

SUSCEPTIBILITY OF DIFFERENT LEGUME SEEDS TO *Callosobruchus maculatus* (Fab.) INFESTATION

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ABSTRACT

Three different commercial species of legume seeds, Cowpea, *Vigna unguiculata* (Walp.), Faba beans, *Vicia faba* (L.), Soya beans, *Glycine max* (L.), and Five *Phaseolus vulgaris* (L.) varieties (White beans, Navy beans, Red (Kidney) beans, Pinto beans and Black beans) were tested for their susceptibility to *Callosobruchus maculatus* infestation. The present study aims to assess the susceptibility of the local pulse seeds compared to the imported ones to *C. maculatus* infestation. Number of laid eggs, incubation period, hatchability percentage, number of emerged adults, adult longevity, mean developmental period, weight loss percentage damage, percentage, adult weight and susceptibility index were recorded as main parameters to evaluate susceptibility of the tested legume beans to *C. maculatus* infestation. The highest mean number of eggs was laid on marine beans (20.5 per seed), while Soya beans have received the lowest mean number of eggs (5.25 per seed). Mean incubation period was not significantly differed between all tested legume seeds. The percentage of hatchability between the eight tested legume seeds were significantly differed. The highest percentage of egg hatchability was obtained in cowpea seeds 99.01%, while the navy bean seeds have the lowest percentage of egg hatchability (96.09%). Zero adult emergence in red, navy, white, black and pinto bean seeds this due to the presence of vicine in these seeds which act as a larval inhibitor and prevent the larva to complete its duration. The mean number of emerged adults was 42.25, 35.75 and 9.75 in cowpea, faba and soya bean seeds, respectively. Results indicated that cowpea was the most susceptible legume seeds to *C. maculatus* with the highest value of Susceptible Index (SI) (6.188) followed by faba (5.640) and soya beans was moderately resistant to *C. maculatus* (3.84). The same trend was recorded regarding to percentage of weight loss, percentage of damage and adult weight. Red, navy, white, black and pinto beans were resistant to *C. maculatus* infestation with zero value of Susceptible Index (SI). The resistant of these legume seeds to *C. maculatus* infestation probably due to the texture of seed coat, and their hardness or presence of other toxic compounds in these seeds.

Keywords: *Callosobruchus maculatus* (Fab.), Susceptibility, Infestation, Legume seeds

INTRODUCTION

Family Bruchidae consists of approximately 1300 species, grouped into 56 genera placed within 5 subfamilies. They exist in every continent especially in tropical regions of Asia, Africa and central and South America except Antarctica (Southgate 1979).

Some of the bruchid species have showed high specificity to one or more species of host plant while others are capable to feed and breed on a wide range of hosts (Johnson 1981).

Among storage bruchids, the cowpea beetle *Callosobruchus maculatus* (F.) and pulse beetle *C. chinensis* are considered serious pests. Causing immense damage every year to legume seeds and attacking legume seeds of

during the warm season, also they able to generate exceeding high levels of infestation even when they were passed only one or two generations on the host (Shomar 1963). The cowpea bruchid, *C. maculatus* (F.) is a cosmopolitan field –to- store pest and ranked as the principal post harvest pest of cowpea, *Vigna unguiculata* (L.) Walpers in the tropics (Jackai and Daoust, 1986). It causes substantial quantitative and qualitative losses manifested by seed perforation, and reductions in weight market value and germinability of seeds (Sekou *et al.* 2001). Under traditional storage conditions, 100% infestation of cowpea occurring within 3-5 months of storage is common (Booker, 1967, Caswell and Akibu, 1980). The integration of insecticidal natural products from locally – available plants for use in storage, and the growing of varieties of cowpea with some resistance of *C. maculatus* may lead the sustainable management of the bruchids especially in subsistence agriculture. Now there has been a move between plant breeders and entomologists to improve grain legume crops by breeding varieties that gives higher yields and are resistant to the pests that devastate the current varieties. Pulses are important sources of proteins, fats, carbohydrates, sugars and vitamin B (Aslam *et al.* 2006). As these beetles do not feed as adults, their reproductive potential, longevity, and growth are determined entirely by resources accumulated during development. Hence, differences in bean quality are likely to be especially important. Indeed, previous studies on *C. maculatus* (Fab.) have shown that host size (Credland *et al.* 1986), host species (Wasserman 1986) can all affect larval survival and development time, and also the fecundity of emerging adults. Credland *et al.* (1986) reported that there is a strong relation between female fecundity and emergence size. Colegrave (1995) indicated that there is a correlation between developmental conditions and the female lifetime. It appears that not many research studies on the susceptibility of this imported pulse seeds compared to the common local pulse seeds in were done in Egypt. The objective of the present study is to determine the susceptibility of the local pulse seeds compared to the imported ones to *C. maculatus* infestation.

MATERIALS AND METHODS

Cowpea beetle *C. maculatus* was obtained from a colony maintained in Plant Protection Research Institute, Agricultural Research Center, Cairo-Egypt. The colony of cowpea beetle reared on a cowpea seeds in 1-liter glass jars and incubated in an environmental controlled condition at temperature $30^{\circ}\pm 2^{\circ}\text{C}$ and 60 ± 5 RH% in the research laboratory, department of plant protection, Al-Azhar University, Cairo-Egypt. Three different commercial species of legume seeds, Cowpea, *Vigna unguiculata* (Walp.), Faba beans, *Vicia faba* (L.), Soya beans, *Glycine max* (L.), and five *Phaseolus vulgaris* (L.) varieties (White beans, Navy beans, Red (Kidney) beans, Pinto beans and Black beans) were tested for their susceptibility to *C. maculatus* infestation.. The last four varieties are not commercially used and grow in Egypt and obtained from a commercial market in the US. The legume seeds were cleaned by washing in ether and left to dry at room temperature and then stored in refrigerator (to kill any pests present) until use.

Two pairs of newly emerged *C. maculatus* adults were placed in a small transparent plastic tubes (100g) contained ten weighed seeds of each tested legume seeds, tubes covered with muslin for aeration. Tubes with each tested legume seeds and *C. maculatus* adults were replicated four times and held in the incubator at temperature $30 \pm 2^\circ\text{C}$ and 60 ± 5 RH%. Number of deposited eggs and hatched ones were counted followed by daily check for the adult emergence. The emerged adults were counted from each tube and the developmental period was estimated from the time of eggs laying up to the appearance of first adult.

The total number of emerged adults was counted and percentage of adult emergence was calculated in relation to the number of hatched larvae. The developmental period of immature stages was taken as criteria for calculating the susceptibility index according to Howe (1971) and Dobie (1974) as following:

Susceptibility Index (SI) = $(\text{Log } S/T) \times 100$

Where, S = adult emergence (%)

T = developmental period (days)

The values of susceptibility index were categorized into five ranks according to Mensah (1986) as following:

A: The values between 0.0 – 2.5 are considered resistant variety (r).

B: The values between 2.6 – 5.0 are considered moderately resistant variety (mr).

C: The values between 5.1 – 7.5 are considered moderately susceptible variety (ms).

D: The values between 7.6 – 10.0 are considered susceptible variety (s).

E: The values > 10.0 are considered highly susceptible variety (hs).

After the adults emerged the seeds were weighed after excluding the frass and dust.

The weight loss was calculated using weight loss % (Khare and Johari, 1984) as following:

Weight loss (%) = $(\text{Initial dry weight} - \text{final dry weight}) / \text{Initial dry weight} \times 100$

Also, the damaged contains eggs and holes and undamaged seeds were recorded and the percentage of damage was calculated according to Abd El-Salam 2005 as follows:

% Seed damaged = $(\text{Number of seed damaged} / \text{total number of seed damaged and undamaged}) \times 100$

Resulted data of the tested parameters number of eggs laid, incubation period, hatchability percentage, number of emerged adults, adult longevity, mean developmental period, weight loss percentage, damage percentage and susceptibility index were subjected to statistical analysis by Analysis of variance (ANOVA) test using a computer software SAS (SAS Institute 1988). Means were detected and compared by Duncan multiple range test at 0.05% probability level (Duncan, 1955).

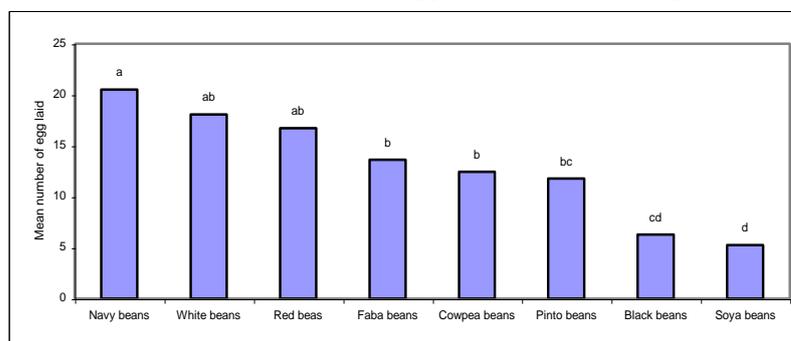
RESULTS AND DISCUSSION

Three different commercial species of legume seeds, Cowpea, *Vigna unguiculata* (Walp.), Faba beans, *Vicia faba* (L.), Soya beans, *Glycine max* (L.), and Five *Phaseolus vulgaris* (L.) varieties (White beans, Navy beans, Red (Kidney) beans, Pinto beans and Black beans) were tested for their susceptibility to *C. maculatus* infestation. The last four varieties are not commercially used and grow in Egypt and obtained from a commercial market in the US. Table (1) showed that the oviposition rate of *C. maculatus* under a non-choice condition showed a significant variation ($P = 0.0001$) the eight tested bean seeds.

Table (1): Response of the developmental stages of *C. maculatus* on different legume seeds.

Legume Seeds	No. of eggs Laid	Incubation period	% Hatchability	No. of emerged adults	Adult Longevity	Mean Developmental Period (MDP)
Red beans	16.72±2.27	4.312±0.16	97.427±10	-	-	-
Navy beans	20.50±5.10	5.145±0.43	96.097±1.03	-	-	-
White beans	18.075±2.52	4.832±0.65	97.557±0.62	-	-	-
Cowpea	12.45±0.37	4.197±0.21	99.01±0.62	42.25±2.25	8.86±0.33	26.292±0.62
Faba beans	13.62±2.82	4.547±0.44	98.765±0.68	35.75±3.87	9.187±0.50	27.497±0.42
Black beans	6.275±2.62	4.725±0.64	97.727±0.46	-	-	-
Pinto beans	11.77±3.42	4.512±0.22	97.427±0.26	-	-	-
Soya beans	5.25±1.20	4.975±0.14	98.95±0.23	9.75±2.25	8.105±0.84	28.735±0.30
SS	813.22	2.941	28.416	15308.86	572.426	5665.037
MS	116.17	0.420	4.059	2186.98	81.775	809.291
F-Value	7.291	1.568	5.441	188.76	389.562	4984.190
P = 0.05	0.0001	0.192	0.0008	0.0001	0.00001	0.00001

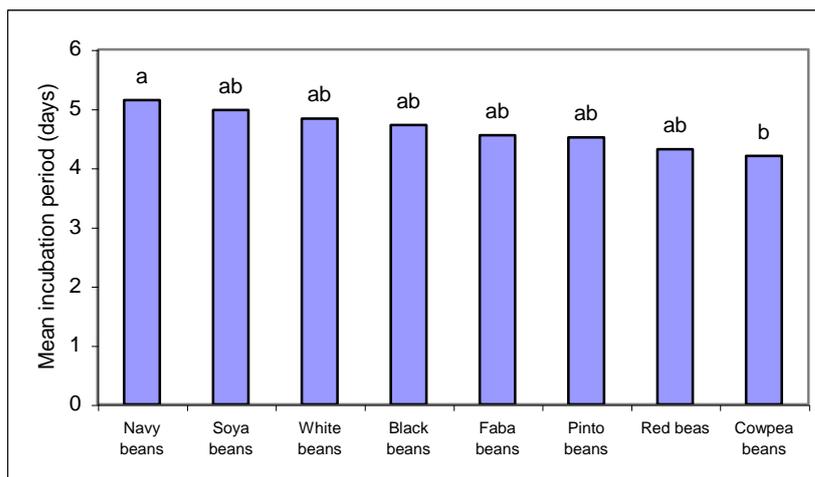
The highest mean number of eggs laid was on marine beans 20.5 per seed, while Soya beans have received the lowest mean number of eggs laid 5.25 per seed (Figure 1).



Means followed by same letter are not significantly different ($P = 0.05$)

Figure 1: Mean number of eggs laid of *C. maculatus* on different tested pulse seeds.

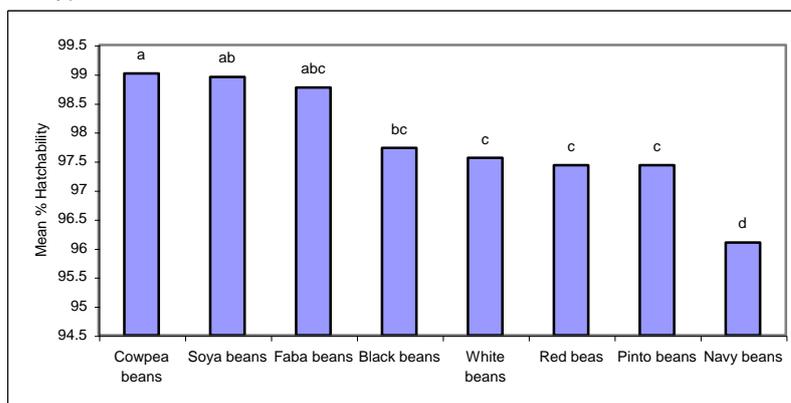
Mean incubation period was not significantly differed between all tested bean seeds ($P = 0.192$). Results indicated that the shortest incubation period was recorded in cowpea seed 4.197 days, while the longest incubation period was recorded in the Navy bean seeds 5.145 days (Figure 2).



Means followed by same letter are not significantly different ($P = 0.05$)

Figure 2: Mean incubation period of *C. maculatus* on different tested pulse seeds.

Results in table (1) indicated that there were a significant variation ($P = 0.008$) of the percentage of hatchability between the eight tested bean types.



Means followed by same letter are not significantly different ($P = 0.05$)

Figure 3: Mean percentage of hatchability of *C. maculatus* on different pulse seeds.

The highest percentage of egg hatchability was obtained in cowpea seeds 99.01%, while the Navy bean seeds have the lowest percentage of egg hatchability 96.09%.

Larvae failed to complete its duration with 100 percent of mortality resulted to zero adult emergence in Red, Navy, White, Black and Pinto beans (Table 1). Obtaining the 100% mortality during the larval stage appears to be due to the presence of vicine in seed coat affects the perforation rate of insect larvae

and seemed to be the main mortality factor (Desroches *et al.* 1995). Also, Smitanond (1991) reported that the isolation and characterization of a glycosylprotein from Red (kidney) beans *P. vulgaris* which is a larval growth inhibitor of the bruchid *Callosobruchus chinensis* could be the explanation of larval mortality. The mean number of emerged adults was 42.25, 35.75 and 9.75 in cowpea, faba and soya bean seeds, respectively. Results indicated that the mean number of adults emerged varied significantly ($P = 0.0001$) in all tested legume beans (Table 1). Results in Table (1) referred that the mean adult longevity was 9.187, 8.860 and 8.105 days, respectively. Statistical analysis showed a significant variation of adult longevity between the tested legume seeds ($P = 0001$). The shortest mean developmental period (MDP) of *C. maculatus* larvae occurred in cowpea 26.292 days, whereas longer MDP 27.497 and 28.735 days were recorded in faba and soya bean, respectively (table 1). Statistical analysis indicated that a significant variation of mean developmental period in all tested legume seeds ($P = 0.0001$).

Table (2) indicated that cowpea was the most susceptible legume seeds to *C. maculatus* with the highest value of Susceptible Index (SI) 6.188. Same trend was observed in cowpea regarding to percentage of weight loss, percentage of damage and adult weight 65.324%, 97.5%, 0.00149g, respectively. Faba bean was moderately susceptible to *C. maculatus* with 5.640 SI value. Weight loss%, damage% and adult weight were 32.177%, 8.50%, and 0.00145g, respectively. Soya beans considered a moderately resistant to *C. maculatus* with the low SI value 3.3384. Weight loss%, damage% and adult weight were 10.675%, 5.50%, and 0.00132g, respectively.

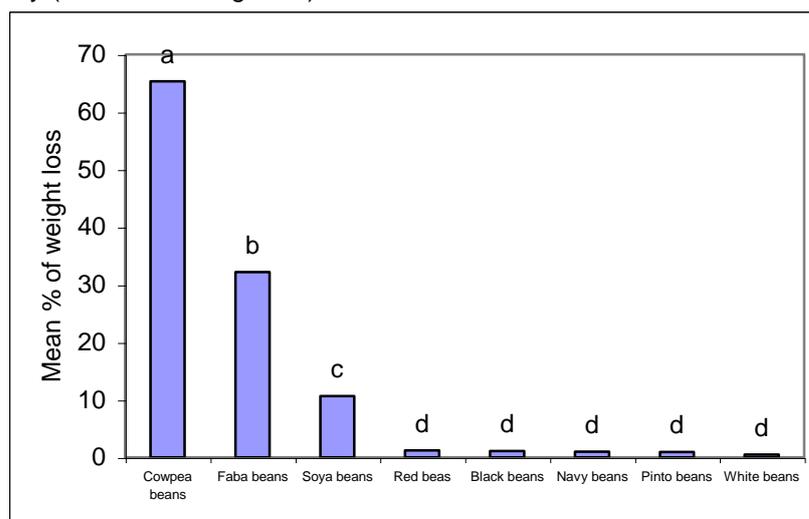
Table (2): Susceptibility of different legume seeds to *C. maculatus* infestation.

Legume Seeds	% Weight loss (Seeds)	% Damage	Adult weight (g)	Susceptibility Index (SI)
Red beans	1.233±0.308	0.00	-	0.00 (r)
Navy beans	0.978±0.082	0.00	-	0.00 (r)
White beans	0.510±0.331	0.00	-	0.00 (r)
Cowpea	65.3240±0.726	9.75±0.37	0.00149±0.00051	6.188±0.236 (ms)
Faba beans	32.177±6.713	8.50±0.50	0.00145±0.00147	5.640±0.265 (ms)
Black beans	1.092±0.448	0.00	-	0.00 (r)
Pinto beans	0.933±0.509	0.00	-	0.00 (r)
Soya beans	10.657±0.491	5.50±1.0	0.00132±0.00061	3.384±0.403 (mr)
SS	8782.718	516.304	1.5223	210.502
MS	1254.674	73.757	2.1748	30.071
F-Value	227.691	449.571	359.598	490.547
P = 0.05	0.00001	0.00001	0.00001	0.00001

These results agreed with Aly *et al.* (2004) who reported a similar trend of cowpea and faba weight loss. The percentage of weight loss in cowpea seeds ranged from 42- 61.6%, while weight loss percentage was slightly lower in faba bean ranged from 8.3 - 28.3%.

Also, El-Degwi and El-Orabi (1997) reported that 6 - 20.6% and 14.6 - 24.1% losses of faba bean and cowpea, respectively when infested with *C. maculatus* under laboratory conditions. In addition, El-Shazly and El-Shabrawy (2000) recorded 12.15% weight loss in cowpea seeds, while this percentage reduced to 8.63% in faba bean attacked by *C. maculatus*. Red, Navy, White, Black and Pinto bean are considered a resistant to *C. maculatus* with zero value of susceptible index and damage% and adult weight. These results are in harmony with the results of Aly *et al.* (2004) reported that *C. maculatus* caused more damage to cowpea seeds ranged from 39.3 to 58.5% according to seed varieties, compare to faba beans that have less damage ranged from 5.2 – 17.4%.

Also results indicated very low values of weight loss% were obtained in Red, Black Navy, Pinto and White bean 1.233, 1.092, 0.978, 0.933, and 0.5100%, respectively (Table 2 and Figure 4).



Means followed by same letter are not significantly different ($P = 0.05$)

Figure 4: Mean weight loss percentage of *C. maculatus* on different pulse seeds.

Statistical analysis showed that there were significant differences ($P = 0.0001$) in susceptibility index, weight loss%, damage% and adult weight between all tested legume seeds. Seed coat and the texture of seed coat, its hardness or presence of other toxic compounds could explain these results (Desroches *et al.* 1995). Further research needed to study the potential effect of the extracted *P. vulgaris* toxic compound glycosylprotein (vicine) as a natural protectant to the legume beans from *C. maculatus* infestation which can be implicated in the integrated pest management of the stored legume insect pests.

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حساسية بعض بذور البقوليات للاصابة بحشرة خنفساء اللوبيا (*Callosobruchus maculatus* (FAB.))

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في هذه الدراسة تم اختبار ثلاث أنواع من بذور البقوليات المحلية هي اللوبيا *Vigna unguiculata* (Walp) وال فول البلدي (*Vicia Faba* (L.)) وفول الصويا *Glycine Max* كما تم ايضا تم اختبار خمسة أصناف من الفاصوليا (*Phaseolus Vulgaris* (L)) (الفاصوليا البيضاء والبحرية والحمراء والبنيتو وكذلك السوداء) للاصابة بحشرة خنفساء اللوبيا والهدف من هذا البحث هو تقييم حساسية الاصناف المحلية للاصابة بحشرة الدراسة مقارنة بالاصناف الغير متاحة بالأسواق المصرية ... تم تقدير عدد البيض الذي وضع بواسطة الأثاث وفترة حضانة البيض ونسبة الفقس عدد الحشرات الكاملة الناتجة وفترة عمر الحشرة الكاملة كذلك معدل الحساسية للاصابة . كان اعلى متوسط لعدد البيض على الفاصوليا البحرية وذلك بمعدل ٢٠,٥ لكل حبة بينما كان فول الصويا أقل الاصناف الذي وضع عليه البيض وذلك بمتوسط ٥,٢٥ لكل حبة وكانت الفروق في متوسط فترة حضانة البيض غير معنوية في كل الاصناف المختبرة . بينما كان هناك فرق معنوي جداً في نسبة الفقس بين أصناف البقوليات المختبرة حيث سجلت اعلى نسبة فقس على اللوبيا (٩٩,٠١ %) بينما كانت أقل نسبة على الفاصوليا البحرية (٩٦,٠٩ %) . لم يتم خروج اي حشرة في أصناف الفاصوليا الحمراء والبحرية والبيضاء والسوداء كذلك البنيتو حيث فشلت جميع اليرقات في استكمال نموها بنسبة ١٠٠ % في هذه الاصناف وقد يعزى ذلك الى وجود مادة الفاييسين في بذور البقوليات المقاومة السابق ذكرها والذي ادى الى موت اليرقات في اعمارها الاولى كان متوسط عدد الحشرات الكاملة الناتجة ٢٥ ، ٤٢ ، ٧٥ ، ٩ ، في بذر اللوبيا والفول البلدي وكذلك فول الصويا على التوالي واطهرت النتائج ان بذر اللوبيا كانت اكثر البقوليات حساسية للاصابة بخنفساء اللوبيا وذلك لتسجيلها لاعلى معدل حساسية (٦,١٨٨) ويليها بذور الفول البلدي (٥,٦٤٠) ثم بذور فول الصويا (٣,٨٤) نفس الاتجاه في النتائج قد تم تسجيله بالنسبة لنسبة الفقس وكذلك نسبة الفقد ووزن الحشرة الكاملة .