Pak. J. Bot., 43(1): 659-668, 2011.

QUANTITATIVE LOSSES AND PHYSICAL DAMAGE CAUSED TO WHEAT KERNEL (*TRITICUM AESTIVUM* L.) BY KHAPRA BEETLE INFESTATION

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Abstract

Wheat and other cereals sustain huge quantitative and qualitative losses each year all over the world due to the attack of storage pests. The damage caused by Khapra beetle is comparatively greater than other pests because of its ability to increase rapidly even under unfavourable environmental conditions. A study was therefore initiated to probe into quantitative and physical losses of stored wheat caused by Khapra beetle infestation. We observed more than 20 % loss in weight of seeds after a storage period of 6 months under natural conditions, initially infested with only 10 pairs of Khapra beetle larvae. There was found a strong positive relationship among the progeny development and number of damaged grains as well as with weight loss as depicted by values of the correlation coefficient i.e., 0.95 and 0.87, respectively. Moisture contents also exhibited a strong positive correlation with the loss in weight of wheat grains (R=0.83). The beetle larvae also showed varying levels of preference to different wheat varieties. Wafaq-2001 was found to be the most resistant among the commonly grown varieties of Pakistan. It was therefore concluded that the wheat grains of promising varieties stored for edible and seed purposes must be protected from the attack of Khapra beetle infestation to maintain nutrition, viability of wheat grains and indirectly to ensure the food security.

Introduction

Khapra beetle (*Trogoderma granarium* Everts) and other beetles of the Dermestidae family are considered to be the most destructive stored product pests (Burges, 2008; Mark *et al*., 2010). The pest has been given status as an A2 quarantine organism for EPPO (Anon., 2007). Besides, it is also of quarantine concern for *Canadian Public Procurement Council* (CPPC), Comite Regional de Sanidad Vegetal del Cono Sur (COSAVE), Junta Del Acuerdo De Cartagena (JUNAC), *Organismo Internacional Regional de Sanidad Agropecuaria,* National Plant Protection Organizations (NAPPO) and *Organismo Internacional Regional de Sanidad Agropecuaria* (OIRSA). The World Trade Organization (WTO) committee on Sanitary and Phytosanitary (SPS) measures has prohibited the importation of wheat and similar grains as well as the flours and meals thereof in order to protect domestic production and to prevent the introduction and spread of this notorious pest. This restriction is applicable to products transported from various countries harboring this pest (Anon., 1981). The import restrictions are supported by the facts that feeding by Khapra beetle larvae reduces the quality, grade and weight of grain.

In India, average damage level ranged from 6-33 percent of grain in a single storage season, with maximum damage of 73 percent (Rahman *et al*., 1945). Loss of weight in wheat ranged from 2.2 to 5.5 percent. Under optimal conditions, 15% infestation level caused 2.6% loss in weight and 24% in viability of wheat grains in few months of storage (Prasad *et al*., 1977). Khan & Kulachi (2002) collected 220 samples of wheat grains from different locations of D.I. Khan. They recorded average losses to the tune of 3.4 and 6.5% *by* count-and-weight *(*C&W) and Thousand-grain Mass (TGM) methods, respectively within 5 months` storage. They also observed presence of black pointed, broken/ shriveled and green/immature grains as well as foreign matter, which were counted as 0.6, 3.1, 0.7 and 30.3 percent, respectively. In Pakistan, first schematic survey of losses was conducted by Chaudhary (1980) who reported an aggregate loss of 15.3% during various post-harvest operations of wheat in the country. Jilani (1981) observed 10 to 15% post harvest losses of food grains in Pakistan, which were chiefly caused by the attack of insect pests. His report was later on supported by findings of Ahmed (1984). In the same year Ahmad & Afzal (1984) recorded 22.7% post harvest loss of wheat in Pakistan, out of which 9.5% occurred during storage period and the remaining 13.2% during harvesting and threshing times. On country wide basis, storage losses of wheat in Pakistan ranged between 3.5 to 25% (Irshad & Baloch, 1985). Mohammad (1986) reported that losses of wheat grains stored for 4 months in house type godowns of Pakistan are 2.03, 8.18 and 1.35% determined by Standard Volume Weight (SVW), TGM methods of loss assessment, respectively. The respective figures for six months wheat storage resulted in average weight losses of 1.99, 6.33 and 2.01%, respectively, whereas for 7 months storage period, losses were recorded as 3.02, 9.41 and 2.06%, determined by S.V.W, T.G.M. and G.M. methods, respectively (Khan, 1986). Baloch (1986) recorded 4 and 7% annual storage losses at farm level and in the public sector, respectively.

The weight loss of grain and cost of necessary treatment may result in less profit for the wholesalers. The present investigations were therefore undertaken to estimate quantitative and physical damages induced in seeds of commonly grown wheat varieties of Pakistan by the notorious Khapra beetle. The results will enable the scientists to adopt appropriate control measures leading protection of wheat seeds as well as to ensure food security by minimizing the storage losses.

Materials and Methods

The present investigations were carried out in Stored Grain Research Laboratory of Entomology Department of Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi during 2004-2008. The materials and methods employed in this study are as under:

Collection of wheat seeds: Seeds of 9 wheat varieties commonly grown in Pakistan viz., BWP-97, Manthar, Bhakkar-2000, BWP-2000, GA-2002, Inquilab-91, DWR-97, Panjnad and Wafaq-2001 were collected from the Regional Agricultural Research Institute, Bahawalpur, Punjab, Pakistan. One kg sample from each variety was taken and fumigated with Aluminium phosphide tablets to nullify the possibility of previous infestation if any. The samples were then cleaned by sieving through 3/8, 3/16, 1/8 and 1/12 inch mesh sieves. From this cleaned wheat, working samples weighing 25 grams from each variety were drawn (Proctor, 1994) and subjected to analyses for determination of moisture contents, insect damage, broken and healthy grains.

Collection and mass rearing of insects: Mixed age cultures of *T. granarium* were collected from farm houses as well as wheat stores of the Punjab Food Department located at various places in Rawalpindi district. The cultures were reared on healthy wheat grains apparently free from insect infestation. To further ensure exclusion of any undetected population of insects, the wheat samples were subjected to phosphine fumigation before using the grains as rearing medium (Jood & Kapoor, 1992). The fumigated grains were put in three glass jars (15 cm × 15 cm × 25 cm), each containing one kg wheat. The jars were covered with muslin cloth with the help of rubber band and placed in the laboratory at 30±2°C and 65±5% relative humidity for conditioning. The grains were used as rearing medium when their moisture contents had reached 10-12% after fortnight. *(*Pingale & Girish, 1967). From the mixed age cultures collected from various destinations, Khapra beetle pupae were separated and kept in an incubator at 32±2°C and 65±5% R.H for adult emergence, which were used for mass rearing after 24-48h. Ten pairs (10 males + 10 females) of adults were introduced in the jars containing clean, healthy and fumigated wheat grains with 10-12% moisture contents. The cultures were maintained in the incubator at 32±2 °C and 65±5% R.H. for a period of three months for mass rearing. Later on, uniform size larvae of khapra were used in the experiment.

Exposing wheat seeds to Khapra beetle infestation: Wheat grains weighing 200 grams from each variety were put in 250 ml glass jars and 20 uniform size larvae of about 1-2 weeks old were taken from the culture and introduced in each jar as triplicates. All the jars were then covered with muslin cloth with the help of rubber bands. The jars were later placed in an incubator under semi-warehouse conditions at 25±2°C, 55±5% R.H. and 12:12 h continuous light: dark conditions for a period of six months. Doors of the incubator were opened for a period of 30 minutes on alternate days to ensure proper aeration and to avoid accumulation of Carbon dioxide produced as a result of biotic respiration. After a period of 6 months, the jars were taken out and further analyses were made for progeny development as well as physical and biochemical changes induced by the insect infestation.

Determination of moisture contents: Moisture of the grain was determined to investigate its correlation with the levels of *T. granarium* infestation, progeny development, weight loss, weight of frass, insect's damaged, broken and healthy grains. For this purpose, a U.S. made grain moisture tester (Farmex-MT3) was used.

Progeny development: After weighing, the entire grain samples from each variety were sieved through 1/8, 1/12, 3/16 and 3/8 inches mesh sieves. Live as well as dead larvae, pupae and adults were counted from each replication of the respective wheat variety by laboratory magnifying glass $\times 10^3$.

Weight loss: The infested grains in each jar were subjected to sieving to separate grain dust, exuviae and other excretions added due to Khapra beetle infestation. A sample weighing 25 gram was drawn from the cleaned wheat for assessment of percent weight loss. For this purpose number and weight of damaged and undamaged grains were recorded and put in the following equation for determination of weight loss (Gwinner *et al*., 1996).

$$\text{Percent weight loss} = \frac{(W\mu \times Nd) - (Wd \times N\mu)}{W\mu \times (Nd + N\mu)} \times 100$$

$W\mu$ = weight of undamaged grains
$N\mu$ = number of undamaged grains
W_d = weight of damaged grains
N_d = number of damaged grains

Weight of Frass: While determination of weight loss, the weight of exuviae, flour dust, dead as well as alive adult and immature stages of Khapra beetle and those of other excretions produced during infestation were measured and collectedly termed as weight of Frass of the respective sample of each variety.

Comparative resistance of wheat varieties: The rate of progeny development indicated by the number of larvae produced in each sample, percentage of infested grains and weight loss were considered as an expression of comparative resistance of each variety to the Khapra beetle infestation.

Insect damaged, broken and healthy grain count: After removing the frass, sample of cleaned grain weighing 25 gram was drawn from each replication of the respective wheat variety. The grains were classified and counted for percent insect damaged, broken and healthy one by using the following equations.

$$\text{Percent Insect damaged grains} = \frac{\text{No. of insect damaged grains}}{\text{Total number of grains in the sample}} \times 100$$

$$\text{Percent Broken grains} = \frac{\text{No. of broken grains}}{\text{Total number of grains in the sample}} \times 100$$

$$\text{Percent healthy grains} = \frac{\text{No. of healthy grains}}{\text{Total number of grains in the sample}} \times 100$$

Statistical procedures: Data so calculated was subjected to **s**tatistical analysis using multi-factorial completely randomized designs (CRD) in Minitab and MSTATC packages (Anon., 1990) and the means were compared by Duncan's Multiple Range tests at 99 % level of confidence (Gomez & Gomez, 1984).

Results and Discussion

Moisture contents: Jood *et al*., (1996) observed that 75% infestation level of *T. granarium* and *R. dominica* caused a significant ($p<0.05$) increase in moisture contents of the infested grains as in the present investigations Analyses of variance of the data in Table 1 has depicted significant differences between moisture content of grain before and after infestation. The moisture percentage values recorded before infestation revealed non-significant differences in DWR-97, Panjnad and Wafaq-2001, with moisture content of 8.44, 8.34 and 8.08%, respectively. Similarly non-significant differences were recorded for percent moisture content values of BWP-97 and Manthar (10.47 and

10.41%, respectively). Moisture content in case of Inquilab-91, GA-2002 was though statistically similar with each other as well as with BWP-2000, but was found higher than those of DWR-97, Panjnad and Wafaq-2001. Wheat variety Bhakkar-2000 exhibited a distinct moisture content of 9.87 %, lower than BWP-97 and Manthar. There was found a significant increase in moisture content of all the wheat varieties after subjecting them to artificial infestation with *T. granarium* larvae for a period of 6 months i.e. between March-August. The Table 1 also reflects that Wafaq-2001 showed minimum increase in moisture content from 8.08 to 8.41% after infestation. The other varieties Inquilab-91, DWR-97 and Panjnad statistically proved similar with each other in respect of increase in moisture content but ranked lower than those of BWP-97, Manthar, Bhakkar-2000 and BWP-2000. Maximum increase in the moisture content was recorded in Bhakkar-2000 with 2.09% increase after infestation. The results are also in line with the findings of Ravan *et al*., (1987), Jood & Kapoor (1993) and Jood *et al*., (1993). The increase in moisture contents over time may be due to the absorbance of atmospheric moisture by the grain, biotic respiration as well insect excretion.

Progeny development, weight loss and weight of frass: It is evident from Table 2 that maximum progeny development was observed in wheat variety BWP-97 showing 792.7 larvae per 200 gram wheat. Progeny development in case of Manthar-2000 was although statistically similar to that recorded in BWP-97 but was numerically lower having 752 larvae per 200 gram wheat. The varieties Bhakkar-2000 and BWP-2000 were also statistically similar in respect of progeny development with 668.7 and 624 larvae, respectively. Minimum progeny development of 189.7 was recorded in case of Wafaq-2001.

Weight loss caused after 6 months of artificial infestation by Khapra larvae revealed BWP-97 as one of the most susceptible wheat varieties with 20.25% loss followed by Manthar, Bhakkar-2000, BWP-2000, GA-2000, Inquilab-91, DWR-97, Panjnad and Wafaq-2001 with weight loss of 19.53, 18.27, 16.24, 15.47, 15.37, 14.1, 13.81 and 6.2%, respectively. Data pertaining to the weight of frass also depicted approximately similar ranking except weight of frass recorded in case of DWR-97 was higher as compared to that recorded in case of Inquilab-91. There was found a positive correlation between progeny development, infestation percentage, weight loss and weight of frass. The results are in conformity with the previous findings made by Ahmad *et al*., (1986) and Navarro *et al*., (1978) who observed a high degree of positive correlation between the progeny development and the infestation level, grain damage and weight loss of the infested grains. Bhardwaj *et al*., (1977) observed 2.5 % weight loss against 5.1 % infestation. Khattak *et al*., (2000) while working on the effect of *T. granarium* infestation on twelve rainfed wheat lines also found that correlation between progeny development, damage and weight loss was positive and highly significant ($p<0.01$). This is because grain is a living entity, which is affected by biotic and a biotic factors resulting in qualitative and quantitative loss (Ahmad, 1995; Singh *et al.,* (1997). Khan & Kulachi (2002) also reported a positive correlation between the progeny development *T. granarium*, *T. castaneum* and *R. dominica* and the losses caused by them to weight of grains. Their results were reconfirmed during an experiment conducted to evaluate losses caused by *T. granarium* and *R. dominica* to different wheat varieties (Syed *et al*., 2006).

Table 1. Moisture content of wheat varieties before and after exposing to infestation of Khapra beetle larvae at 25±2°C, 55±5% R.H. and 12:12h continuous light : dark conditions for a storage period of 6 months.

Wheat varieties	Moisture content (%)	
	Before infestation	After infestation*
BWP-97	10.47a	11.79 a
MANTHAR	10.41a	11.32 ab
BHAKKAR-2000	9.87 b	11.96 a
BWP-2000	9.00 c	10.37 cd
GA-2002	8.59 cd	10.67 bc
INQUILAB-91	8.55 cd	9.69 de
DWR-97	8.44 d	9.63 e
PANJNAD	8.34 d	9.48 e
WAFAQ-2001	8.08 d	8.41 f

Means followed by the same letter in each column are not significantly different by Duncan's multiple range test (p=0.01)

Table 2. Progeny development, weight loss and weight of frass in wheat varieties exposed to artificial infestation for at 25±2°C, 55±5 % R.H. and 12:12h continuous light: dark conditions for a storage period of 6 months.

Wheat varieties	Progeny development (No.)	Weight loss (%)	Weight of frass (gm)
BWP-97	792.70 a	20.25 a	24.47 a
MANTHAR	752.00 a	19.53 a	23.21 a
BHAKKAR-2000	668.70 b	18.27 ab	20.64 b
BWP-2000	624.00 b	16.24 bc	19.25 b
GA-2002	551.70 c	15.47 bc	17.03 c
INQUILAB-91	440.00 d	15.37 bc	13.58 d
DWR-97	472.00 d	14.10 c	14.57 d
PANJNAD	280.70 e	13.81 c	8.663 e
WAFAQ-2001	189.70 f	6.223 d	5.857 f

Means followed by the same letter in each column are not significantly different by Duncan's Multiple Range Test (p=0.01)

Insect damaged, broken and healthy grains recorded after infestation: The results pertaining to percent damage to wheat grains caused by *T. granarium* larvae in different wheat varieties under natural storage conditions are presented in Table 3. Mean values did not reveal statistically significant differences among various varieties of wheat in respect of broken and healthy grains before infestation. Significant differences were, however, found among the wheat varieties in respect of insect damaged grains before subjecting them to artificial infestation. Before infestation maximum number of damaged grains were recorded in variety BWP-97 (2%) followed by Inquilab-91, DWR-97, GA-2002 and Manthar with 1.66, 1.66, 1.33 and 0.66% infestation, respectively. The remaining four varieties did not show any sign of grain damage caused due to insect infestation. Comparison of mean values after infestation showed highly significant variations among different wheat varieties in respect of insect damaged, broken and healthy grains. As is evident from Table 3, maximum insect damaged grains (43.37%) were found in BWP-97 which statistically resembled to Manthar with 42.87% damage.

Table 3. Insect damaged, broken and healthy grains observed in wheat varieties exposed to Khapra larvae at 25±2°C, 55±5 % R.H. and 12:12h continuous light: dark conditions for a period of 6 months.

Wheat varieties	Insect damaged grains %		Broken grains (%)		Healthy grains (%)	
	Before infestation	After infestation*	Before infestation	After infestation*	Before infestation*	After infestation*
BWP-97	2.00 a	43.37 a	2.66 b	16.72 a	95.34 b	39.91 f
MANTHAR	0.66 bc	42.87 a	2.00 bc	15.02 ab	97.33 a	42.10 f
BHAKKAR-2000	0.00 c	38.44 b	4.00 a	13.65 b	96.00 b	47.91 e
BWP-2000	0.00 c	34.21 c	1.66 c	11.27 c	98.34 a	54.52 d
GA-2002	1.33 ab	34.45 c	1.66 c	11.17 c	97.00 a	54.38 d
INQUILAB-91	1.66 a	30.27 d	2.66 b	10.43 c	95.68 bc	59.30 c
DWR-97	1.66 a	35.94 bc	3.33 a	6.913 d	95.00 c	57.15 cd
PANJNAD	0.00 c	24.67 e	2.00 bc	5.237 d	98.00 a	70.10 b
WAFAQ-2001	0.00 c	19.97 f	3.33 a	4.973 d	96.67 b	75.06 a
Mean	**0.81**	**33.79**	**2.59**	**10.60**	**96.59**	**55.60**

Means followed by the same letter in each column are not significantly different by Duncan's Multiple Range Test (p=0.01)

Table 4. Correlation matrix showing relationship of different physical and biochemical changes of wheat grains induced by larval infestation of Khapra beetle (*T. granarium)* larvae at 25±2 °C, 55±5 % R.H. and 12:12h continuous light: dark conditions for a period of 6 months.

Parameters	Progeny Development						
Progeny development	1.000	Moisture content					
Moisture content	0.900	1.000	Weight of frass				
Weight of frass	1.000	0.900	1.000	Weight loss			
Weight loss	0.920	0.835	0.920	1.000	Weevilled grains		
Weeviled grains	0.968	0.864	0.968	0.911	1.000	Damaged/ Broken	
Damaged/broken grains	0.954	0.920	0.954	0.876	0.888	1.000	Healthy grains
Healthy grains	-0.989	-0.907	-0.989	-0.923	-0.986	-0.952	1.000

Whereas minimum percentage of insect damaged grains was found in Wafaq-2001 with 19.97 %. A similar trend was observed in case of broken grains and *vice versa* with the healthy grains. These findings are in consistent with those of Badawy & Hassan (1965), Shah (1969), Azeem *et al*., (1976), Hameed *et al*., (1984), Irshad and Baluch (1985), Ahmad *et al*., (1986), Irshad *et al*., (1988), and Khattak *et al*., (2000) who observed a positive correlation between infestation caused by *T. granarium* and damage to wheat grains. Later on, Syed *et al*., (2006) carried out investigation to evaluate the comparative resistance of wheat varieties against infestation of *T. granarium* and *R. dominica* infestation. Their results revealed that grain damage and weight loss percentage was mainly dependent upon the progeny development which was also dependent on the varietal preference of the pests used in the investigation.

Results of the present investigations revealed significant increase in progeny development, weight loss, weight of frass, the number of broken and insect damaged grains with the increase in infestation level. The Khapra larvae fed on both germ as well as endosperm of the grain. In most of the cases, entire grain was consumed and partially eaten

husk was the only remain of the grain. In some grains larvae were found camouflaging themselves inside the shell. A huge quantity of exuviae, flour dust, live and dead larvae and adults was an indication of the infestation severity and level of susceptibility or resistance in different varieties tested during the present investigation. Color change as well as foul odour was also observed in severely infested samples. Correlation matrix (Table 4) better reflects relationship between different variables investigated in the present investigations. Wheat variety BWP-97 proved to be the most susceptible and Wafaq-2001 behaved as the most resistant wheat varieties with minimum weight loss, weight of frass and number of damaged grains. Literature has revealed that the insect resistance mechanisms of cereal grains are complex and depend on physico-chemical and bio-chemical properties of the grain and on the subsequent bio-chemical and physical adaptation of post-harvest insects to these properties (Warchalewski *et al*., 1989; Dobie, 1991; Warchalewski & Nawrot, 1993; Warchalewski *et al*., 1993). Stored grains may have high resistance to insect pests because of the lack of vital nutrients or the presence of compounds that adversely affect insect development (Taylor & Medici, 1966; Medici & Taylor, 1966; Yetter *et al*., 1979; Nawrot *et al*., 1985; Gatehouse *et al*., 1986; Dobie, 1991; Baker *et al*., 1991; Huesing *et al*., 1991; Warchalewski & Nawrot, 1993; Pueyo *et al*., 1995; Zhang *et al*., 1997; Piasecka-Kwiatkowska, 1999; Piasecka-Kwiatkowska & Warchalewski, 2000a, b). In general, insects tend to develop more slowly on resistant grain varieties. Various studies of wheat grain resistance have been made, but there have been few attempts to determine the particular factors that govern resistance (Dobie, 1991). The present studies have revealed Wafaq-2001 as the most resistant variety against *T. granarium*. The results are in conformity with those of Ahmedani *et al*., (2009) who observed minimum impact of khapra beetle infestation on the grains of this variety.

Acknowledgements

The authors are grateful to the Higher Education Commission of Pakistan for providing adequate funding to carry out this research work.

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(Received for publication 15 April 2009)