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Appraising the quantitative and biochemical changes caused by *Trogoderma granarium* (Everts) in different wheat varieties of Pakistan

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Abstract

Laboratory studies were conducted to determine quantitative and biochemical changes as affected by *Trogoderma granarium* (Everts) in five wheat varieties viz., Faisalabad-2008, Sehar-2006, Lasani-2008, Shafaq-2006 and Pasban-90at Entomological Research Institute, Faisalabad during 2011. Wheat varieties were offered to Khapra beetle for the period of four months. Results demonstrated considerable changes in quantitative (weight loss, moisture content, percent insect damaged grains and percent healthy grains) and biochemical (total protein and starch losses) components of stored wheat varieties. Experimental findings revealed maximum weight loss (24.14%) in 1000 grains was observed in wheat varietyFaisalabad-2008 and least (7.04%) in Lasani-08. Maximum decrease of 7.4% in starch contents was observed in Faisalabad-08 and variety Sehar-06 depicted higher (2.90%) loss in protein contents. The correlation between physical and biochemical parameters revealed percent damaged grains had significant and positive effect (r =0.886) with weight loss while negative and significant correlation (r = -0.92) with healthy grains.

Keywords: Wheat varieties, Khapra beetle, weight loss, moisture content, starch and protein losses

Introduction

Wheat *Triticum aestivum* L. (Gramineae) is an important staple food in Pakistan. Post-harvest losses around 9.3 to 42% of achievable wheat are due to biotic (insects, molds, rodents and birds) and abiotic (temperature and humidity) variables of the environment (Dhaliwal and Arora, 2001)[1]. Insect pests of the environmental biotic part are the more common entities, which cause 10 to 20% storage losses (Khan *et al*, 2010)[2] and this infestation cause significant changes in nutritional quality and workability of stored grains (Ahmed *et al*, 2008)[3].

Among the stored grain pests khapra beetle *Trogoderma granarium* Everts is the most destructive (Burges, 2008[4]; Mark *et al*., 2010[5]). Its larva starts feeding from embryonic end and consumes the entire kernel/seed, making the grain hollow and leaving behind the husk. Under natural room storage condition for five months, 1045.50 individuals in 25 gm. sample cause 24.6%

loss in wheat grains (Riyad *et al*., 2015)[6].Singh *et al* (2000)[7] observed 5-17 % loss in germination when grains were stored approximately for five months. The fragmentation in flour is major concerns for processing business (Perez *et al*., 2003)[8].Grains infestation with >0.5 percent attack is considered to be unfit for wheat flour which contains more than 10mg uric acid/100g and assume inadmissible for consumers (Raju, 1984)[9].Seventy five percent level of infestation in sorghum, wheat and maize grains diminishes sugars, true protein contents, crude fat and total carbohydrates; while crude fiber, total protein and moisture content is increased (Jood *et al*.,1993[10], 1996[11], Jood & Kapoor, 1993[12];) while, uric acid level was reported to exceed the acceptable limit due to infestations of *T. granarium* and other insect pests. Ahmdani *et al*(2009)[13] in a study towards varietal variation in nutritional composition of wheat kernel (*Triticum* aestivum L.), recorded a decrease in total protein and carbohydrates after subjecting them to khapra beetle infestation. Ahmdani *et al*(2011)[14] reported positive correlation among the progeny development and number of damaged grains as well as with weight loss. They further found moisture contents also exhibiting a strong positive correlation with the loss in weight of wheat grains.

No such work of quantitative and biochemical losses caused by most destructive khapra beetle to these commonly grown varieties are done in Pakistan. Losses in quality and weight of the grains and cost needed to avoid losses may cause considerable loss to wholesalers. Findings of this research may help the researchers to select proper control measures to avoid losses in addition to secure food quality.

This study was therefore, conducted with the following objectives;

1. Estimation of quantitative changes in wheat kernel induced by *T. granarium* infestation.
2. Estimation of nutritional changes with special reference to contents of starch and crude protein.

Materials and methods

The study was conducted on five wheat varieties viz., Faisalabad-2008, Lasani-2008, Sehar-2006, Shafaq-2006 and Pasban-90in Stored Grain Research Laboratory, Entomological Research Institute, Ayub Agriculture Research Institute, Faisalabad during 2011.

The seeds of these varieties were obtained from Wheat Research Institute, AARI, Faisalabad. The sample collected were treated with Aluminium phosphide tablets to invalidate the likelihood of previous infestation if any.

Mass rearing of Khapra beetle

Pupae of Khapra beetle were collected from the mix aged culture collected from Punjab Food Department stores located at Faisalabad and kept in an incubator run 30± 2°C at 65±5 % R.H for rearing. Ten pairs (10 males+10 females) of newly emerged adults were taken and released in the glass jars having sound, cleaned and uninfested grains of wheat variety Inqlab-91 with 10 % moisture contents. The mouth of jars was closed with muslin cloth and put in an incubator at a temperature of 30±2°C and 65±5 % relative humidity to provide ideal conditions for mass rearing of beetle.

Inoculation of wheat varieties

Samples of 200 grams wheat grains were taken from each variety and placed in 1000 ml glass jars as five replicates. Twenty newly emerged adults of uniform size were taken and introduced in jars at the same time as followed by Parugrug and Roxas (2008)[15]. All jars were covered with muslin cloth and tied up with rubber bands. These

jars were placed in an incubator run at 30± 2°C and 65±5 %.

After four months post liberated period, the jars were removed from the incubator. The data on the following parameters were recorded.

Loss in grain weight

The damaged grains of each jar of each variety were cleaned by sieving to remove exuviae, dust and insect excretions. Grain weight loss was calculated by 1000 grain mass method (Proctor and Rowley, 1983)[16].

Moisture content in grains

Moisture content of the grains was determined to know the impact of moisture content on losses caused by insect infestation by using grain moisture meter (Farmex-MT3).

Damaged and sound grains

Following a period of four months sample weighing 25 gram of cleaned grains was drawn from every replication. The grains were isolated and counted for damaged and sound grains with the help of following equations.

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

$$\text{Healthy grains (\%)} = \frac{\text{No. of healthy grains}}{\text{Total number of grains in the sample}} \times 100$$

Determination of starch and protein contents

Starch and protein contents of wheat grains infested with khapra beetle were determined by using AOAC methods (Helrich, 1990)[17]. Wheat samples infested with *T. granarium* larvae were cleaned and sieved to remove dust and insect body parts. Then 50 gram sample of wheat grains as 3 replicates was taken for biochemical analysis to determine total protein and starch contents.

Protein is the most important nutrient that supplies essential and non-essential amino acids in human diet. Crude protein was determined by following AOAC Method No. 979.09. Sample was added to the flask containing H_2SO_4, hydrogen peroxide and heated in the presence of a catalyst. The flask was then cooled and diluted with distilled water. NaOH was added to the digested sample and free ammonia was distilled and titrated. Protein was determined by multiplying the total nitrogen by a conversion factor of 5.7 for wheat.

Starch was determined by using AOAC method No. 979.10. Analysis of starch was done in two steps. In first step starch was hydrolyzed with an enzyme (amyloglucosidase) into glucose and second involved the measurement of glucose levels.

Statistical procedure

All the experiments were carried out in five replications in Completely Randomized Design. Data regarding weight loss, moisture content, percent healthy grains, percent damaged grains, total protein and starch contents were subjected to ANOVA. The means were separated to find out the difference among different wheat varieties by using LSD test at 5% level of probability. Pearson's correlations(r) among physical and biochemical parameters were also determined.

Results and discussion

Results pertaining to weight loss (table 1) during 4 months of storage period revealed that all the varieties were significantly different from each other. Maximum weight loss was estimated to be 24.13% in variety Faisalabad-2008 which depicted statistical difference from other varieties. Lasani-2008 showed minimum (7.033%) weight loss. These results regarding weight loss are not in agreement with the work of Ahmadani *et al* (2011)[14]who observed 20% loss during 6 months storage period. Riyad *et al*

(2015)[6]observed 24.6 % loss in wheat grains during 5 months storage period. These results are somewhat consistent to our findings.

Moisture content of grains before and after infestation depicted significant difference. Before infestation only Pasban-90 (10.43%) showed statistical difference in moisture content of wheat grains whereas all the other varieties showed non-significant difference in moisture content. All the varieties enunciated significant differences in moisture content when offered to attack of khapra beetle. Highest moisture content (12.23%) was observed in Faisalabad-2008 that varied significantly from Sehar-2006 and Shafaq-2006 with moisture content of 10.40and 9.47%, respectively but found to have no statistical difference from Pasban-90 and Lasani-2008 having moisture content values of 11.80 and 10.53%, respectively. The results are similar to the findings of Ravan*et al*., (1987)[18]and Hossain *et al*., (2011)[19] where a continuous increasing trend was observed in grain moisture with interval of time. The increase in moisture content may be due to the aerobic activity of both the wheat grain and *T. granarium*. CO_2, H_2O and energy is produced during aerobic respiration. This water produced during respiration might be the reason of increasing grain moisture content. Another important factor is insect excretion that might have contributed to increased moisture content. Also the energy produced as a result of oxidative metabolism increases the grain temperature which in turn provides favorable environment for insect growth.

It is evident from the Table 1. That Faisalabad-2008 observed to have maximum number of insect damaged grains with 47.31% infestation that varied significantly from other varieties. Minimum insect damaged grains were found in Pasban-90 with 24.6% infestation. These findings are in consistent with those of Irshad and Baluch (1985)[20], Irshad *et al* (1988)[21], Khattak *et al*(2000)[22] and Syed *et al* (2006)[23]whose studies depicted linear assosiation between damaged grains and infestation caused by khapra beetle.

The data in Table 1 revealed that, highest sound grains (62.95%) was recorded in Pasban-90 followed by 58.50%, 53.64%, 47.79% and 37.16% in Shafaq-2006, Lasani-2008, Sehar-2006 and Faisalabad-2008, respectively. These results are in accordance with Khattak *et al*., 2000[22] and Syed *et al*., 2006[23], who reported that, weight loss (%) and damaged grain (%) were directly related upon infestation period and insect density.

Table 1: Quantitative changes in wheat grains caused by *T. granariun* before and after infestation.

Wheat varieties	Weight loss (%)	Moisture content (%)		Damaged grains (%)		Sound grains (%)	
		Before infestation	After infestation	Before infestation	After infestation	Before infestation	After infestation
Pasban-90	9.93d	10.43a	11.80a	0.0c	24.6e	97.90a	62.95a
Sehar-06	12.26b	8.73b	10.40b	0.0c	35.34c	98.85a	47.79d
Faisalabad-08	24.13a	9.43b	12.23a	0.23b	47.31a	93.14c	37.16e
Lasani-08	7.033e	9.0b	10.53a	0.0c	38.54b	98.44a	53.64c
Shafaq-06	10.20c	9.17b	9.47c	0.51a	32.32d	96.35b	58.50b
LSD	0.077	0.70	0.62	0.20	2.83	1.17	3.10

Means sharing similar letter (s) are not significantly different at P=0.05

Biochemical losses

Means of protein and starch content in grains before and after infestation by *T. granarium* larvae presented in Table 2. exhibited statistical differences. Total protein contents generally decreased after *T. granarium* infestation. A maximum decrease of 7.50% was observed in the variety Faisalabad-2008 followed by Lasani-2008, Shafaq-2006, Sehar-2006 and Pasban-90 with 5.06, 3.20, 1.45 and 0.67% loss respectively. It has been observed that *T. granarium* larva mainly a germ feeder attacks moreon wheat endosperm which is protein in nature that is why protein contents decrease after larval feeding. These results are in conformity with those of Jood and Kapoor,. 1992a[24] whose findings revealed that *T. granarium* larvae cause more decrease in protein digestibility of maize and wheat as compared to *R. dominica* or a mixed population of these two species. Protein loss may be attributed to high temperature and increased moisture contents of stored wheat varieties because at high temperature, protein metabolism is high and proteolytic activity breaks peptide bonds into free amino acids due to which decrease in protein content occurs Bakker-Arkema *et al*,2007[25]. The decrease in protein content can also be attributed tomycotoxins produced by mould growth at high temperature and moisture content. These mycotoxins produce aflatoxins which utilize nutrients like protein for their growth and survival and hence cause a decrease in total protein.

Starch is a major component of most of the cereals and is responsible in providing major amount of nutrients and vast amount of energy in the human food. The results indicated that starch content of grain decreased throughout the storage duration. The maximum decrease (2.89%) of starch content was observed in case of Sehar-2006 followed by Lasani-2008, Pasban-90 and Faisalabad-2008 with 1.86, 1.12, 0.69 and 0.25 % loss respectively. Endosperm or bran portion of grains is least affected because larvae of *T. granarium* show more preference towards germ portion. These results are supported by the work of Strelec *et al*, 2010[26] where starch was found reduced when exposed to elevated temperatures (40 and 25 °C) and relative humidity of 45% during one year of storage. The reduction in starch percentage of grain could also be due to the improved growth of insects and fungi. It has been reported that 75% level of insect infestation in sorghum, wheat, and maize seeds caused a considerable decline in starch percentage (Jood and Kapoor 1993[10], Jood *et al*., 1996[11]).

Table 2: Biochemical changes induced by the infestation of *T. granarium*.

Wheat varieties	Total Protein		Protein loss (%)	Starch contents		Starch loss (%)
	Before infestation	After infestation		Before infestation	After infestation	
Pasban-90	14.87a	14.77a	0.67d	51.80a	51.17d	1.12c
Sehar-06	13.77c	13.57d	1.45d	54.17a	52.60a	2.89a
Faisalabad-08	14.67b	13.57d	7.50a	51.90c	51.77c	0.25d
Lasani-08	14.50b	13.77c	5.06b	53.87a	52.87a	1.86b
Shafaq-06	14.50b	14.03b	3.20c	52.53b	52.17b	0.69cd
LSD	0.18	0.10	1.17	0.49	0.37	0.56

Means sharing similar letter (s) are not significantly different at P=0.05

Table: 3 Correlation between Quantitative and Biochemical parameters

Parameters	Weight loss					
Weight loss	1.000	Damaged grains				
Damaged grains	0.886	1.000	Sound grains			
Sound grains	-0.8247	-0.9260	1.000	Moisture content		
Moisture content	-0.0922	0.2523	-0.3935	1.000	Total protein	
Total protein	-0.9868	0.8501	0.8194	0.1672	1.000	Starch content
Starch content	0.7212	0.3678	-0.2095	0.6324	-0.7113	1.000

The results (Table 3) showed that damaged grains had significant and positive correlation with weight loss while negative and significant correlation with sound grains. All other physical and biochemical parameters had non-significant correlation among themselves. These results match with Ahmdani *et al.,* 2009[14] who recorded negative correlation between healthy and damaged grains. Literature has revealed that insects of stored grains may have a tendency of slow development on resistant varieties due to the absence of some essential nutrients needed for insect reproduction and survival (Warchalewski and Nawrot, 1993[27], Zhang *et al*., 1997[28] and Piasecka-Kwiatkowska 2000a[29], b[30]).

Conclusion

Findings of the present in vitro study have revealed Pasban-90 as the most resistant variety against *T. granarium* with minimum weight loss, minimum insect damaged grains and maximum healthy grains. Variety Faisalabad-08 appeared as comparatively susceptible with maximum decrease in protein contents, maximum weight loss and least healthy grains. These results are not in conformity with those of Ahmedani *et al*., (2009)[14] and Ahmadani *et al*., 2011[15] who observed Wafaq-2001 as the most resistant variety against *T. granarium.* It is therefore, concluded that variety Faisalabd-08 needs more attention from storage point because it was suffered more from *T. granarium* infestation having maximum protein loss that may affect its germination.

References

1. Dhaliwal, G. S. and R. Arora. Integrated Pest Management: Concept and Approaches, Kalyani Publishers, New Delhi, India, 2001, pp. 427.
2. Khan, I., S. Afshan, N. Din, S. Khattak, S. K. Khalil and Y. H. Y. Lou. Appraisal of different wheat genotypes against Angoumois grain month, *Sitotrogacerella* (oliv). *Pak. J*., 2010, 42: 161-168
3. Ahmed, A., T. Ahmed, M. A. Arain and M. Ahmed. Management of bagged wheat godowns to control the stored grain insect pests. *Pak. J. Entomol*., 2008, 30: 31-35.
4. Burges, H.D. Development of the khapra beetle, *Trogoderma granarium*, in the lower part of its temperature range. *J. Stored Prosd Res*., 2008, 44: 32-35.
5. Mark, A. C., D. L. Severtson, C. J. Brumley, A. Szito, R. G. Foottit, M. Grimm, K. Munyard and D. M. Groth. A rapid non-destructive DNA extraction method for insects and other arthropods. *J. Asia-Pacific Entomol*., 2010, 13: 243-248.
6. Riyad R. Al-Iraqi., Zahra I. Dallal-Bashi and Raed S. Al-Safar. Population Density of Khapra Beetle, *Trogodermagranarium* Everts (Dermestidae: Coleoptera), on Grains and Spikes of Wheat and Barley. Jord. *J. of Agric. Sci*, 2015, 11(2): 393-398.
7. Singh, S. C., K. Parsad and R. Kumari. Studies on losses in wheat in relation to storage structure in the

villages of Barh under Patna district of Bihar State. Uttar Pradesh *J. Zool*., 2000, 20 (2): 197-198.

8. Perez-Mendoza, J., J.E. Throne, F.E. Dowell and J.E. Baker. Detection of insect fragments in wheat flour by near-infrared spectroscopy. *J. Stored Prod. Res*., 2003, 39: 305-312.
9. Raju, P. The staggering storage losses - causes and extent. Pestic., 1984,18:35-7.
10. Jood, S., A.C. Kapoor and R. Singh. Biological evaluation of protein quality of sorghum as affected by insect infestation. Plant Foods Hum. Nutr., 1993a, 43: 105-114.
11. Jood, S., A.C. Kapoor and R. Singh.. Effect of insect infestation and storage on lipids of cereal grains. *J. Agric. Food Chem*., 1996, 44:1502-1506.
12. Jood, S. and A.C. Kapoor. Protein and uric acid contents of cereal grains as affected by insect infestation. Food Chem., 1993b, 46:143-146.
13. Ahmedani, M.S., Haque, M.I., Afzal S.N., Aslam M. and Naz, S. Varietal changes in nutritional composition of wheat kernel (*Triticumaestivum* L.) caused by khapra beetle infestation. *Pak. J. Bot*., 2009, 41: 1511-1519.
14. Ahmedani, M.S., Haque, M.I., Afzal S.N., Naeem M., T. Hussain and S. Naz. Quantitative losses and physical damage caused to wheat kernel (*Triticumaestivum L*.) by khapra beetle infestation. *Pak. J. Bot*, 2011, 43(1): 659-668.
15. Parugrug ML, Roxas AC. Insecticidal action of five plants against maize weevil, *Sitopliluszeamais*Motsch, (Coleoptera:Curculionidae). KMITL SciTechnol J, 2008, 8(1):24-38.
16. Proctor D.L. and Rowley J.Q. The Thousand Grain Mass (TGM): Abasis for better assessment of weight losses in stored grain. Trop. stored Prod. Inf, 1983, 45, 19-23.
17. Helrich, K. Official Methods of Analysis. Association of Official Analytical Chemists, Inc. Suite 400. 2200 Wilson Boulevard, Arlington, Virginia 22201, 1990, USA. pp.1298.
18. Ravan, S., M. Ahmad, M. Hassan and M. R. Khan. Relative Susceptibility of Milled and Polished Grains of Different varieties of Rice to Rice weevil. *Pak. Entomol*., 1987, 9:5-8.
19. Hossain M. S., R.C. Kabiraj, M. A. Hasan, M. R. U. B. Shaheen and M. A. K. Al-Azad. Effect of biotic and abiotic factors on quality of black gram seed. Bangladesh Res. Publications J., 2011, 5: 103-110.
20. Irshad, M. and U.K. Baloch. Losses in wheat during storage and their prevention. Prog. Farm., 1985, 5: 17-19.
21. Irshad, M.A., U.K. Baloch, M. Irshad and A.Khan. Losses in wheat in public sector storage in Rawalpindi region during 1984-85. *Pak. J. Agric. Res*., 1988, 9: 136-140.
22. Khattak, S.U., S. Kamal, K. Ullah, S. Ahmad, A.U. Khan and A. Jabbar. Appraisal of rainfed wheat lines against Khapra beetle, *Trogodermagranarium* Everts. *Pak. J. Zool*. 2000, 32:131-134.
23. Syed, T.S., F.Y. Hirad and G.H. Abro. Resistance of different wheat varieties to khapra beetle, *Trogodermagranarium* (Everts) and lesser Grain Borer, *Rhyzoperthadominica* (Fabricus). *Pak. J. Biol. Sci*., 2006, 9:1567-1571.
24. Jood, S., A.C. Kapoor and R. Singh. Biological evaluation of protein quality of maize as affected by insect infestation. *J. Agric. & Food Chem*., 1992, 40: 2439-2442.
25. Bakker-Arkema, F. W.; Brooker, D.; Hall, C. Drying and Storage of Cereal Grains and Oilseeds; Chapman and Hall, New York, 2007, p 56.
26. Strelec I., D. K. Komlenic, V. Jurkovic, Z. Jurkovic and Z. Ugarcic-Hardi. 2010. Quality parameter changes in wheat

varieties during storage at four different storage conditions. Agric. Conspectus Sci. 75: 105-111.

27. Warchalewski, J. R. and J. Nawrot. Insect infestation versus some properties of wheat grain. Roczniki Nauk Rolniczych, Seria E. 1993, 23:85–92.
28. Zhang, N., B.L. Jones and P. Tao. 1997. Purification and characterization of a new class of insect α-amylase inhibitors from barley. Cereal Chem., 74:119–122.
29. Piasecka-Kwiatkowska, D. and J.R. Warchalewski. The cereal protein inhibitors of hydrolytic enzymes and their role. Part I. Protein inhibitors of α-amylase. Zywnosc, 2000a, 2:110–119.
30. Piasecka-Kwiatkowska, D. and J.R. Warchalewski. The cereal protein inhibitors of hydrolytic enzymes and their role. Part II. Protein inhibitors of proteinases. Zywnosc, 2000b, 3:33–38.