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CERTAIN STORAGE PROTEINS AND THEIR ROLE IN THE RESISTANCE OF SOME LEGUME SEED TYPES AND VARIETIES TO INFESTATION WITH THE COWPEA SEEDS BEETLE, *Callosobruchus maculatus* (F.)

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ABSTRACT

Seventeen legume seed varieties belonging to ten types were analyzed for each of total proteins, trypsin inhibitors, vicilins and lectins to study the relationship between the presence of the storage seed proteins in legume seeds and resistance to infestation with the cowpea seeds beetle, *Callosobruchus maculatus* (F.) under no-choice bioassay at constant conditions of 29±1°C and 70 ± 5% RH. The tested types and varieties were cowpea (Fitriat, Forage and Kafr El-Sheikh), fababean (Aquadams, Giza 843 and Sakha 1), pea (Master B and Smooth) common bean (Bronco and Nepraska), lupin (Giza 1 and White lupin), chickpea (Giza 195), soyabean (Giza 111), lentil (Giza 9), guar (Guvar bean) and lablab (Egyptian kidney bean). To clarify the impact of the storage seed proteins in legume seed varieties and types on infestation with *C. maculatus*, larval period, pupal period, least and mean complete developmental periods, number of emerged adults per female, percentage of adults emergence and sex ratio (as percentage of males) were determined. Moreover, susceptibility index, infested seeds (%), mean number of holes per infested seed, seeds weight loss (%), weight of early emerged adult and number of dead individuals inside seeds per female were assessed as infestation parameters. The obtained results showed that, each of total proteins, trypsin inhibitors, vicilins and lectins of different tested legume seed varieties and types are negatively correlated with all aforesaid resistance parameters with the exception of those respecting larval and pupal durations, least and mean complete developmental periods and number of dead individuals inside seeds per female. As regards total proteins of varieties, larval period, least and mean complete developmental periods, susceptibility index and number of dead individuals inside seeds per female reached high significance degree at 0.01 level of probability, whereas pupal period obviously showed significant correlation coefficient at 0.05 level of probability. But, the other studied resistance parameters did not demonstrate significant correlations. Significancy test of correlation relationships respecting trypsin inhibitors clearly proved to be insignificant for all studied characters, except for those of larval stage period, least and mean developmental periods which cleared highly significant correlations and significant ones with susceptibility index, number of holes per infested seed and number of dead individuals inside seeds per female. All tested seed varieties of guar, lupin and common bean revealed completely linear inverse relationship between trypsin inhibitors and susceptibility to cowpea seeds beetle infestation indicating deterring effects for insect development. Also, vicilins content proved that simple correlation coefficient values regarding the abovementioned resistance parameters reached high significancy degree excepting those concerning larval stage period, percentage of infested seeds, seeds weight loss percentage and weight of emerged adult that showed significant correlation as well as insignificant ones were recorded with sex ratio as percentage of adult males and number of dead individuals inside seeds per female. All tested legume seed varieties of soyabean- Giza111 and lentil- Giza 9 revealed almostly linear inverse relationship between vicilins and susceptibility to cowpea seeds beetle infestation and showed retarding effects for insect development. As concerns lectins of the infested legume seed varieties, the correlation relationships proved to be highly significant with larval stage period, least and mean complete developmental periods, susceptibility index and number of dead individuals inside seeds per female, while in case of number of emerged adults per female, adults emergence percentage, number of holes per infested seed and seeds weight loss percentage the correlation was significant. The correlation of other resistance parameters did not attain significancy degree.

Key words: Legume seed types and varieties, *Callosobruchus maculatus* (F.), resistance, storage seed proteins, trypsin inhibitors, vicilins, lectins.

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INTRODUCTION

Legumes are a good source of dietary proteins and essential nutrients. However, post-harvest insect infestation severely affects quality and store ability of the product (Steele *et al.*, 1985). Legumes are known to contain a variety of non-nutrient compounds such as lectins, proteinase inhibitors, arcelins, vicilins and other compounds which could be exploited as part of an array of constitutive defensive attributes against attack by stored seed pests, particularly those belonging to the family Bruchidae (Janzen *et al.*, 1986; Gatehouse *et al.*, 1990). Storage proteins are mostly the classically known globulins, which are insoluble in water and are typically present in leguminous seeds. Many water-soluble albumins have also been classified are reserve proteins, other kinds of seed proteins such as lectins and lectin- like proteins, which are also used for their nitrogen and carbon, are associated with defence mechanisms that plants have developed against the action of pests and pathogens. Also, the defensive protein group of the variant vicilins was obtained from African cowpea (*Vigna unguiculata* L.) genotypes that were resistant to the cowpea seeds beetle, *Callosobruchus maculatus* (F.) (Macedo *et al.*, 1993 and 1995). These legume seed storage proteins are digeometric molecules of globulin nature classified as 7S globulins according to their sedimentation coefficient and have variable degrees of glycosylation (Shutov *et al.*, 1995). In addition, trypsin and chymotrypsin inhibitors, besides their well-known function as storage proteins, are also important in imparting resistance (Liener and Kakade, 1980; Ryan, 1990; Sales *et al.*, 2000).

Therefore, the present work has been conducted to study the relationship between the content of certain storage seed proteins antimetabolites in legume seeds and resistance to infestation with the cowpea seeds beetle, *Callosobruchus maculatus* (F.) under no-choice bioassay at constant conditions of 29±1°C and 70± 5% RH. These results enable geneticists and plant breeders to introduce these antimetabolite proteins responsible for resistance against many stored product pests in transgenic programs which attempt to improve the recommended legume seed varieties having high yield and good quality aiming to gain a more store safe control method against this insect pest instead of pesticides causing serious bad effects on human and domestic animals owing to their residues post-treatment.

MATERIALS AND METHODS

Varietal Resitance Under No-Choice Bioassay

Stock culture of the cowpea seeds beetle, *Callosobruchus maculatus* (F.) was obtained from Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt, where the colony was maintained on cowpea seeds (*Vigna unguiculata* L.) Walpers) over about 15 years. Cultures were set up by introducing 75 pairs of the newly emerged beetles on 350 g of cowpea seeds (Fitriat variety) brought from a local market in Zagazig, Egypt which previously sterilized in an electrical oven regulated at 60°C for 24 hours according to Umoetok *et al.* (2013) in one-kg glass jars and tightly covered with muslin, held in place by rubber bands and allowed to oviposit for 3 days at which time the adults are removed with a sieve. The glass jars were labelled and kept in an electrical incubator running at 29±1°C and 70± 5% RH for about five generations before using the insects in the experiments of the present study. In this investigation, ten legume types with 17 varieties *i.e.,* cowpea (Fitriat, Forage, Kafr El-Sheikh), fababean (Aquadams, Giza 843, Sakha 1), pea (Master B, Smooth), common bean (Bronco, Neprasca), lupin (Giza 1, White lupin), chickpea (Giza 195), soyabean (Giza 111), lentil (Giza 9), guar (Guvar bean), lablab (Egyptian kidney bean). Cowpea, fababean, common bean, pea, chickpea, soyabean, lupin, lentil and guar seeds were supplied by the Field Crops and Horticulture Research Institutes, Agricultural Research Center, Dokki, Giza, Egypt. While, those of lablab, Aquadams fababean were supplied by the local markets in Cairo and Smooth pea from Zagazig. All seeds used were frozen at -20°C for continuous one month to kill any internal insects and then conditioned for three weeks at 29±1°C and 70±5% RH (Hassan, 1975).

To study the susceptibility of the tested seed varieties, fifty grams of each variety were placed separately in fourth-kg glass jars. Three pairs of

C. maculatus adults newly emerged (0-24 hours) were carefully sexually separated (Southgate, 1958; Bandara and Saxena, 1995) and introduced to each jar. For each variety, three replicates were used and kept at 29±1°C and 70 ± 5% RH. Adults of the replicates were let in the jars until death. For each treatment, the initial dates of larval penetration (1^{st} instar) into seeds and formation of seed testa windows were recorded. The first emergence date of F_1 was recorded and the total number of emerged adults was daily counted and sexed. These adult insects from each jar are collected, numbered until there are no more emergences. The number of beetles emerged per day is noted down and the mean developmental period of the insects is calculated on that basis. The newly emerged adults have been numbered and weighed for each variety of legume seeds. The average weight of a beetle per variety of legume seeds is calculated as the following: average weight of a beetle = total weight of the beetles/ number of beetles weighed. Numbers of total seeds, uninfested as well as infested and holes were determined for each replicate. Susceptibility index for each variety was calculated according to Howe (1971). Each infested seed sample was weighed at the beginning of the experiment and again at its end after all the insects and all the created dust had been removed to calculate the loss in weight. Finally, the infested seeds of each variety were soacked in water for one day and then carefully dissected to record the number of dead individuals (larvae, pupae and adults) inside seeds. From the aforesaid records, some biological aspects and infestation parameters which may be considered as resistance indicators could be calculated *i.e.,* larval and pupal periods, least and mean complete developmental periods, number of emerged adults per female, percentage of adults emergence, sex ratio of emerged adults (% of males), susceptibility index, percentage of infested seeds, number of holes per infested seed, percentage of seeds weight loss, emerged adult weight and number of dead individuals inside seeds per female.

Chemical Analyses

Seeds of tested varieties were finely ground to meal and extracted (1: 10, *W/V*) with 50 mM Tris–HCl buffer, pH 7.5, for 3 hr. at room temperature. After centrifugation for 30 min at 12000 rpm and 4°C, the supernatant was used in the assays. Total protein concentrations in seed samples supernatant after homogenization were determined using a commercial available kit according to Koller (1984). Trypsin inhibitors activity was determined by the method described by Kakade and Liener (1969) using casein as substrate. Vicilins and lectins were determined by the methods described by Macedo *et al*. (1993) and Yufang *et al*. (2010), respectively.

Statistical Analysis

All the obtained results were statistically analyzed according to completely randomized design and factorial experiments. The appropriate methods were used for the analysis of data according to Little and Hills (1975) and the proper "F" value was calculated as described by Fisher (1950) and Snedecor (1957). To make all possible individual comparisons between the means of different treatments which proved to be statistically significant, least significant range test according to Duncan (1955) was done. Simple correlation coefficient values were calculated and significantly tested according to Hendy (1969). In addition to the abovementioned methods, SPSS computer program was also used for the analysis of the present results.

RESULTS AND DISCUSSION

Seeds of the tested seventeen varieties belonging to ten legume types, cowpea, fababean, pea, common bean, chickpea, soyabean, lupin, lentil, guar, and lablab were chemically and quantitatively analyzed for total proteins (colourmetrically), trypsin inhibitors, vicilins and lectins to clarify the correlation relationship between these storage seed proteins of the tested seed varieties and some biological aspects as well as infestation parameters of the cowpea seeds beetle, *C. maculatus* which may be taken as resistance indicators. The biological aspects are represented by larval period, pupal period, least and mean complete developmental periods, number of emerged adults per female, adults emergence (%), sex ratio measured as (%) of males (Table 1). The infestation parameters were susceptibility index (%), infested seeds (%), number of holes per infested seed, seeds weight loss (%), weight of newly emerged adult and number of dead individuals inside seeds per female (Table 2).

Table 1. Effect of storage seed proteins of certain legume seed types and varieties on some biological aspects of the cowpea seeds beetle, *Callosobruchus maculatus* (F.) under no-choice bioassay at constant conditions of 29±1°C and 70 ± 5% RH

Legume type and variety	Total proteins (mg/g)	Trypsin inhibitors (µg /g)	Vicilins (µg /g)	Lectins (µg /g)	Larval period (day)	Pupal period (day)	Least develop. period (day)	Mean develop. period (day)	No. of emerged adults/ female	Adults emergence (%)	Sex ratio (% of males)
Cowpea											
–Fitriat	3.19g	1.96i	57.21i	1.60p	10.00h	3.00f	18.00f	23.49f	42.22ab	80.08ab	49.49abc
-Forage	2.31h	3.12k	93.41j	2.14o	10.00h	3.00f	17.00f	22.83f	29.67bc	81.73ab	56.57ab
-Kafr El-Sheikh	4.29f	3.83j	66.52k	0.80q	10.00h	3.00f	18.00f	23.28f	49.45a	94.06a	61.73a
Average	3.26H	2.97G	72.38H	1.51G	10.00E	3.00G	17.67B	23.20B	40.45A	85.29A	55.93A
Fababean											
–Aquadams	4.43f	4.60h	118.59g	3.14i	14.00e	3.00f	22.00e	31.32cde	6.33de	13.35d	44.44bc
-Giza 843	4.50f	4.83h	102.32h	2.70l	16.00c	3.67f	23.33d	26.47ef	18.44cd	46.55c	51.73ab
-Sakha 1	4.43f	5.31g	105.48h	2.88j	16.00c	3.33f	23.67d	26.50ef	11.78de	91.15a	54.31abc
Average	4.45G	4.91E	108.80F	2.91E	15.33B	3.33F	23.00B	28.10B	12.18CD	50.35C	50.61BC
Pea											
- Master B	4.55f	4.81h	102.03h	2.60m	15.00d	7.00d	25.67c	37.96b	38.78ab	74.70ab	51.47abc
- Smooth	5.43e	4.74h	103.31h	2.74k	15.00d	7.33cd	26.33c	30.58de	29.33bc	75.40ab	44.31bc
Average	4.99F	4.78E	102.67FG	2.67F	15.00B	7.17D	26.00B	34.27A	34.06A	75.05AB	47.89C
Common bean											
-Bronco	6.23d	8.91b	189.62b	6.12b	0.00i	0.00g	0.00g	0.00g	0.00e	0.00d	0.00d
-Neprasca	10.98a	9.27a	254.10a	6.17a	0.00i	0.00g	0.00g	0.00g	0.00e	0.00d	0.00d
Average	8.61A	9.09A	221.86A	6.14A	0.00F	0.00H	0.00C	0.00C	0.00E	0.00D	0.00E
Lupin											
-Giza 1	5.83de	8.34c	149.28d	4.95e	0.00i	0.00g	0.00g	0.00g	0.00e	0.00d	0.00d
-White lupin	5.38e	7.31d	155.14c	5.05d	0.00i	0.00g	0.00g	0.00g	0.00e	0.00d	0.00d
Average	5.61E	7.83B	152.21B	5.00B	0.00F	0.00H	0.00C	0.00C	0.00E	0.00D	0.00E
Chickpea											
-Giza 195	4.40f	4.19i	96.86i	2.45n	12.00f	8.67c	23.00de	27.59ef	15.56cde	61.59bc	38.92c
Average	4.40G	4.19F	96.86G	2.45F	12.00C	8.67C	23.00B	27.59B	15.56BC	61.59BC	38.92D
Soyabean											
-Giza 111	7.98a	7.15e	148.24d	4.75f	20.00a	15.67a	39.67a	45.50a	7.11de	13.43d	50.25abc
Average	7.98C	7.15C	148.24C	4.75C	20.00A	15.67A	39.67A	45.50A	7.11DE	13.43D	50.25BC
Lentil											
-Giza 9	4.86d	4.10i	125.68f	3.43h	11.00g	13.00b	28.00b	37.18bc	5.55de	18.52d	54.04abc
Average	4.86F	4.10F	125.68E	3.43D	11.00D	13.00C	28.00B	37.18A	5.55DE	18.52D	54.04AB
Guar											
-Guvar bean	8.34b	7.29d	137.55e	4.66g	0.00i	0.00g	0.00g	0.00g	0.00e	0.00d	0.00d
Average	8.34B	7.29C	137.55D	4.66C	0.00F	0.00H	0.00C	0.00C	0.00E	0.00D	0.00E
Lablab											
- Egyptian kidney bean	7.05b	6.89f	98.93i	5.20c	19.67b	5.33e	29.00b	35.97bcd	19.33cd	43.68c	51.16abc
Average	7.05D	6.89D	98.93G	5.20B	19.67A	5.33E	29.00B	35.97A	19.33B	43.68C	51.16ABC
F. test for types	**	**	**	**	**	**	*	*	**	**	**
F. test for varieties	**	**	**	**	**	**	**	**	**	**	**
r_1					0.908**	0.579	0.932**	0.891**	-0.493	-0.697	-0.102
r_2					0.843**	0.646*	0.916**	0.813**	-0.427	-0.553	-0.172
r_3					0.978**	0.391	0.843*	0.783*	-0.463	-0.605	-0.246
r_4					0.936**	0.463	0.832**	0.731**	-0.523	-0.471	-0.093
r_5					0.518	0.858*	0.897**	0.887**	-0.800*	-0.901**	0.283
r_6					0.600*	0.719**	0.810**	0.790**	-0.826**	-0.762**	-0.231
r_7					0.859*	0.463	0.826*	0.816*	-0.851	-0.748	0.065
r_8					0.829**	0.542	0.826**	0.785**	-0.695*	-0.695*	-0.245

r_1, r_3, r_5 and r_7 indicate the correlation coefficient values between the contents of total proteins, trypsin inhibitors, vicilins and lectins and some biological aspects of the seven infested legume types and r_2, r_4, r_6 and r_8 for varieties, respectively.
* indicates significancy degree at 0.05 level of probability.
** indicates high significancy degree at 0.01 level of probability.
Capital letters were used to distinguish the significancy between different tested legume types and small ones for varieties.
Means followed by similar letters indicate that the differences between means are not significant at 0.05 level of probability.

Table 2. Effect of storage seed proteins of certain infested legume seed types and varieties on some infestation parameters of the cowpea seeds beetle, *Callosobruchus maculatus* (F.) under no-choice bioassay at constant conditions of 29±1°C and 70 ± 5% RH

Legume type and variety	Total proteins (mg/g)	Trypsin inhibitors (µg /g)	Vicilins (µg /g)	Lectins (µg /g)	Susceptibility index (%)	Infested seeds (%)	No. of holes/ infested seed	Seeds weight loss (%)	Emerged adult weight (mg)	No. of dead individuals inside seeds/female
Cowpea										
-Fitriat	3.19i	1.96k	57.21h	1.60j	8.94a	60.37ab	1.57a	7.57b	3.5a	8.56c
-Forage	2.31j	3.12j	93.41g	2.14i	9.50a	20.64ef	1.11abc	4.62c	2.2bcd	11.17bc
- Kafr El-Sheikh	4.29h	3.83i	66.52i	0.80k	8.17ab	51.90abc	1.38ab	10.25a	2.7abc	4.23cd
Average	3.26G	2.97G	72.38G	1.51G	8.87A	44.30AB	1.35A	7.48A	2.8A	7.99C
Fababean										
–Aquadams	4.43fg	4.60f	118.59c	3.14d	4.11def	47.19abc	0.99bc	3.70cd	3.0ab	14.73b
-Giza 843	4.50f	4.83d	102.32d	2.70g	6.57bc	62.89a	1.55a	1.50def	1.7d	15.73b
-Sakha 1	4.43fg	5.31c	105.48cd	2.88e	5.50cd	26.93def	1.30abc	1.60def	2.3bcd	4.07c
Average	4.45E	4.91C	108.80C	2.91D	5.39B	45.67A	1.28AB	2.26BC	2.3AB	11.51BC
Pea										
-Master B	4.55e	4.81cd	102.03d	2.60g	5.45cd	34.22cde	1.28abc	2.54cdef	1.7d	8.37c
-Smooth	5.43c	4.74e	103.31d	2.74fg	6.14cd	37.06cde	1.28abc	4.49c	2.9ab	7.27c
Average	4.99C	4.78D	102.67D	2.67E	5.79B	35.64AB	1.28AB	3.52B	2.3AB	7.82C
Chickpea										
-Giza 195	4.40g	4.19g	96.86f	2.45h	5.09cde	24.93ef	1.44ab	0.88ef	2.0cd	13.33b
Average	4.40F	4.19E	96.86F	2.45F	5.09B	24.93BC	1.44A	0.88CD	2.0B	13.33B
Soyabean										
-Giza 111	7.98a	7.15a	148.24a	4.75b	2.85d	9.02fg	0.81c	2.76cde	1.7d	33.60a
Average	7.98A	7.15A	148.24A	4.75A	2.85C	9.02CD	0.81C	2.76BC	1.7B	33.60A
Lentil										
-Giza 9	4.86d	4.10h	125.68b	3.43c	3.10ef	2.94g	1.03abc	0.10f	1.7d	0.00d
Average	4.86D	4.10F	125.68B	3.43C	3.10C	2.94D	1.03BC	0.10D	1.7B	0.00D
Lablab										
-Egyptian kidney bean	7.05b	6.89b	98.93e	5.20a	4.90cde	44.23bcd	1.04abc	1.03ef	1.6d	38.11a
Average	7.05B	6.89B	98.93E	5.20B	4.90B	44.23AB	1.04BC	1.03CD	1.6B	38.11A
F. test for types	**	**	**	**	**	**	**	**	**	**
F. test for varieties	**	**	**	**	**	**	**	**	**	**
r_1					-0.693	-0.339	-0.853*	-0.396	-0.779*	0.910**
r_2					-0.729**	-0.257	-0.556	-0.358	-0.291	0.768**
r_3					-0.618	-0.133	-0.748	-0.403	-0.718	0.902**
r_4					-0.702*	-0.276	-0.586*	-0.516	-0.485	0.727*
r_5					-0.914**	-0.758*	-0.806*	-0.519	-0.696	0.550
r_6					-0.847**	-0.665*	-0.761**	-0.669*	-0.657*	0.530
r_7					-0.717	-0.274	-0.835*	-0.560	-0.870*	0.954**
r_8					-0.739**	-0.391	-0.706*	-0.691*	-0.525	0.882**

r_1, r_3, r_5 and r_7 indicates the correlation coefficient values between the contents of total proteins, trypsin inhibitors, vicilins and lectins and some infestation parameters of the seven infested legume types and r_2, r_4, r_6 and r_8 for varieties, respectively.

* indicates significancy degree at 0.05 level of probability.

** indicates high significancy degree at 0.01 level of probability.

Capital letters were used to distinguish the significancy between different infested legume seed types and small ones for varieties.

Means followed by similar letters indicate that the differences between means are not significant at 0.05 level of probability.

Analysis of variance of the aforementioned resistance indicators obviously proved that the differences between these characteristics recorded with the seventeen tested legume seed varieties belonging to ten types are highly significant at 0.01 level of probability except the least and mean complete developmental periods which are significantly varied in case of legume types (Tables 1 and 2).

Total Proteins

Regarding the relationship between resistance indicators (biological aspects and infestation parameters) toward cowpea seeds beetle and total contents of certain storage seed proteins of 17 legume varieties belonging to 10 types, the data presented in Table 1 show that all tested legume types and their varieties were infested with *C. maculatus* with different levels of infestation except common bean (Bronco and Neprasca), lupin (Giza 1 and White) and guar (Guvar bean) that have relatively high levels of total proteins. As concerns, the correlation coefficient values between total proteins of the infested seven seed types and their varieties and all the studied biological aspects, it is worthy to mention that developmental periods (larval, pupal and both least and mean complete developmental periods) showed positive coefficients except the number of emerged adults per female, adult emergence percentage and sex ratio as (%) of males which cleared negative relationships and insignificant correlation with pupal period for types and significant in case of varieties. Moreover, there are highly significant ones with larval period, least and mean complete developmental in both types and varieties.

As regards the correlation relationships between total proteins and the considered infestation parameters of the seven infested legume seed types as well their varieties, the results clearly proved that they were negatively correlated except the number of dead individuals inside seeds which was positive (Table 2). The correlation coefficients respecting legume types were insignificant with all resistance indicators except those in respect to number of holes per infested seed and emerged adult weight which proved to be significant and number of dead individuals inside seeds which was high significantly influenced. In general, from the present results in Tables 1 and 2, it can be concluded that individuals reared on seed varieties having the highest total proteins of 5.38-10.98 mg/g (lupin- White lupin and Giza 1, common bean- Bronco and Neprasca and guar- Guvar bean) did not complete their larval and pupal development in these seeds. Also, soyabean- Giza 111 variety contain relatively high level of proteins (7.98 mg/g) showed the prolongest larval, pupal, least and complete developmental periods of 20.00, 15.67, 39.67 and 45.50 days, respectively. On the contrary, cowpea varieties (Fitriat, Kafr El-Sheikh and Forage) proved to have the lowest total proteins content of 3.19, 4.29 and 2.31 mg/g, successively, showed the shortest least and mean developmental periods of 18.00, 23.49 days for the first variety, 18.00, 23.28 for the second and 17.00, 22.83 for the third one. The other tested varieties contain median values of proteins ranging from 4.40 to 7.05 mg/g exhibiting least as well mean developmental periods in ranges of 23.00-25.67 and 27.59-37.96 days, consecutively. The previous conclusion is confirmed by the other considered resistance parameters since the variety that contain high level of proteins of 7.98 mg/g (soybean- Giza 111 variety) showed relatively the lowest values of susceptibility index (2.85%) and mean number of emerged adults per female (7.11). On the other hand, cowpea varieties containing the lowest proteins content of 2.31 mg/g for Forage variety, 3.19 mg/g for Fitriat variety and 4.29 mg/g for Kafr El- Sheikh proved to have the highest values of susceptibility index (9.50, 8.94 and 8.17%); mean number of F_1 progeny/ female (29.67, 42.22 and 49.45) and relatively high percentages of seeds weight loss (4.62, 7.57 and 10.25) for the abovementioned three cowpea varieties, successively. The infested legume seed types indicated the same abovementioned trend of resistance showing the lowest susceptibility index (2.85%) with soyabean containing the highest content of 7.98 mg/g protein and the highest one (8.87%) with cowpea having the lowest protein content of 3.26 mg/g.

These results are in agreement with those obtained by some authors such as Singh *et al.* (1995) who stated that there is no direct bearing of protein content of cowpea genotypes on their susceptibility to cowpea seeds beetle, *C. maculates* infestation. Fawki *et al.* (2012) indicated that there is a negative correlation between cowpea seeds total proteins and the susceptibility index.

The contrasting findings obtained by Chakravarthy and Sahni (1972) exhibited that the varieties susceptible to pest or disease were found to proven higher amount of proteins. Also, Umarao and Verma (2003) in India indicated that the genotypes with least proteins were least susceptible to *C. chiensis* and *C. maculatus*. The same conclusion was also attained by Khaled *et al.* (2009) and Quali- N' goran *et al.* (2014).

Trypsin Inhibitors

The correlation relationships between the considered biological aspects of cowpea seeds beetle and trypsin inhibitors in all the seven tested legume seed types and their varieties were determined as shown in Table 1. From the results, it is apparent that there is a positive correlation coefficient with all aforesaid biological aspects except the number of emerged adults per female, adults emergence percentage and sex ratio as (%) of males which proved to be negative. Significancy test of these correlation relationships clearly proved to be significant with least and mean complete developmental periods, highly significant for larval stage period in legume types and in varieties for larval, least and mean complete developmental periods and insignificant in both types and varieties for number of emerged adults per female, adults emergence percentage and sex ratio as (%) of males.

While, in case of the correlation relationships between trypsin inhibitors of the seven infested legume seed types as well their varieties and the considered infestation parameters, the results compiled in Table 2 evidently demonstrate that they were negatively correlated except the number of dead individuals inside seeds which was positive correlation. The correlation relationships were insignificant with all infestation parameters of legume types except to number of dead individuals inside seeds which was highly significant. In case of varieties, there are significant correlation between trypsin inhibitors and susceptibility index, number of holes per infested seed and number of dead individuals inside seeds. All tested seed varieties of guar, lupin and common bean revealed completely linear inverse relationship between trypsin inhibitors and infestation indicating deterring effects for insect development. Seeds of common bean- Neprasca and Bronco varieties contain the highest levels of trypsin inhibitors of 9.27 and 8.91 µg /g, consequently, they were not infested with the bruchid under study (Table 1). The same aforesaid conclusion was detected also with lupin and guar varieties that having high levels of trypsin inhibitors ranging from 7.29 µg /g (guar- Guvar bean) to 8.34 µg /g (lupin- Giza 1). Infestation parameters revealed negative and insignificant correlation relationships except those respecting susceptibility index and number of holes per infested seed which reached significant degree at 0.05 level of probability. Also, seeds of soyabean- Giza 111 variety containing relatively high level of trypsin inhibitors of 7.15 µg /g showed the lowest value of susceptibility index of 2.85%. Whereas seeds of cowpea- Fitriat, Forage and Kafr El-Sheikh varieties having relatively low trypsin inhibitors of 1.96, 3.12 and 3.83 µg /g (2.97 µg/g in average) gave relatively high susceptibility degrees of 8.94, 9.50 and 8.17% (8.87% in average), respectively.

The present results are in full agreement with those obtained by many authors (Prasad *et al.*, 1996; Marconi *et al.*, 1997; Ignacimuthu *et al.*, 2000; Amirhusin *et al.*, 2007; Sumikawa *et al.*, 2010) who reported that significant positive correlations were found between legume seeds resistance to bruchids and trypsin inhibitors.

Vicilins

As obviously shown in Table 1, the vicilins of the seven infested legume seed types and their varieties demonstrate positive correlation coefficient with all the considered biological aspects with the exception of number of emerged adults, adults emergence (%) and sex ratio in varieties which proved to be negatively correlated. Significancy test of the present results clearly indicate that simple correlation coefficient values regarding the abovementioned relationships for legume types reached high significancy degree at 0.01 level of probability, excepting those of pupal stage period and number of emerged adults per female which proved to be significantly correlated, while insignificant correlations were detected with larval stage period and sex ratio as (%) of males. In case of legume varieties, it is worthy to mention that the correlation relationships were highly significant with all biological aspects and legume vicilins except larval stage period which was significant and insignificant with sex ratio of emerged adults.

Regarding the correlation relationships between vicilins of the seven infested legume seed types and their varieties and the infestation parameters, the results presented in Table 2 obviously demonstrate that they were negatively correlated except the number of dead individuals inside seeds which was positive. The correlation coefficients were insignificant with all infestation parameters of legume types except each of infested seeds percentage and number of holes per infested seed which were significant and highly significant with susceptibility index, while in case of varieties there are significant correlations between vicilins and infested seeds percentage, seeds weight loss percentage and emerged adult weight. Also, susceptibility index and number of holes per infested seed were high significantly correlated and insignificant with number of dead individuals inside seeds. From the results in Tables 1 and 2, it is evident that the tested legume seed varieties of soyabean (Giza 111) and lentil (Giza 9) revealed almostly linear inverse relationship between vicilins and susceptibility to cowpea seeds beetle infestation and simultaneously a retarding impact for insect development was detected exhibiting the prolongest mean developmental periods of 45.50 and 37.18 days as well the lowest percentages of adults emergence of 13.43 and 18.52 for the first and second varieties of the legume hosts, successively. Meanwhile, seeds of soyabean (Giza 111) and lentil (Giza 9) seeds containing relatively high level of vicilins of 148.24 and 125.68 µg /g showed low values of susceptibility index of 2.85 and 3.10%, successively. Contrarily, seeds of cowpea-Fitriat, Kafr El-Sheikh and Forage varieties having relatively low vicilins of 57.21, 66.52 and 93.41 µg /g revealed relatively high susceptibility degrees of 8.94, 8.17 and 9.50 %, respectively. In respect to common bean (Bronco, Neprasca), lupin (Giza1 and White) and guar (Guvar bean) which contain the highest content of vicilins ranging from 137.55 % (for Guvar bean) to 254.10% (for common bean- Neprasca) were not completely infested with the tested insect. Among the infested legume seed types, it can be observed that cowpea seeds were the most susceptible host to infestation with cowpea seeds beetle (S.I. = 8.87%) and the insect developed more faster (17.67 and 23.20 days) for least and mean complete developmental periods, respectively, had the lowest content of vicilins (72.38 µg /g). On the other hand, soyabean seeds were relatively highly resistant to this bruchid infestation (S.I.= 2.85 %) and the insect developed more slowly (39.67 and 45.50 days) for least and mean developmental periods, consecutively, had relatively high content of vicilins (148.24 µg /g). In this respect, seeds of common bean, lupin and guar whereon the insect is uncapable to complete its development and proved to be immune had high vicilins of 221.86, 152.21 and 137.55 µg/g for the three aforementioned legumes, respectively. This conclusion confirmed that vicilins play a major role in imparting resistance to leguminous seeds against this species of bruchids in stores.

The toxicity of variant vicilins has been correlated to their resistance due to proteolysis by exogenous proteases and their binding affinity to the *C. maculatus* midgut epithelial cells surface as obviously mentioned by some investigators such as Sales *et al*. (1992) and Macedo *et al.* (1993). The same authors added also, it has been shown that the variant *V. unguiculata* vicilins bind to the apical membrane of midgut cells in larvae of the same aforesaid bruchid. Vicilin molecules have also been considered to interact with chitin of this bruchid (Firmino *et al*., 1996; Sales *et al*., 1996) and with the peritrophic membrane in larvae of the lepidopteran *Diatraea saccharalis* (Mota *et al*., 2003). This putative mode of action of toxic vicilin, being dependent on binding to glycoprotein constituents on the surface of midgut microvilli, resembles the proposed mode of action of certain lectins (Zhu-Salzman *et al*., 1998; Fitches *et al*., 2001 a and b).

These results are in accordance with those obtained by some authors (Sales *et al*., 1992; Macedo *et al*. 1993; Ignacimuthu *et al*., 2000; Mota *et al*., 2003; Sales *et al*., 2005; Oliveira *et al*., 2014) working on legume seeds and their varieties, who reported that antinutrients like the vicilins (55 KD polypeptides) played a role in imparting resistance to bruchids. Moreover, Sales *et al.* (2005) added that *C. maculatus* emerged from resistant cowpea seeds variety (IT81 D- 1045) excreted 7 times higher vicilins and 0.4 time less trypsin inhibitors than that emerged from susceptible seeds variety (Epac 10).

Lectins

The results compiled in Table 1 show the correlation relationships between lectins in seven seed types and their varieties and some biological aspects of cowpea seeds beetle. Statistical analysis of the obtained results clearly indicated that all considered biological aspects of types and varieties were positively correlated with lectins except the number of emerged adults per female and adults emergence percentage. Significancy test of the present results clearly proved that simple correlation coefficient values regarding the above-mentioned relationships for legume types reached significancy with larval stage period, least and mean complete developmental periods and insignificant with the other biological parameters, while in case of legume varieties the correlation relationships were highly significant with all biological aspects and significant with number of emerged adults per female and adults emergence percentage and insignificant with the other studied biological parameters.

Regarding the correlation relationships between lectins of the seven infested legume seed types as well their twelve varieties and infestation parameters, they were negatively correlated except the number of dead individuals inside seeds which indicated positive correlation (Table 2). The correlation coefficients were insignificant with all parameters of legume types except the number of holes per infested seed and emerged adults weight which proved to be significant and highly significant with number of dead individuals inside seeds, while in case of varieties there are highly significant correlations between lectins and susceptibility index and number of dead individuals inside seeds, while those as concerns number of holes per infested seed and seeds weight loss were significant correlations and insignificant with the other infestation parameters. The present results in Tables 1 and 2 apparently prove that legume seeds of varieties whereon the cowpea seeds beetle is unable to complete its development (S.I. = 0.00%) *i.e.*, common bean- (Bronco and Nepraska), lupin (Giza1 and White) as well guar (Guvar bean) varieties contain relatively high levels of total lectins ranging between 4.66 µg /g for guar (Guvar bean) and 6.17 µg/g for common bean (Nepraska). The aforesaid trend did not occur in case of lablab (Egyptian kidney bean) seeds wherein lectins is relatively high (5.20 µg/g), they moderately infested showing 4.90 % susceptibility index. The seeds of soyabean (Giza 111) contain relatively high lectins (4.75 µg/g) and therefore showed the lowest susceptibility index (2.85%). Finally, it must be mentioned that low lectins followed by the high susceptibility to this species of bruchid beetles, this is obviously shown in case of cowpea (Fitriat, Forage and Kafr El-Sheikh) varieties having the lowest lectins of 1.60, 2.14 and 0.80 µg/g (1.51 µg /g in average) gave the highest values of susceptibility index of 8.94, 9.50 and 8.17% (8.87% in average), respectively.

The role of lectins in legume plants is controversial (Grant and Driessche, 1993; Beric *et al*., 1997). These glycoproteins usually occur in small quantities in legume seeds, *e.g.,* soyabean, beans, but do not occur in lupins (Jamroz and Kubizna, 2007). The above-named authors stated also that lectins from various sources differ from each other in the degree of antinutritional activity. They are the main toxic substances in the raw beans, *(Phaseolus vulgaris*), which is considered as one of the major human foods in some areas. It is well known that some lectins found in legume seeds can cause serious health problems in both humans and animals. Many of them, but not all, are capable to agglutinate red blood cells followed by haemolysis and even death of an animal. Also, *in vitro* lectins show the red blood cells agglutinating properties as a result of binding of sugar or glucoproteides (Liener, 1989; Pusztai *et al*., 1989; Koninkx *et al*., 1993 a and b). The toxicity of lectins depends on their capacity to adhere to the specific sugar receptors on mucus layer of intestine. Lectins can affect the intestinal mucosa and induce acute etherities, diarrhea, bloating and some other disturbances in the gastrointestinal tract. So, in effect, they can inhibit the growth and development of insects and animals. In acute, long-lasting incidence lectins can cause even death of insects and animals. Applied parenterally, lectins can affect the immune response and increase the resistance against insect infestation (Bardocz *et al*., 1999; Czerwinski *et al*., 2004).

Similar results were obtained by some researchers (Osborn *et al*., 1988; Cardona *et al*., 1990; Guzman Maldonado *et al*., 1996; Gatehouse *et al*., 2006; Machuka *et al*., 2014) who indicated that seeds of the kidney bean (*P. vulgaris*) are toxic to developing larvae of the bruchid beetle *C. maculatus*, a major storage pest of many legumes. They suggested that the lectin glycoproteins have been shown to have an inhibitory effect on the larval development of the aforesaid bruchid, whereas lectins appear to have no effect on the bean beetle, *Acanthoscelides obtectus*.

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بعض بروتينات البذور المخزونة ودورها في مقاومة بعض أنواع وأصناف بذور البقوليات للإصابة بخنفساء بذور اللوبيا *Callosobruchus maculatus* (F.)

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تم تحليل مكونات البروتين الكلي، مثبطات التربسين، الفيسيلينات والليكتينات لبذور سبعة عشرصنفاً تنتمي لعشرة أنواع بقولية لدراسة العلاقة ما بين محتوى بعض البروتينات المخزونة في بذور البقوليات والمقاومة للإصابة بخنفساء بذور اللوبيا تحت ظروف التقييم الحيوي الإجباري وفي ظروف ثابتة من حيث درجة الحرارة (٢٩±١°م) والرطوبة النسبية (٧٠±٥%)، تمثلت الأنواع والأصناف المختبرة في ثلاثة أصناف من اللوبيا (فطريات، علف و كفر الشيخ)، ثلاثة أصناف فول (أكوادامس، جيزة ٨٤٣ وسخا ١)، صنفان بسلة (ماستربى وملساء)، صنفان فاصوليا (برونكو ونبراسكا)، صنفان ترمس (جيزة ١ وترمس أبيض)، صنف حمص (جيزة ١٩٥)، صنف فول صويا (جيزة ١١١)، صنف عدس (جيزة ٩)، صنف جوار (جوفار) وصنف اللبلاب (اللوبيا المصرية كلوية الشكل)، لإظهار تأثير البروتينات المخزونة في أصناف البذور وأنواعها، تم تقدير بعض الصفات البيولوجية ومنها فترة طوري اليرقة والعذراء، أقل و متوسط فترة النمو الكاملة، عدد الحشرات الكاملة الخارجة فى الجيل الأول، النسبة المئوية لخروج الحشرات الكاملة، ونسبتها الجنسية (النسبة المئوية للذكور). بالإضافة إلى ذلك، فقد قُدرت بعض مقاييس الإصابة والتى تمثلت في معامل الإصابة، النسبة المئوية للبذور المصابة، عدد ثقوب خروج الحشرات الكاملة في البذرة المصابة، النسبة المئوية للفقد فى وزن البذور، وزن الحشرات حديثة الخروج وعدد الأفراد الميتة داخل البذور، أوضحت النتائج المتحصل في حالة البروتينات الكلية، مثبطات التربسين، الفيسيلينات والليكتينات لأصناف بذور البقوليات المختبرة المختلفة وجود معامل ارتباط سلبي مع كل معايير المقاومة فيما عدا فترة الطور اليرقي والعذراء، أقل ومتوسط فترة النمو الكاملة وعدد الأفراد الميتة داخل البذور لكل أنثى، علاوة على ذلك، فقد أظهرت فترة الطور اليرقي، أقل ومتوسط فترة النمو الكاملة، معامل الحساسية وعدد الأفراد الميتة داخل البذور لكل أنثى ارتباط عالي المعنوية مع محتوى البروتينات الكلية على مستوى احتمال ٠,٠١، بينما كانت فترة طور العذراء ذات معامل ارتباط معنوي على مستوى احتمال ٠,٠٥، ولكن في حالة معايير المقاومة المدروسة الأخرى فقد ثبت أنها لم تصل إلى الارتباط المعنوي. فيما يخص مثبطات التربسين أثبت اختبار المعنوية لعلاقات الارتباط وجود ارتباط غير معنوي مع كل معايير المقاومة فيما عدا ما يخص فترة الطور اليرقي، أقل ومتوسط فترة النمو الكاملة التي أوضحت وجود ارتباط عالي المعنوية وارتباط آخر معنوي مع معامل الحساسية، عدد الثقوب لكل بذرة مصابة وعدد الأفراد الميتة داخل البذور لكل أنثى، أوضحت النتائج وجود علاقة عكسية تامة ما بين محتوى أصناف الجوار، الترمس والفاصوليا من مثبطات التربسين والقابلية للإصابة بخنفساء بذور اللوبيا وبالتالي حدوث تأثير مانع لتطور الحشرة، أيضاً، تبين أنه في حالة الفيسيلينات وصول قيم معامل الارتباط البسيط والتي تخص معايير المقاومة التي سبق ذكرها إلى درجة معنوية عالية، فيما عدا ما يخص فترة الطور اليرقي، النسبة المئوية للبذور المصابة، النسبة المئوية للفقد في وزن البذور ووزن الحشرات الكاملة حديثة الخروج حيث كان الارتباط معنوي، بينما كان الارتباط غير معنوي مع النسبة الجنسية كنسبة مئوية للذكور وعدد الأفراد الميتة داخل البذور المصابة لكل أنثى، أوضحت بذور صنفي فول الصويا- جيزة ١١١ والعدس- جيزة ٩ أنها ذات علاقة عكسية خطية بين ما تحتويه من الفيسيلينات والقابلية للإصابة بخنفساء بذور اللوبيا وبالتالي يظهر التأثير المؤخر لنمو وتطور الحشرة، أما في حالة الليكتينات الكلية لأصناف بذور البقوليات الإثنى عشر والتي أُصيبت بالحشرة، وصلت علاقات الارتباط إلى درجة معنوية عالية مع مقاييس فترة الطور اليرقي، أقل ومتوسط فترة النمو الكاملة، معامل الحساسية وعدد الأفراد الميتة داخل البذور لكل أنثى، بينما في حالة عدد الحشرات الكاملة الخارجة لكل أنثى، النسبة المئوية لخروج الحشرات الكاملة، عدد الثقوب لكل بذرة مصابة والنسبة المئوية للفقد في وزن البذور كان الارتباط معنوياً، أما في حالة بقية مقاييس المقاومة الأخرى فلم تصل معاملات الارتباط الخاصة بها إلى درجة المعنوية.

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