in free- and no- choice methods

All tested broad bean seed varieties were found to be higher susceptible to *C. chinensis* than *C. maculatus* and caused higher weight loss (%). Similar results were obtained by [Desroches et al. \(1995\)](#) who reported that *C. chinensis* caused higher losses on different broad bean seed varieties during storage than those caused by *C. maculatus*. All studied infestation parameters (susceptibility index, weight loss and seed damage %) influenced by broad bean seed varieties. These findings are harmony with those recorded by some authors such as [Ali & Smith \(2001\)](#) who mentioned that susceptibility index for *C. chinensis* varied among the different broad bean varieties. [Nchimibi- Msolla & Misanga \(2002\); Mebeasilassi \(2004\)](#) added that weight loss due to *C. maculatus* depends on varieties of legume seeds. [El- Rodeny et al. \(2018\)](#) found that the tolerant broad bean genotypes to infestation with *C. maculatus* exhibited the reduced rate of susceptibility index value and weight loss%.

The present data cleared that Giza 3 was the most susceptible variety to infestation with *C. chinensis* and *C. maculate*, while the least susceptible one was Giza 716 in free- choice method. These results partially agreed with that recorded by [El- Rodeny et al. \(2018\)](#) who found that the broad bean line 2 and Sakha 3 were tolerant against *C. maculatus* in storage.

4.2. Seeds physical characters of broad bean varieties

The results indicated that the differences between certain physical characters of eight broad bean varieties were significant. The obtained results are partially agree by the findings of [El-Sitiny, Mona \(2016\)](#) who mentioned that there were significant differences among the physical characters of some broad bean varieties except seed length per width ratio. Different trends were found by [Hassan Shazali \(1990\)](#) who reported that the thickness of the seed coat of the tested broad bean cultivars was not differed significantly.

4.3. Relationship between seeds physical characters of broad bean varieties and some biological and infestation parameters of *C. maculatus* and *C. chinensis*

Seed physical characters affected differently on the biological and infestation parameters of *C. maculatus* and *C. chinensis*. Statistical analysis cleared that there were negative correlation coefficients between seed hardness and number of laid eggs, progeny number, SI, weight loss and seed damage % of *C. chinensis* and *C. maculatus* in free-choice method and seed damage % of *C. maculatus* in no-choice methods. These findings are partially supported by [Ali & Smith \(2001\)](#) who found that the seed coat of broad bean cultivars affected the adults emergence of *C. chinensis*. In no- choice method, simple correlation coefficients exhibited positive values between seed hardness and number of laid eggs, progeny number, SI, and seed damage % for *C. maculatus* and *C. chinensis*. Similar results were recorded on different legume varieties infested with *C. maculatus* such as [Sarwar \(2012\)](#) on chickpea genotypes; [Amusa & Ogunkanmi \(2021\)](#) on cowpea accessions who reported that seed hardness was positively correlated with eggs laid number, number of F₁ progeny and seed damage %.

As for the effect of seed size, susceptibility index, weight loss (%) and seed damage (%) correlated positively with seed size in no-choice method. These results are in accordance with Modi et al. (1994), Keneni et al. (2011) on cowpea varieties infested with *C. chinensis*; Lephale et al. (2012) on cowpea varieties infested with *C. maculatus* and Lambrides & Imrie (2013) on mungbean accessions infested with *C. chinensis* and *C. maculatus* who mentioned that the tested insects preferred to infest the big size seeds. Mohamed et al. (2019) added that cowpea varieties with big sized seeds were more preferable for laying eggs, adult emergence of *C. maculatus* and suffered high weight loss (%). In case of the effect of seed weight, number of laid eggs correlated positively with seed weight in no-choice method. Similar results was recorded by Carrillo-Perdomo et al. (2019) who reported that the little weight of the seeds was correlated with lower infestation rates in the different experiments. On the other hand these results disagreed with the findings of Swella and Mushoboy (2009) who stated that there was no association between the seeds preferred for oviposition and the culture on which the bruchids were reared.

5. Conclusions

The obtained results clearly demonstrated that none of the studied eight broad bean varieties were resistant or tolerant to both *C. chinensis* and *C. maculatus*, indicating different levels of high susceptibility in free- and no-choice methods. The differences in the studied biological and infestation parameters among the varieties were significant except the developmental period. All tested varieties were found to be higher susceptible to *C. chinensis* than *C. maculatus*, whereas all studies biological and infestation parameters recorded highly values for *C. chinensis* in the two tested methods. Differences between seed physical characters of varieties were significant. Seed hardness correlated negatively with laid eggs, progeny and susceptibility index of both insects in free-choice method and positively in no-choice method. Seed coat thickness correlated negatively with weight loss and seed damage (%) of *C. maculatus* and positively of *C. chinensis*. The Giza 716 variety suffered less damage than the other tested varieties in free-choice tested. To reduce seed losses the cultivation of the least susceptible variety (Giza 716) is encouraged and considered for breeding purposes to avoid insecticide usage.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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