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Evaluation of certain Egyptian wheat cultivars against rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) using biochemical and biophysical traits

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Abstract

Sitophilus oryzae **(L.) is the most destructive pest of stored wheat, and is widely distributed in tropical regions. In the present study, the susceptibility of 11 Egyptian wheat cultivars, including seven durum wheat and four bread wheat cultivars were evaluated against *S. oryzae* were on the basis of percentage of weight loss, and damaged grain. The comparison between tested wheat cultivars in respect to wheat biophysical traits (kernel weight, kernel hardness and moisture), and biochemical variants (starch, wet gluten and protein content) was assayed. The correlation between these biophysical and biochemical traits and the percentage of weight loss and damaged grain was estimated. The results revealed that bread wheat cultivars (Misr-1, Misr-2, Giza-171, and Gimmiza-10) were highly susceptible to *S. oryzae* infestation than durum wheat (Beni Sweaf-1, Beni Sweaf-3, Beni Sweaf-4, Beni Sweaf-5, Beni Sweaf-6, Sohag-3, and Sohag-5). The most susceptible cultivar was the bread wheat cultivar Gemmieza-10, and the most resistant was durum wheat cultivar Beni Sweaf-3. The tested cultivars significantly varied in there biophysical and biochemical traits. A significant positive correlation was observed between percentage of damaged grain and grains content of starch, and wet gluten. However, negative correlations were found in grain hardness index and percentage of damaged grain. Protein content, grain diameter, kernel weight, %moisture (a) and (b) did not show a significant correlation to damaged grain.**

Keywords: stored grain pests, wheat cultivars susceptibility, biophysical and biochemical traits, Sitophilus oryzae.

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1. Introduction

Wheat can be considered the third cereal crop in terms of world production. According to FAOSTAT, wheat production exceeded 711 million tonnes in 2013. Also, wheat occupies more than 220 million hectares of land worldwide (Anonymous, 2015). Durum wheat (*Triticum turgidum* subsp. *durum*) is one of the most important cereal crops. About half of the total durum wheat-producing area is located in developing countries, where it is used for making products such as pasta and couscous (Herrera-Foessel et al., 2014). In 2014, the total cultivated wheat area in Egypt, including bread and durum wheat, was 8.9 million acres and the total production was 9.3 million tonnes (FAO, 2014; CAPMAS, 2014) which is away less than the consumed wheat. Cereal grain losses during storage can reach to 50% of total harvest in some countries, a worldwide loss quality of grain is caused by insects (Fornal et al., 2007). Many pests attack wheat plant in the field and the stored grain in the storage and cause a severe damage. *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is a serious cereal pest that attacks stored wheat grain.The adults fed on endosperm, hence declining the carbohydrate contents. The larvae feed on the germ of the grain and reducing great amount of protein and vitamins (Belloa et al., 2000). Rice weevil female chews a hole in the kernel, lays the egg inside, and seals the hole with a gelatinous secretion which protects the eggs (Arbogast, 1991). Weevil has a life cycle of 34.8 days in average at 27˚C and 69% relative humidity (Osman et al., 2012). It has direct and indirect impact on the grain. The direct impact comes as a result of weevils feeding which decreasing the quantity and the available grain for human consumption. The indirect impact is due to affecting the quality of the grain which may leads to lowering its price or rejection of grain. In general, the performance of cereal cultivars against weevils infestation varied from cultivar to another (Khan et al., 2014; Shafique & Ahmad, 2003). In the past few decades possibility of identifying resistant cultivars of wheat to stored grain pest have received attention. Wheat cultivation in respect to cultivars has been undergoing a marked change in recent past. New cultivars are coming up every year, which resulted a change in the intensity of insect infestation during storage. In recent year the laboratory studies established that many cultivars have shown some resistances against *S. oryzae* (Chauhan et al., 2005; Tiwari & Sharma, 2002; Ram & Singh, 1996). However, a few studies have been implemented on evaluating the resistance of recent growing Egyptian wheat cultivars against *S. oryzae*. Many factors can help the development of stored grain pests and cause losses ranging from 5-30%, (Khan et al., 2014). High moisture content of grain, relative humidity and high environmental temperature during storage provide suitable condition for insect’s production (Dars et al., 2001; Ahmad et al., 1998). The resistant cultivars, especially in village cooperative and farmer’s stores, can be kept for longer time without use of pesticides (Semple, 1985). Thus, this study aims to evaluate some Egyptian spring bread and durum wheat for *S. oryzae* resistance by studying the effect of some biochemical and biophysical traits on the feeding of adult weevils.

2. Materials and methods

2.1 Insect rearing

Insects were reared in 1 L capacity glass gars with a 7 cm diameter which were covered with muslin cloth and tied by rubber bands. The gars containing grain of mixture wheat varieties were kept in a controlled environment (28 ± 1 °C, and 70% RH). The grain periodically sieved, and the insects placed in rearing gars with fresh grain.

2.2 Source of tested Egyptian wheat cultivars

Eleven wheat cultivars including 7 durum wheat (Beni Sweaf-1, Beni Sweaf-3, Beni Sweaf-4, Beni Sweaf-5, Beni Sweaf-6, Sohag-3, and Sohag-5) and 4 bread wheat (Misr-1, Misr-2, Giza-171, and Gimmiza-10) were obtained from institution of wheat diseases research, Agriculture research centre, Dokki, Giza Egypt.

2.3 Experimental procedures

The experiments were conducted in small glass jar (5cm diam. and 7.5 cm length), the jars were covered with mesh cloth. Thirty grams of wheat grain were placed in each jar. The infestation was conducted by adding 30 newly emerged adults to each jar (1 weevil/1gm grain). Thirty grams of whole grain were accurately weighted and placed in small glass jar (5cm diam. and 7.5 cm length), 30 newly emerged adults of each tested insect species were introduced to glass jars, which were covered with muslin cloth and tied by rubber bands. The adults were left for one month then removed, and the remaining grain was used for calculating the % Weight loss and % damaged grain. As following formulas:

$$\text{Weight loss (\%)} = \frac{\text{Weight of control sample} - \text{weight of infested sample}}{\text{Weight of control sample}} \text{ X } 100$$

$$\text{Damaged grain (\%)} = \frac{\text{No. of damaged grain}}{\text{Total No. of grain}} \text{ X } 100$$

2.4 Biochemical traits measurements

2.4.1 Starch content

The colorimetric technique was used for estimation of starch percentage based on method by (Magel, 1991).

2.4.2 Protein content

The protein content and moisture% (a) of the flour was determined using Percon Inframatic 8620 near infrared spectrometer (Perten Instruments NA, Inc., Reno, NV) and the data was processed using Perten's NetPlus software.

2.4.3 Gluten estimation

Gluten determination was done as described by (Imran et al., 2013) which carried out using a Perten Glutomatic System, based on International Code Council (ICC) Standard No. 155, No 158 and American Association for Cereal Chemist (AACC) method 38-12 (Operational Manual Glucomatic System). This method uses a glutomatic gluten washer with a sieve size of 170 mesh (88 microns) and a gluten centrifuge which provides information on the quantity and quality of gluten obtained. 10g of wheat flour was transferred into the wash chamber and shaken to obtain a homogenous flour layer. 4.8 ml of 2% sodium chloride was added from the dispenser into the chamber at a slight tilt. The chamber was then agitated to spread the water evenly over the flour. The remaining mixing and washing sequence was accomplished automatically within the washer. Liquid containing starch was collected in a beaker placed below the washer and the gluten mass remained on the sieve. The gluten mass was centrifuged in a special sieve cassette in order to force the wet gluten to pass through the sieve. The centrifuge allowed for the collection of both parts of the gluten remaining on the sieve and that which passed through the sieve. The total weight of wet gluten was recorded and expressed as a percentage of the mass of the original sample. The following formula was used for the calculation:

Wet gluten content (%) = [Total gluten (g) x 100/ Weight of wheat flour sample (10g)]

2.5 Biophysical traits determination

The colorimetric technique was used for estimation of starch percentage based on method by (Magel, 1991).

2.5.1 Determining the kernel hardness, weight, diameter, and moisture by SKCS 4100

Grain moisture, kernel diameter, kernel weight, and hardness index were determined using the Perten Single Kernel Characterization System (model 4100, Perten Instruments, Reno, NV) (Psotka, 1999; Gaines et al., 1996). Hardness Measurement by SKCS A Single Kernel Characterization System (SKCS) instrument, (model 4100. Perten Instruments, Reno, NV) was used for assessment of single wheat kernel hardness, moisture, and kernel diameter and weight. Duplicates of each wheat cultivar were submitted to the SKCS under normal operating parameters and conditions, with a selection of 300 seeds per measurement. During operation, the instrument isolated individual wheat kernels, measured, weighed, and crushed them in a progressively narrower gap formed by a toothed rotor and a crescent. The crushing force and electrical conductivity between the rotor and electrically isolated crescent were recorded. Those data were then processed by the integrated computer software to provide the means and standard deviations for weight, size, moisture, and hardness index (HI).

2.5.2 Moisture percentage

Moisture was measured with two different methods first moisture (a)% which measured by using Percon Inframatic 8620 near infrared spectrometer (Perten Instruments NA, Inc., Reno, NV) and moisture (b)% which measured by using SKCS A Single Kernel Characterization System (SKCS) instrument, (model 4100. Perten Instruments, Reno, NV).

2.6 Statistical analysis

The experiment was laid out in complete randomized design (CRD) with three replications. The obtained data were analyzed by ANOVA test and significant means were separated by Duncan's multiple rang test using a computer program of SPSS 14.0.

3. Results

Results were evaluated on the basis of

percent weight loss, percent grain damaged of wheat grain because of the infestation by *S. oryzae* for grain of 11 Egyptian wheat cultivars. Also, biophysical and biochemical traits for these cultivars were measured and the potential correlation between these traits and *S. oryzae* infestation was calculated.

3.1 Estimation of *S. oryzae* infestation

The percentage of weight loss for grainand damaged grain were chosen as an associated parameter to *S. oryzae* infestation. Percentage of Weight loss and damaged grain weremeasured for11 Egyptian wheat cultivars including (7 durum wheat cultivars and 4 bread wheat cultivars) (Table 1).

3.1.1 Weight loss percentage

Significant differences were found among tested cultivars for percentage of weight loss for grain (ANOVA-One way, df=10, F=42.86, P<0.01) (Table 1). All durum wheat cultivars showed less loss weigh percentages compared with bread wheat cultivars. Gemmeiza-10 showed significantly the highest percentage of weight loss for grain (6.11%) compared to all tested cultivars. While Beni Sweaf-3 had the lowest percentage of weight loss (0.83%) than Beni Sweaf-5, Misr-1, Misr-2, Giza-171 and Gemmeiza-10 with a range of 1.96-6.11% of Weight loss. But no significant differences were found between Beni Sweaf-3 and other durum cultivars.

3.1.2 Damaged grain percentage

The infestation by *S. oryzae* had similar influence on the tested cultivars in respect to percentage of damaged grain as that in weight loss. It showed significant differences among cultivars (ANOVA-One way, df=10, F=612.20, P<0.01). The infestation resulted a significantly more damage for bread wheat cultivars compared to that in durum. The highest percentage of damaged grain (22.95%) was observed in Gemmeiza-10 compared to all tested cultivars. While, Beni Sweaf-3 followed by Beni Sweaf 6 had significantly lowest percentage of damaged grain (5.28% and 5.68%, respectively) than other tested cultivars.

3.2 Biophysical parameters

Four biophysical traits were measured including; moisture (a), moisture (b), kernel diameter, kernel weight, and hardness index for the tested 11 Egyptian wheat cultivars including (7 durum wheat cultivars and 4 bread wheat cultivars) (Table 1).

3.2.1 Percentage of moisture (a)

There is no significant differences were noticed between tested cultivars in moisture (a) (ANOVA, one-way, df=10, F= 1.19, P>0.05). Minor differences were observed among tested cultivars.Giza-171 showed low percentage for moisture (a) followed by Sohag-5 (12.93% and 12.96%, respectively) than Misr-1and Beni Sweaf-1 (13.63% and 13.66%). While, Beni Sweaf-1 had high percentage of moisture (a) (13.66%) than Giza-171 and Sohag-5.

3.2.2 Percentage of moisture (b)

The estimation of moisture using SKCS produced similar results to moisture (a). No significant differences were found among tested cultivars (ANOVA, one-way, df=10, F= 2.10, P>0.05). High percentage of moisture (b) was recorded for Giza-171 followed by Gemmeiza-10 (13.32% and 13.37%) compared to Beni Sweaf-1, Beni Sweaf-3, Beni Sweaf-4, Beni Sweaf-5 and Misr-1 with a range of 12.61%-12.78%. Otherwise, Misr-1 had the lowest percentage of moisture (b) (12.61%).

Table 1: Susceptibility of wheat cultivars to the infestation of *S. oryzae* and the variation between biophysical traits.

Wheat cultivar	Biophysical traits (Mean± SE)						
	Weight loss (%)	Damaged grain (%)	Moisture (a) (%)	Moisture (b) (%)	Diameter (mm)	Weight/ kernel (mg)	Hardness index
Beni Sweaf-1	1.79±0.66	7.35±0.12	13.66±0.27	12.78±0.14	3.06±0.06	43.40±0.13	97.61±0.22
Beni Sweaf-3	0.83±0.30	5.28±0.09	13.33±0.22	12.67±0.16	2.89±0.03	42.61±0.15	98.82±0.23
Beni Sweaf-4	1.57±0.40	7.00±0.38	13.56±0.25	12.69±0.15	3.25±0.06	52.51±0.17	93.98±0.24
Beni Sweaf-5	1.96±0.55	8.98±0.55	13.51±0.25	12.73±0.13	2.89±0.03	39.20±0.13	96.99±0.26
Beni Sweaf-6	1.55±0.17	5.68±0.09	13.39±0.24	12.82±0.14	3.18±0.06	48.31±0.16	93.91±0.24
Sohag-3	1.19±0.66	7.40±0.12	13.23±0.21	12.89±0.17	3.45±0.07	56.49±0.14	94.53±0.23
Sohag-5	1.58±0.66	6.40±0.12	12.96±0.20	12.95±0.18	3.29±0.06	51.01±0.15	100.0±0.26
Misr-1	3.42±0.10	13.78±0.09	13.63±0.26	12.61±0.14	3.44±0.07	57.93±0.16	72.95±0.22
Misr-2	2.26±0.51	11.08±0.14	13.15±0.22	12.84±0.17	3.31±0.06	50.42±0.17	77.54±0.23
Giza-171	4.66±0.82	16.10±0.06	12.93±0.20	13.32±0.19	3.03±0.05	43.66±0.15	67.05±0.22
Gemmeiza-10	6.11±0.86	22.95±0.03	13.23±0.23	13.37±0.18	3.12±0.06	45.72±0.16	48.45±0.20

3.2.3 Diameter (mm)

Wheat grain diameter (mm) was measured for all tested cultivars. Significant differences were found among tested cultivars (ANOVA, one-way, df=10, F= 11.48, P<0.01). Results revealed that Misr-1 and Sohag-3 had high and almost similar grain diameter (3.44 mm and 3.45 mm) compared to tested cultivars except Sohag-5, Misr-2 and Misr-1 (3.29 mm, 3.31 mm and 3.44 mm, respectively). Low and similar grain diameter was recorded for Beni Sweaf-3 and Beni Sweaf-5 (2.89 mm) compared to other cultivars except for Beni Sweaf-5 and Giza-171 (2.89 mm and 3.03 mm).

3.2.4 Weight kernel (mg)

Wheat kernels were weighted for tested cultivars, and results showed that there is a significant difference in weight kernel among wheat cultivars. High kernel weight was recorded for Misr-1 and sohag-3 (57.93 mg and 56.49 mg) compared to the other tested cultivars. However, Beni Sweaf-5 had significantly the lowest kernel weight (39.20 mg).

3.2.5 Hardness index

Significant differences were found between cultivars in hardness index. All durum wheat showed significantly higher hardness index than bread wheat. Sohag-5 showed the highest hardness index value (100 hardness indexes) compared to all cultivars except Beni Sweaf-5, Beni Sweaf-1 and Beni Sweaf-3 (96.99, 97.61 and 98.82 respectively). While, Gemmeiza-10 showed the lowest hardness index value (48.45).

3.3 Biochemical parameters measured for eleven Egyptian wheat cultivars

Biochemical parameters including starch, protein, gluten index and wet gluten content were assayed for 11 Egyptian wheat cultivars (Figure 1).

3.3.1 Starch percentage

Starch analysis showed significant differences between tested cultivars.

Gemmeiza-10 and Misr-2 recorded the highest values (78.41% and 78.13 %) than all tested cultivars. While, Beni Sweaf-3 had the lowest value for starch (54.16 %) compared to tested cultivars. Almost similar starch values were recorded for Beni Sweaf-5, Sohag-3 and Sohag-5 (58.61%, 58.83% and 59.85 % respectively).

3.3.2 Protein percentage

Measurement of protein in the tested wheat cultivars resulted a significant difference among the tested cultivars. Results indicated that Misr-1 showed the highest protein percentage (17.19%) compared to all tested cultivars. While, Beni Sweaf-1 had the lowest protein percentage (11.7%). Almost similar percentage of protein was recorded for Beni Sweaf-6, Giza-171, Sohag-3 and Beni Sweaf-5 (13.19%, 13.35%, 13.55% and 13.93% respectively). Similar results for Beni Sweaf-4, Sohag-5 and Gemmeiza-10, where percentages of protein were 14.23%, 14.81% and 14.82 respectively. The same trend was recorded for Beni Sweaf-3 and Misr-2, where percentages of protein were 15.28 % and 15.70%.

3.3.3 Percentage of wet gluten content

The content of wet gluten was measured for the tested cultivars, and the results revealed that there is a significant difference among tested cultivars. Misr-1 and Misr-2 showed a high value for wet gluten content (37.3% and 35.6%) compared to all tested cultivars. While Beni Sweaf-1 had the lowest value for wet gluten content (20.2%). Similar wet gluten content values were recorded for Beni Sweaf-3 (22.5%) and Beni Sweaf-5 (22%).

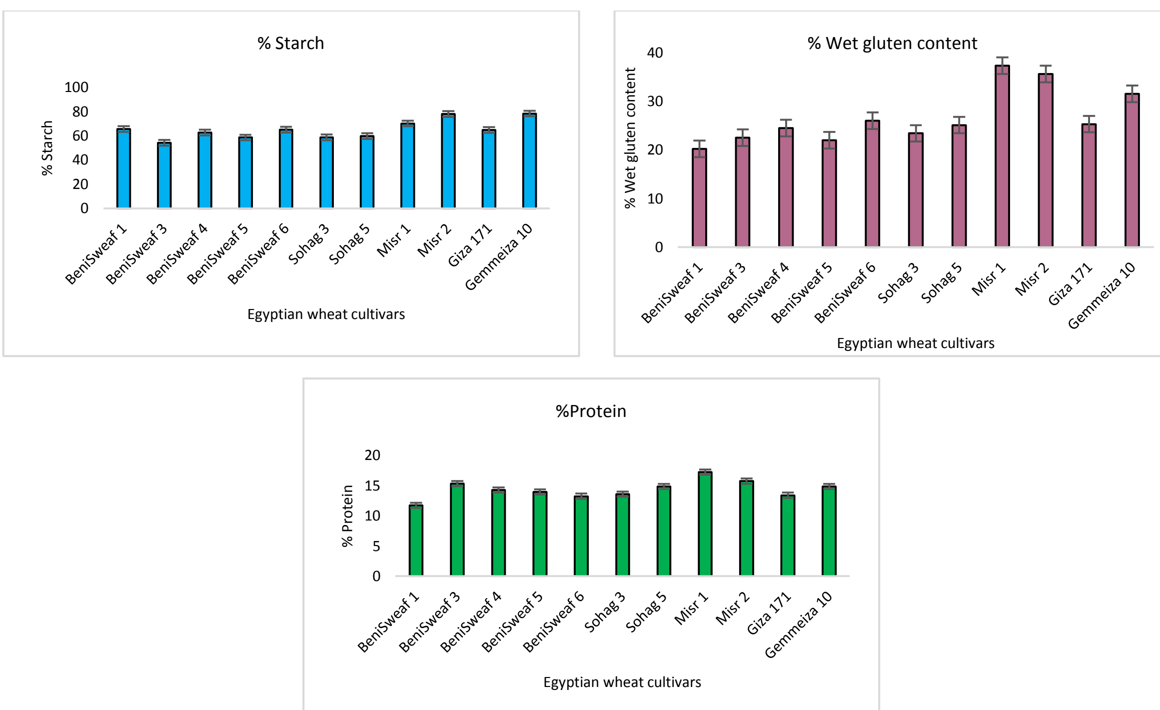

Figure (1): A comparison between biochemical traits of eleven Egyptian wheat cultivars.

3.4 Correlation between percentages of damaged grain for eleven Egyptian wheat cultivars and their biophysical and biochemical parameters

Highly significant positive correlation was observed between percentage of damaged grain and percentage of weight loss (r = 0.968**, P<0.01). So, the percentage of damaged grain was chosen to correlate with the biophysical and biochemical parameters of the eleven Egyptian wheat cultivars as showed in Table (2). In respect to the biochemical traits, a significant positive correlation was noticed between % of damaged grain and starch (r = 0.713**) and wet gluten (r = 0.551**), while no significant correlation was found with the protein content. On the other hand, high significant negative correlation was noticed between hardness index and percentage of damaged grain (r=-0.973**). However, no significant correlation was found between moisture (a), moisture (b), diameter (mm), and weight kernel (mg) and percentage of damaged grain.

Table (2): Correlation between percentages of damaged grain for eleven Egyptian wheat cultivars and their biophysical and biochemical traits.

Biophysical traits	r value	P value
Weight loss (%)	0.968**	P<0.01
Moisture (a) (%)	-0.136 ns	P>0.05
Moisture (b) (%)	0.286 ns	P>0.05
Diameter (mm)	0.016 ns	P>0.05
Weight/ kerenl (mg)	-0.049 ns	P>0.05
Hardness index	-0.973**	P<0.01
Biochemical traits		
Starch (%)	0.713**	P<0.01
Protein (%)	0.262 ns	P>0.05
Wet gluten content (%)	0.551**	P<0.01

*Significant at 0.05 Probability level; ** Significant at 0.01 Probability level; ns is not significant.

4. Discussion

This study was conducted on 11 recent Egyptian wheat cultivars including (7 durum wheat and 4 bread wheat). The main aim is to provide more information about the susceptibility of the tested cultivars to *S. oryzae* based on two measured parameters percentage of damaged grain and weight loss of infested grain. On the other hand, the study indicated a correlation between some biophysical and biochemical traits of the tested wheat grain and the infestation by *S. orzyae*. Overall, high percentage of weight loss and damaged grain was observed in bread wheat as compared with the durum wheat. Similar results was reported by Mahmoud et al. (2011), who found high susceptibility of bread wheat compared to durum wheat against the infestation by *Sitophilus granarius* (L).The same authors also reported Gemmeiza-7 as the most susceptible cultivar and Beni Sweaf-4 as the most resistance one, which partially in harmony with this study finding. According to the obtained data there is a significant difference among wheat cultivars in respect to the grain content of starch, protein, and wet gluten as biochemical traits and in their biophysical traits (hardness index, kernel diameter, and kernel weight). There is no significant variation in grain content of moisture among all the tested cultivars. The obtained data indicated no actual association between grain content of protein and the percentage of damaged grain. This finding is supported with Rao and Sharma (2003) who reported that grain protein content could only had a slight role in wheat resistance to the weevils. In contrary, (Ram & Singh, 1996) and (Tiwari & Sharma, 2002) stated that *S. oryzae* weevil correlated negatively with protein. Wet gluten had a weak correlation to weevil infestation, that in harmony with a study by (Tiwari & Sharma, 2002) who stated that no impact of gluten content on the resistance and susceptibility to insect species. The obtained data revealed that wheat grain content of starch positively correlated with weevil infestation that could be due to the enzymology of digestive such as amylase for some *Sitophilus* weevils required initial stages of large food polymers such as starch (Franco et al., 2002; Baker, 1986). Also, our results are in harmony with Singh and McCain (1963) who noticed positive correlation between sugar and starch content of the kernels and weevil reproduction. Highly significant negative correlation between grain hardness index and percentage of damaged grain was observed. This observation tremens dously clear when comparing the hardness index of durum wheat cultivars as a hard red spring wheat and the bread wheat as a soft red spring wheat cultivars, and the percentage of damaged grain of these cultivars. Many studies have also reported hardness index as negatively correlated with *S. oryzae* infestation (Tiwari & Sharma, 2002; Ram & Singh, 1996; Sinha et al., 1988; Rout et al., 1976). However a study by Rao and Sharma (2003), who screened different wheat germplasm against the infestation by *S. oryzae* and reported no clear relationship between kernel hardness and weevil resistance. There is no significant correlation was found between percentage of damaged grain and grain size of tested cultivars. However, a study by Ram and Singh (1996), Tiwari and Sharma (2002) indicated positive correlation between grain size and resistance to *S. oryzae* weevils. The conflict in the results could be due to the variation in tested cultivars since none of the previous studies included durum wheat in their studies. Some of durum wheat had almost the same size as bread wheat, but tremendously varied in other traits that widely reported as a correlated parameter to weevil infestation such as hardness index. Grain weight also showed no impact on damaged grain. There is no clear evidence that grain weight had an impact on host resistance to insects (Tiwari & Sharma, 2002). Grain content of moisture has reported one of the factors that may affecting the susceptibility to *S. oryzae* (Hussain & Nasr, 2015; Rashad et al., 2005; Hameed et al., 1984). However, no correlation was detected between grain content of moisture, that measured by two different instruments, and percentage of damaged grain that could be due to the minor differences in moisture content among the tested cultivars. Further investigation should be done to confirm the importance of moisture content. It could be concluded that none of the tested cultivars were completely resistant to infestation by the granary weevil but their susceptibility to the infestation varied considerably and the tested cultivars significantly varied in their biophysical and biochemical traits.

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