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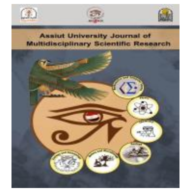

Effects of different cowpea cultivars on some biological parameters of the cowpea seed beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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ABSTRACT

The cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) is an economically serious pest of stored legume seeds, particularly cowpea seeds. Choice and no-choice test carried out to assess the resistance of five common cowpea cultivars; Cream7, Kaha1, Sakha1, Tiba, and Kafr El-Sheikh1, against *c. maculatus*. The cowpea seed beetle laid more eggs on Kafr El-Sheikh1 and Kaha1 seeds in the choice test and there was no preference for the pest in the no-choice test. The development time was longest on Tiba and shortest on Kafr El-Sheikh1 cultivars in both choice and no-choice tests. The total number of emerged adults was significantly reduced when the insects were kept on Sakha1, Kaha1, and Tiba cultivars in the no-choice test. However in the choice test, the smallest number of emerged adults was found in the insects kept on Cream7, Sakha1, and Tiba seeds. The smallest percentage of seed weight loss was found in Sakha1 in both choice (14.19%) and no choice (19.58%) tests. There was a reduction in seed weight loss, fecundity and adult emergence rates, and prolonged larval development time in *C. maculatus* that were grown in the

Sakha1 cultivar. Consequently, our results suggest that Sakha 1 cultivar is the most resistant against cowpea beetle, among the tested ones and can thus be recommended to farmers and used in IPM programs to mitigate the spread and viability of the cowpea beetle.

INTRODUCTION

Globally more than two billion tons of pulse grains are propagated and stored every year to meet the nutritional demands of humans and animals [1]. Stored grains are infested by different insect pests mainly coleopteran and lepidopteran pests [2]. These pests induce damage to the grains not only in the field but also in the stored area resulting in both qualitative and quantitative losses [3]. It has been estimated that between twenty-five and thirty percent of the world's grain crops are lost each year during storage because of the various types of pests that can affect the quality of the grains and the unsuitable facilities used by the farmers [1]. Some of these include the rice weevil, the lesser grain borer, and the rust red flour beetle [4].

The cowpea seed beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), is an endophagous, economically important pest of legumes, such as lentils, soybean, chickpeas, and cowpeas [4]. In particular, this pest causes serious damage to cowpea (*Vigna unguiculata* (L.) Walp), (Leguminosae: Papilionidae) which has been recorded as a big supply of dietary protein in many nations of the world especially in tropical regions such as Africa, South America, and Central America [5, 6].

The cowpea beetle has many generations each year, and after mating, the adult female oviposits fertilized eggs on the cowpea seeds [7]. Larvae hatch from the eggs, start to feed on the seed coat, and enter the seed into the endosperm. Within the seed, the juvenile stages eat, pupate, and eventually convert into adult stages [8]. After biting through the seed coat, adults emerge from the seed, ready to mate and lay eggs [8]. The adult cowpea beetle begins the infestation in the field, but the majority of the injury, which may be direct or indirect occurs in the storage area [9].

The direct damage is caused by feeding on the grains while the indirect damage includes contamination of grains with the beetle fecal matter, exuviae, or secondary infestation with molds and other microorganisms [3]. The developing larvae of *C. maculatus* which are considered the only feeding stage induce significant loss in grain weight, seed germination rate, market value, and seed quality due to insect parts, odors, molds, and heat damage [10]. The percentages of losses due to this pest vary according to the type of legumes and the interval time between harvest and post-harvest period [11].

Given the significance of the harm it causes to the cowpea seeds, numerous techniques for controlling this pest have been developed worldwide including biocontrol, chemical insecticides, plant extracts, and cultural control methods [1]. Pest populations were controlled using chemical pesticides, but the extensive use resulted in serious problems such as resistance, pollution of the seeds and hence to humans, pest resurgence, and secondary pest outbreaks [12].

Alternatively, plant-derived materials such as plant extracts and essential oils have been used [13, 14]. Soundararajan [15] used a hymenopteran parasitoid, *Dinarmus basalis* (Rond), a biological control agent for controlling *C. maculatus*. Finally, one of the most promising approaches to lessen the effect of this insect pest has been the development of resistant cowpea cultivars [16].

Cowpea cultivars have a wide range of distinctive seed features (color, texture, size, hardness, and chemical composition) that have been associated with bruchid resistance [17]. Several researches have been undertaken to find cowpea varieties and features linked to resistance to *C. maculatus*. For example, seed texture has been found as a factor determining oviposition preferences [18], whereas *C. maculatus* appears to avoid seeds with hard seed teguments. [19, 20].

In Egypt, farmers use different cowpea varieties such as Dokki 331 and Cream7. Mahmoud *et al.* [21] indicated that Dokki 331 was more susceptible to *C. maculatus* than Cream 7. Mohamed *et al.*[22] showed different susceptibility levels of two bruchids; *C. maculatus* and *C. chinensis* against five cowpea varieties in Egypt.

Because there are many new breeding cowpea varieties in Egypt, the current study was conducted to assess the susceptibility of five cowpea seed cultivars against C. maculatus in the choice and no-choice tests to select the resistant cultivars as a promising way to control this insect pest. Different parameters were determined such as fecundity, developmental time, adult emergence, and loss in grain weight.

MATERIALS AND METHODS

1.1. Biological materials

Five cowpea seed cultivars (Sakha1, Tiba, Kaha1, Kafr El-Sheikh1, and Cream7) were kindly given from Horticulture Research Institute (HRI), Agricultural Research Center (ARC), Dokki, Egypt. The seeds were cultivated for two seasons in 2021 and 2022 in the Experimental Farm of Faculty of Agriculture, Assiut University, Assiut, Egypt without any use of insecticides. Seeds of these five cultivars are morphologically different in shape, color, and size. After harvesting the seeds were collected, packaged, and then frozen at -20˚C for 15 days, to prevent the transmission of any infestation that might be happened from the field.

1.2. Rearing of insects

The cowpea beetle, *C. maculatus,* was collected from stored cowpea seeds from (HRI) mentioned above. For mass rearing, the insects were reared on seeds of cowpea (*Vigna unguiculata* L.) under incubator conditions (29 ± 2 ^{0}C, 60 ± 5% RH, and 12 h photoperiod). The insects were reared in a clear one-liter jar capacity wrapped by a fine net for ventilation. Both male and female cowpea insects were placed in the containers containing 300 g of seeds cultivar. The adults were eliminated from the jars after one week to ensure mating and oviposition. The population of *C. maculatus* was reared on seeds of cowpea for three generations in the laboratory and the insects of the 4th generation were used to perform the present experiments.

1.3. Experimental tests

1.3.1. No-choice experiment

In this experiment, the preference of *C. maculatus* beetle for five cowpea seed cultivars (Sakha1, Tiba, Kaha1, Kafr El-Sheikh1, and Cream7) was evaluated in the laboratory. In no-choice experiments, two couples of *C. maculatus* adults aged 24 h were introduced to 100 ml jar containing 10 g of solely cowpea seeds of the tested cultivar. The jars were covered well with net cloth and closed with rubber bands and then were kept under incubator conditions (29±2˚C, 60±5% R.H, and 12 h photoperiod). After four days the adults were eliminated from the jars, and the number of eggs was counted. Based on these data, fecundity was calculated as the total number of eggs laid. The jars were watched daily for adult emergence which started in this experiment after 15 days of egg laying. This experiment was maintained in the laboratory until the appearance of cowpea beetle adults ceased. Additionally, the development time that was calculated as the time from egg laying till the emergence of adults, number of holes, and weight loss of cowpea seeds also were recorded. This experiment was replicated three times for each cowpea cultivar.

1.3.2. Choice experiment

For the choice test, two g for each cowpea cultivar (Sakha1, Tiba, Kaha1, Kafr El-Sheikh1, and Cream7) were placed and mixed in a 100 ml jar (described above) forming a total weight of 10 g of seeds. Two pairs of *C. maculatus* aged 24h were put in the jars, and the experiment was replicated three times. Four days later, the adults were eliminated, and the seeds of each cultivar were separated in a petri-dish to count the number of laid eggs on each cultivar. After that, the seeds for each cultivar were placed in a test tube covered with a piece of cotton and were examined daily from day fifteen (the day at which the adults started to emerge from the seed) for the adult emergence. The fecundity and adult emergence were calculated for each cowpea cultivar as mentioned above for the non-choice test. This experiment was done in the insect laboratory in an incubator under the same environmental conditions mentioned above.

RESULTS

2.1. Host preference and fecundity of *C. maculatus*

Females laid most eggs throughout the first two days after emergence from the cowpea seeds. The total number of eggs laid by *C. maculatus* was found to be not significant in all cowpea cultivars in the no-choice test (F=0.95, df =14, P= 0.48) (Fig.1a). On the other hand, the total number of eggs deposited by the cowpea beetle differed significantly among the cowpea cultivars in the choice test (F=5.31, df =14, P= 0.02) (Fig. 1b). There was a significant increase in a number of eggs laid on both Kafr El-Sheikh1 and Kaha1 seeds compared to Sakha1 cultivar (Fig. 1b). The maximum numbers of eggs were laid on Kafr El-Sheikh1 cultivar in both tests while the least numbers of eggs were found on Sakha1 cultivar in both tests (Fig. 1a, b).

(a) No choice **(b) Choice**

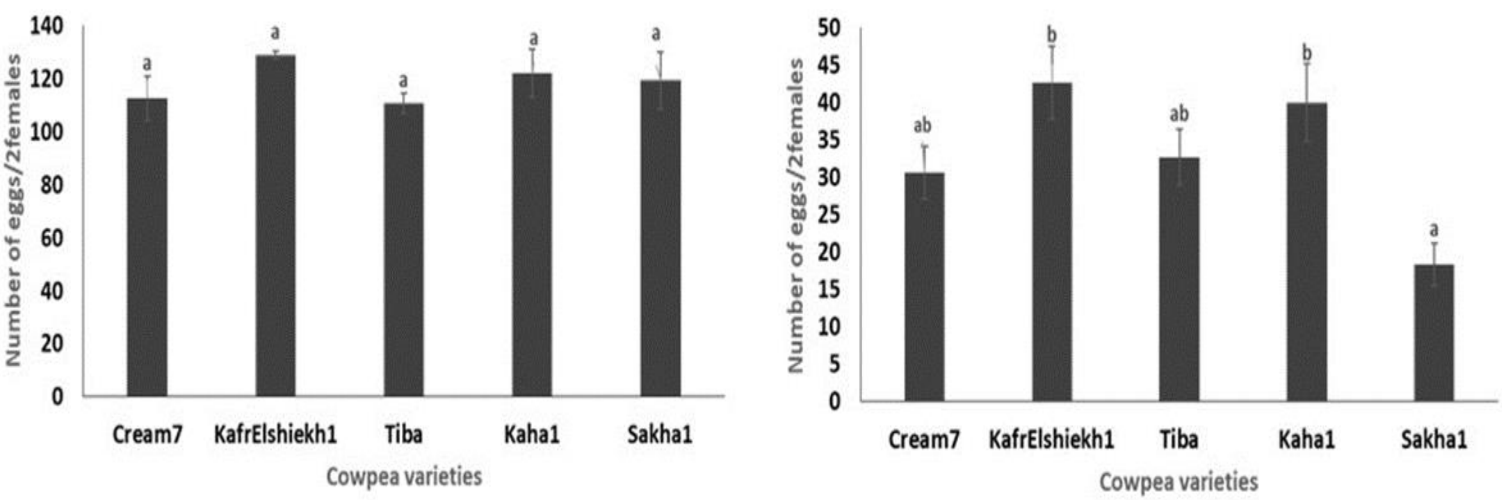
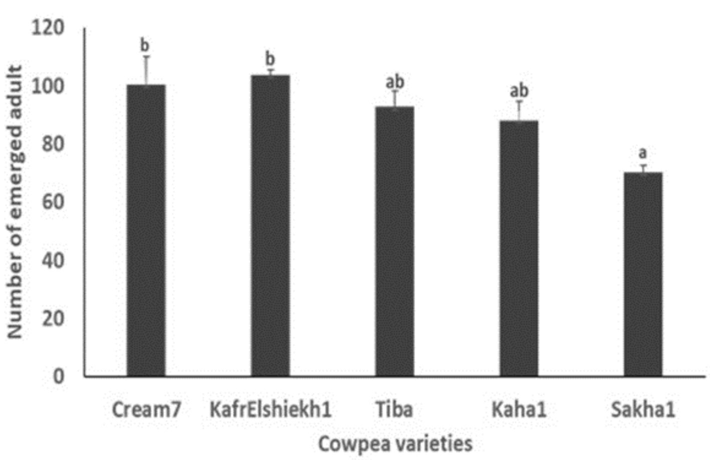


Fig. 1. Host preference and fecundity (means ± SE) of *C. maculatus* on five cowpea cultivars in the no-choice (a) and the choice (b) scenarios. Means followed by the same letters are not significantly different, as determined by Tukey's post hoc test at (P < 0.05). Three replications were made.

2.2. Total number of *C. maculatus* adults emerged from different cowpeas cultivars

The *C. maculatus* adults started to emerge from the cowpea seed cultivars after fifteen days of laying eggs. The number of adults emergence showed significant differences among cowpea cultivars in both no-choice (F=4.96, df =14, P= 0.02) and choice (F=5.12, df =14, P= 0.02) tests (Fig. 2 a, b, respectively). In the no-choice scenario, the total number of emerged adults was significantly higher in Kafr El-Sheikh1 and Cream7 cultivars when compared to Sakha 1 (Fig. 2a). Because the ANOVA test p-value did not show significant differences between cowpea cultivars we used the Tukey test for the comparison procedure to identify which pairs of cowpea cultivars differed significantly. In the Tukey test, the comparison between cultivars in the no-choice test showed a significant difference between Kafr El-Sheikh1 and Sakha1 (p=0.02), Cream7 and Sakha1 (P=0.03), and no significant difference (NS) was observed between other cultivars. While the choice test showed a significant difference between Kafr El-Sheikh1 and Sakha1 (p=0.02), Kaha1 and Sakha1 (p=0.03), and no significant difference (NS) was observed between other cultivars.

(a) No choice



(b) Choice

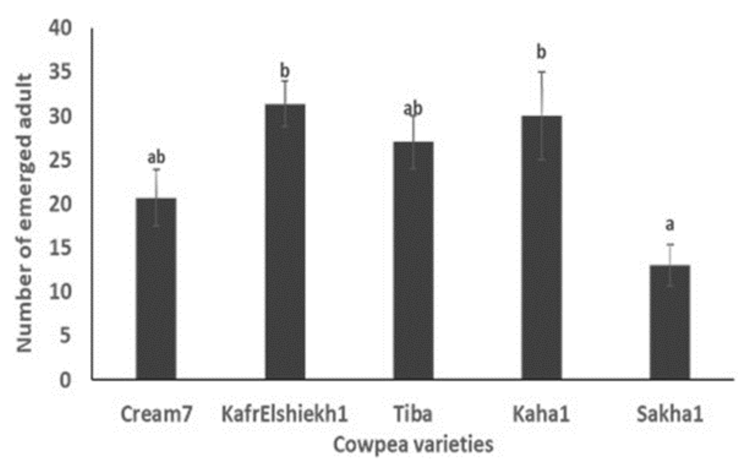


Fig. 2. Effect of different cowpea cultivars on adult emergence of C. *maculatus* in both the no choice (a) and the choice test (b). Means followed by the same letters are not significantly different, as determined by Tukey's post hoc test at (P < 0.05). Three replications were made.

2.3. Effect of different cowpea cultivars on *C. maculatus* development time

The KRUSKAL WALLIS H test was applied for the data of development time as the data did not follow the ANOVA parametric analysis. The data on the effects of different cowpea cultivars on the development period of *C. maculatus* are shown in Figure 3. We noticed significant variation in the average development time of *C. maculatus* on cowpea cultivars in the no-choice test (Fig. 3a; H = 72.77; df = 4; P < 0.001). Tiba cultivar showed the longest average development time at 23.27 days, while Kafr El-Sheikh1 showed the shortest period at 21.5 days. In the choice test (Fig. 3b; H = 47.88; df = 4; P < 0.001), the mean development time was significantly reduced in cowpea cultivars compared to Tiba one. The longest and shortest development period was recorded in Tiba and Kafr El-Sheikh1 cultivars recording 22.81 and 20.43 days, respectively (Fig. 3 b).

(a) No choice **(b) Choice**

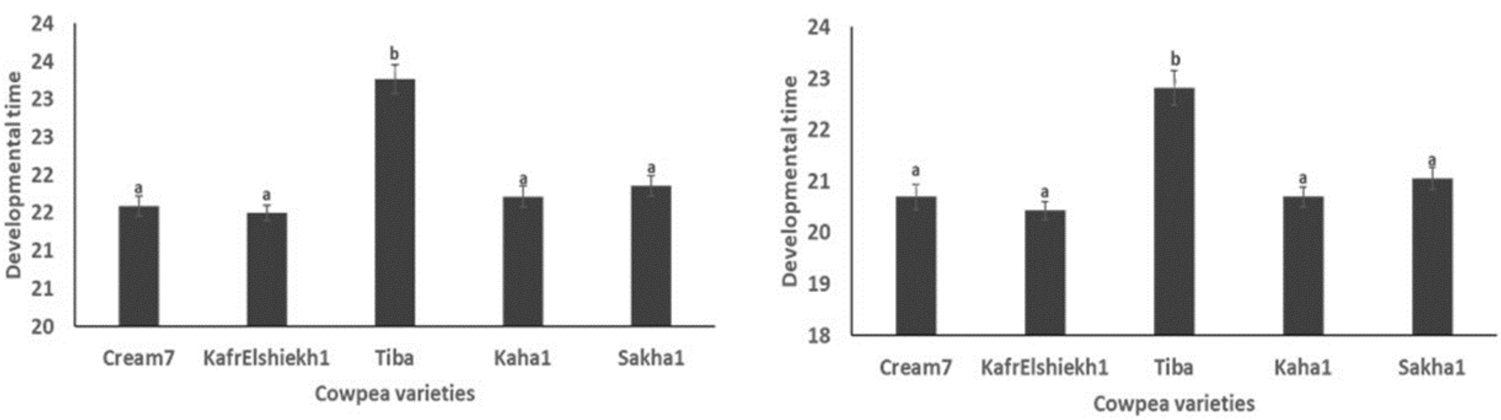
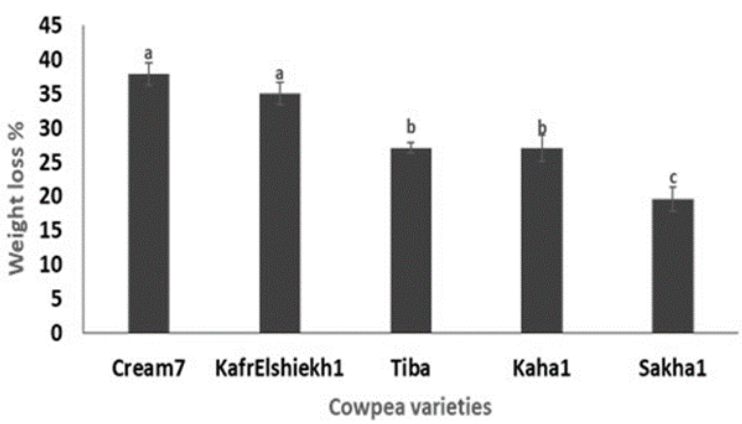


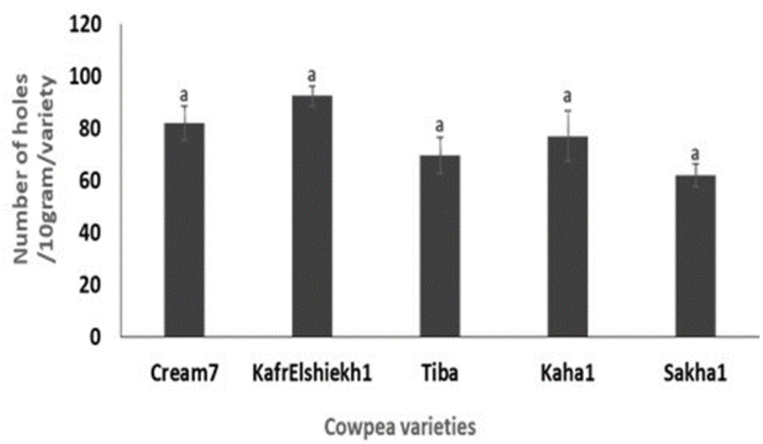
Fig. 3. Effect of different cowpea cultivars on development time of C. *maculatus* in both no choice (a) and the choice test (b). Means followed by the same letters are not significantly different, as determined by Kruskal-Wallis (H) test at (P < 0.05). Three replications were made.

2.4. Effect of feeding by *C. maculatus* on seed weight loss of different cowpea cultivars

The seed weight loss among the different cowpea seeds differed significantly among the groups in the no-choice test (F = 21.25; df = 14; P < 0.001; Fig. 4 a, b). Cream7 showed the highest significant loss in seed weight (37.90%) with a mean number of holes (82), whereas Sakha1 showed the least loss in seed weight (19.58%) with a mean number of holes (62). Similarly, the weight of seeds differed significantly among the treatments in the choice test (F = 9.93; df = 14; P <0.001; Fig. 4 c,d). The Kafr El-Sheikh1 revealed the highest significant loss in seed weight (43.07%) with a mean number of holes (28), whereas Sakha1 (14.19%) revealed the least loss in seed weight with a mean number of holes (9.67).

No choice (a) **(b)**





Choice (c) **(d)**

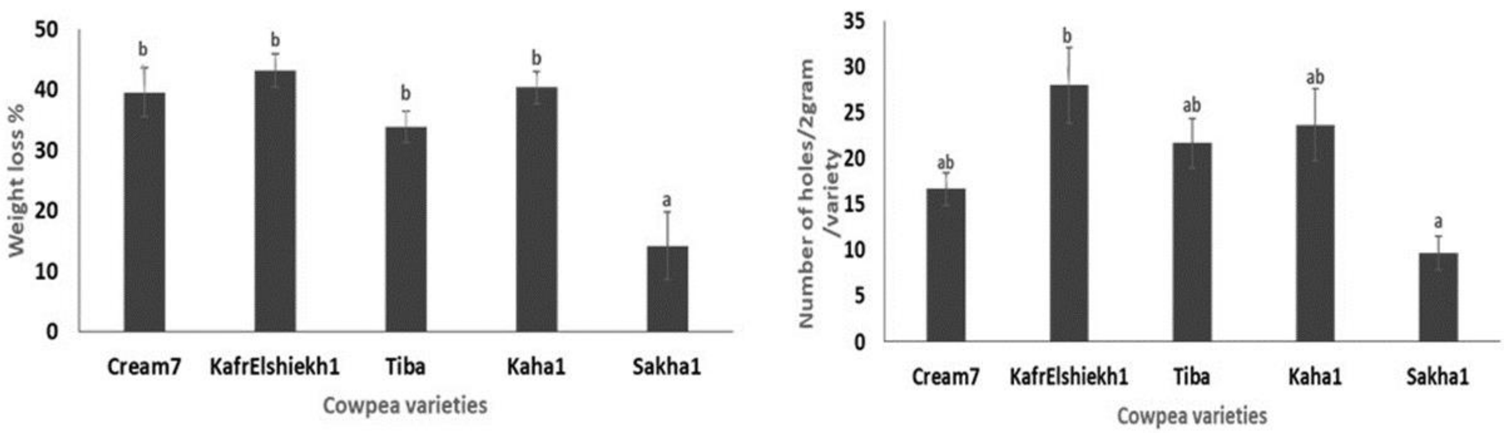


Fig. 4. Weight seed loss % and number of holes (means ± SE) of different cowpea cultivars in the no-choice (a,b) and the choice (c,d) tests. Means followed by the same letters are not significantly different, as determined by Tukey's post hoc test at ($P < 0.05$). Three replications were made.

DISCUSSION

The cowpea, *V. unguiculata* insect-resistant cultivars were used several years ago as an effective and alternative control method to reduce the *C. maculatus* spread and losses in production, in addition to minimize insecticide use, production cost, and environmental pollution [23, 24]. Among the key indices of cowpea resistance to *C. maculatus*, damage are the percentages of fecundity, adult emergence, development time, growth index, and seed weight loss [25].

In this study, we assessed the vulnerability and resistance of five different *V. unguiculata* cultivars to infestation and damage by *C. maculatus*, considering the above-mentioned parameters as the main indicators. Our findings showed reductions in fecundity, adult emergence, and delayed larval development in *C. maculatus* that have been grown on Tiba, Sakha1, Kaha1, Cream7, and Kafr Elshiekh1 cowpea cultivars. In the no-choice experiment, the numbers of eggs laid by *C. maculatus* were not significantly different between the five cowpea cultivars. This may be due to the fact that all the used cowpea cultivars are suitable hosts for the pest. Kebe et al. [8] indicated that *C. maculatus* has a wide range of host plants and the females can use the cowpea seeds available to them at the time of oviposition. Additionally, the same authors demonstrated that the non-significant difference in fecundity percentage in the no-choice test may be due to the small variations in seed size among the five cultivars tested here when other options are present.

In choice test, the total number of eggs laid by *C. maculatus* differed statistically between cowpea cultivars, with more eggs deposited on Kafr El-Shiekh1 followed by Kaha1 cultivars. The considerable disparity in oviposition reported here on different cowpea types might be explained by the size, texture, color, tegument hardness, and/or chemical composition of the seeds [17, 20, 26].

Bruchids favored large-seeded cowpea types when both large and small-seeded cultivars were provided [27]. Additionally, the cowpea beetle, *C. maculatus* was found to lay more eggs on smooth cowpea seeds over wrinkled seeds [26, 27, 28] and this agrees with some cultivars tested in our experiment. Other seed characteristics may also have a role in determining oviposition decision.

The female cowpea beetle *C. maculatus* may use environmental parameters such as seed stimulation such as fragrance, texture, or humidity levels to determine which seed type is desirable. [28]. In 1998, Parr et al. [29] found that Chemical substances on the seed surface, such as fatty acids and alkanes, can have a significant impact in the cowpea beetle's oviposition behaviour. As a result of these chemicals being linked to the seed color and hardness of cowpea cultivars, they may explain the considerable difference in female oviposition in choice tests between Kafr-Elshiekh1, Kaha1 (white and pale creamy color cultivar) and Sakha1 (white with yellow hilum color cultivar). Bele *et al.* [30] discovered a high level of tannins (more concentrated with darker colors) in the teguments of colored cowpea seeds, polyphenols that have been linked to reduced food intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in the tested insects. Insect pests' capacity to recognize. In our results, significant differences in adult emergences and development times of *C. maculatus* were found between cowpea seed cultivars in both choice and no-choice tests. Also the total numbers of holes were lesser than the total numbers of adults emerged from the seeds. This finding could be due to the rivalry among larvae within the seed which prevent the total number of larvae from reaching maturity or more than one adult using the same holes for the emergence as reported by Kébé [8]. Tiba cultivar displayed the most delayed development time when keeping records of the number of adult emergences throughout time. The delay in development could be due to the thick seed coat, which prevents immature larvae from entering the seed and adults from emerging from the seed [31].

The low rate of emergence in Sakha1 shows major larval mortality, which can be analyzed by more than one factor. First, the seed's chemical composition (high in tannins and lignin), Dobie *et al.* [32] and hardness of the seed tegument may prevent the larvae from reaching the cotyledons at the primary stage [33]. Second, the decrease in emergence rates in some seed cultivars could also be explained by the fact that the nutritional values of compounds in the teguments were unfavorable for feeding *C. maculatus* larvae and for their development [8]. Third, a decreased emergence rate in some cowpea types may be investigated as higher larval mortality in the cotyledons, most likely because of the presence of some seed chemicals that cowpea larvae cannot detoxify [10, 34]. Souza *et al.*, and Edde & Amatobi [20, 35] found high quantities of peptides and proteins in the embryonic cot of some cowpea cultivars seeds which may have been related to resistance to insects.

According to Torres *et al.* [36], the level of the injury and consequent decreased weight of cowpea seeds is largely dependent on the number of *C. maculatus* adult emergences on the grain. Thus, the more the number of F1 offspring emerging on a certain cultivar, the greater cultivar's damage and decreased weight, and vice versa. In the current study, Sakha1 had the lowest percentage of weight loss in both tests, which might be related to their intrinsic chemical ingredients that make them unpalatable to the insect. Arcelin in the cotyledons, tannins in the seed coat, and phytohemagglutinin (PHA) within the seed, which comprised-amylase inhibitors, are among the chemical components implicated in storage pest resistance [37, 38]. Moreover, the same authors indicated that the existence of these chemical components in the legume grains explains antibiosis or non-preferential resistance mechanisms against bruchid attack by the legume grains. Seeds of Kafr

Elshiekh1, Cream7 and Kaha1 cultivar lost a substantial percentage of weight due to insect damage, rendering them unfit for selling and consumption by the general population. We discovered that the behavior of *C. maculatus* is affected by the sort of cowpea cultivar, particularly in the choice test. Although all of the examined cultivars permitted *C. maculatus* to grow, their performance was greater on the Kafr Elshiekh1 and Kaha1 types (white and spotted cultivars, respectively) than the other three. Larval mortality was higher in Sakha1 than in the other cultivars, which might be attributed to anti-nutritional substances found in the embryonic cot and/or teguments of these types.

CONCLUSION

The five cowpea cultivars were found to be variable in their effects on C. *maculatus* biology. In terms of the mean number of eggs deposited, development duration, and adult emergence of the insect, as well as cowpea decreased weight, there were substantial variations across the cultivars. The Sakha1 cultivar had the lowest development indices, hosted fewer larvae, and was thus thought to be more resistant to the cowpea seed beetle.

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