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VARIETAL CHANGES IN NUTRITIONAL COMPOSITION OF WHEAT KERNEL (*TRITICUM AESTIVUM* L.) CAUSED BY KHAPRA BEETLE INFESTATION

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Abstract

Wheat and other stored products are vulnerable to attack of Khapra beetle, which deteriorates quality of the infested commodities, depletes nutrition value and makes them unfit for human consumption. The present investigations revealed radical changes in nutritional composition of the grains of different wheat varieties when subjected to artificial infestation with 10 pairs of *Trogoderma granarium* larvae for six months. Maximum changes in protein, carbohydrate, fat, fiber and ash contents were observed in wheat variety BWP-97, whereas minimum changes were recorded in case of Wafaq-2001. Although protein, fat, fiber and ash contents increased with the increase in infestation level, yet the increase was just a percent increase due to depletion of carbohydrate and loss in total weight. Factually these variables decreased with the increase in infestation level.

Introduction

The Khapra beetle is one of the world's most damaging pests of whole and ground cereals, oilseeds, dry fruits, copra and other stored products. Its immense economic significance is due to its potential to cause huge loss in stored grains through voracious feeding and heating of grains, in larval ability to withstand starvation for up to three years as well as in its ability to live on food with very low moisture content. Literature reveals that Khapra beetle is a continuous threat to our food security as well as food safety. The barbed hairs of the larvae that rub off and remain in the grain may prove a serious hazard to human health if swallowed (Morison, 1925). Besides, cast skins may cause dermatitis in people handling heavily infested grains (Pruthi & Singh, 1950). Hence, the insect fragments in wheat flour are major concerns for the milling industry (Perez *et al*., 2003) as consumers demand high quality and wholesome products. Due to the reason FDA has established a defect action level of 75 insect fragments per 50 g of flour. Today flourmills of the United States regularly test their flour to comply with this federal requirement and to deliver sound products to their consumers. Amongst the storage pests, *T. granarium* larvae are reportedly the most serious and the most destructive one though its adults are harmless (Parashar, 2006). The larvae start feeding from embryo point and later consume the entire kernel/seed, which makes the grain hollow and only the husk remains. In case of severe infestations, infested grains are filled with frass, cast skins and excreta, which badly deteriorate quality of the grains. Hence, there is an increasing trend among grain buyers towards zero-tolerance to these contaminants. Countries such as Canada have a legally defined zero tolerance for stored-grain insects (Canada Grain Act, 1975). The larvae are often found on edges of jute stacks and make the infested store unhygienic. The insect infestation in wheat grains reduce germination and produce unpleasant odors,

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dirty appearance and abhorrent taste due to contamination with insect fragments and excrement (Khare *et al.*, 1974). Severe infestations of wheat grain by Khapra beetle may make it unpalatable or un-marketable. Grain quality may decrease due to depletion of specific nutrients. Infestation levels of 75% in wheat, maize and sorghum grains significantly decreased crude fat, total carbohydrates, sugars and true protein contents; while it increases the moisture, crude fiber and total protein contents (Jood & Kapoor, 1993; Jood *et al.*, 1993, 1996a). Besides, substantial loss of vitamins e.g., thiamin, riboflavin and niacin occurs due to the infestation (Jood & Kapoor, 1994). Significant increases or health hazardous non-protein nitrogen, total nitrogen and total protein have also been recorded (Jood & Kapoor, 1993). In addition, level of uric acid has been reported to exceed the acceptable limit due to infestations of *T. granarium* and other insect pests. Total lipids, phospholipids, galactolipids, and polar and non-polar lipids have also been reported to decline significantly at infestation levels of 50% and 75% (Jood *et al*., 1996b). On the other hand, anti-nutrient polyphenol and phytic acid have been reported to increase significantly. The quality deteriorating characteristics of *T. granarium* have assigned them a status of a noxious pest and a technical barrier to trade. Keeping the destructive nature and its significance for the global food security and safety in view, the present investigations were carried out to determine nutritional changes in wheat kernel induced by *T. granarium* infestation.

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. Biochemical and nutritional changes in the infested grains induced by infestation of *T. granarium* larvae were studied at Poultry Research Institute and Romer`s Laboratories, Rawalpindi for crude protein, crude fat, crude fiber and ash contents using ICC (Anonymous, 1994) and AOAC methods (Helrich, 1990). The materials and methods employed on this study are as follows:

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 biochemical analyses to determine nutritional composition of the wheat kernel before infestation.

Collection and mass rearing of insects: Mixed age cultures of the *T. granarium* were collected from farmhouses as well as 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, wheat samples were subjected to phosphine fumigation before using the grains as rearing medium. The fumigated grains were put in three glass jars (20 × 15 cm) each containing one kg of wheat. The jars were covered with muslin cloth with the help of rubber band and were placed in the laboratory at 30±2°C and 65±5% R.H. for conditioning. After a fortnight, when moisture levels of the grains ranged from 10-12%, which is suitable for insect growth and development (Pingale & Girish, 1967), the grains were used as rearing medium. Out of the mixed age cultures collected from various destinations, Khapra beetle were separated at the pupal stage and the pupae were then reared in an incubator at 32±2°C and 65±5% R.H. The newly emerged adults (24-48 hrs after emergence) were used for mass rearing. For this purpose, 10 pairs (10 males + 10 females) of adults were introduced in the jars having cleaned, healthy and fumigated wheat with 12% moisture contents. The cultures were maintained in the incubator at 32±2°C and 65±5% relative humidity for a period of three months for mass rearing. Uniform size larvae of the insect were used in the experiment.

Exposing wheat seeds to Khapra beetle infestation: Wheat grains weighing 200 gm from each variety was taken 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. All the jars were covered with muslin cloth with the help of rubber bands. The jars were placed in an incubator under semi-warehouse environment at 25±2°C, 55±5% R.H. and 12:12 hrs continuous light:dark conditions for a period of six months to realize the field conditions. Doors of the incubator were opened for a period of 30 minutes on alternate days to ensure proper aeration and to avoid accumulation of carbondioxide 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 biochemical changes induced by the insect.

Determination of crude protein, fat, fiber, carbohydrates and ash contents: For this purpose, infested wheat samples were cleaned and sieved to remove insect body parts. Later on, 50 gm grains in triplicate were drawn for determination of crude protein, fat, carbohydrates, fiber and ash contents. Crude protein is a conventional expression for the total content of nitrogenous compounds of the analyzed product, calculated by multiplying the corresponding total nitrogen content by factor of 5.7 for wheat. Whereas, nitrogenous compounds were determined by oxidizing the organic matter of the sample with concentrated sulfuric acid in the presence of a catalyst: the product of the reaction $(NH4)_2SO_4$ was treated by alkali; free ammonia was distilled and titrated (AOAC Method No. 979.09). For determination of crude fiber, wheat samples were gelatinized in the presence of heat stable alpha amylase, and then enzymatically digested with protease and amyloglucosidase to remove digestible protein and starch. Four volumes of ethanol were added to precipitate soluble dietary fiber. Total residue was filtered off and washed with ethanol and acetone. The residue was weighed after drying. The remaining material was analyzed for protein and ash content, respectively. Subtracting the amounts measured for protein, ash and a blank control from the dry weight of the filtered residue yielded a value for total dietary fiber content (ICC Standard No.156, 1994). To determine ash, a five gram well mixed sample was weighed into a shallow, relatively broad dish that had been ignited, cooled in a desiccator and weighed soon after reaching at room temperature. The sample was later on ignited in a furnace at 550°C (dull red) until the light gray ash resulted, which was cooled in a desiccator and weighed after reaching at room temperature (AOAC Method No. 923.03). The crude fat and carbohydrates were determined by using AOAC method No. 920.39 and 979.10, respectively.

Statistical procedures: Statistical analyses was carried out in multi-factorial completely randomized designs (CRD) in Minitab and MSTATC packages (MSTATC, 1990) and the means were compared by Duncan's Multiple Range tests at 99 % level of confidence (Gomez & Gomez, 1984).

Table 1. ANOVA showing biochemical changes of wheat grains caused by Khapra Beetle Infestation.

S.O.V.	DF	S.S	M.S.	F-value	Prob.
Protein before infestation	8	50.654	6.332	3985.002	0.0000
Error	18	0.029	0.002		
Protein after infestation	8	31.924	3.990	3168.873	0.0000
Error	18	0.023	0.001		
Fat before infestation	8	1.229	0.154	74.481	0.0000
Error	18	0.037	0.002		
Fat after infestation	8	2.274	0.284	39.655	0.0000
Error	18	0.129	0.007		
Fiber before infestation	8	1.448	0.181	46.638	0.0000
Error	18	0.070	0.004		
Fiber after infestation	8	7.545	0.943	188.633	0.0000
Error	18	0.090	0.005		
Ash before infestation	8	2.441	0.305	1872.410	0.0000
Error	18	0.003	0.000		
Ash after infestation	8	0.176	0.022	24.289	0.0000
Error	18	0.016	0.001		
Carbohydrate before infestation	8	618.982	77.373	217.536	0.0000
Error	18	6.402	0.356		
Carbohydrates after infestation	8	430.401	53.800	69.751	0.0000
Error	18	13.884	0.771		

Results and Discussion

Analysis of variance presented in Table 1 exhibited highly significant differences among different biochemical parameters before and after infestation of *T. granarium* larvae. Feeding preference of the larvae toward different varieties was found definitely diverse. It is worth mentioning that a positive correlation between larval infestation and protein, fat, fiber and ash contents was found, irrespective of the wheat varieties. On the contrary, there was found a negative correlation between carbohydrate and infestation caused to stored grains irrespective of varieties. Quality of flour made from the infested wheat may depend upon the nature and feeding behaviors of pests attacking the grain.

Crude fiber: It is evident from Table 2 that fiber contents generally increased after infestation on over all weight bases. A maximum increase of 1.4% was observed in BWP-97 followed by Manthar, Inquilab-91, Bhakkar-2000, GA-2002, DWR-97, BWP-2000, Wafaq-2001 and Panjnad with 1.36, 0.92, 0.84, 0.86, 0.587, 0.56, 0.25 and 0.21%, respectively. The difference observed in level of fiber contents may be attributed to the feeding behaviour of the *T. granarium* larvae (Jood *et al.,* 1992). It has been reported that *T. granarium* larvae primarily prefer to feed on germ portion as compared to bran or endosperm. That is why bran portion is least effected under the normal course of infestation resulting in an increase in fiber contents on over all weight basis (Salunkhe *et al.,* 1985). The results are also in line with work of Hameed *et al.,* (1984), Jood & Kapoor (1993), Jood *et al.*, (1993, 1996a), who observed an increase in fiber contents of wheat grains after subjecting to *T. granarium* infestation. They also found an inverse correlation between the susceptibility of the stored grains to insect pests and the fat, protein, fiber and ash contents. Our findings are in consistence to the previous work as Wafaq-2001 with the highest fiber contents of 1.953 proved to be the most resistant wheat variety with only 6.2% weight loss. The results are also in line with findings of Samuels & Modgil (2003).

Crude protein: The results pertaining to crude protein enunciated highly significant differences between the crude protein contents of wheat grains before and after infestation by the *T. granarium* larvae. Maximum increase of 2.83 % was observed in case of BWP-97 and Manthar followed by GA-2002, Panjnad, Inquilab-91, Bhakkar-2000, Wafaq-2001 and BWP-2000 with 1.65, 1.64, 1.11, 1.10, 0.57 and 0.01%, respectively (Table 2). In case of Panjnad, no change in content of crude protein was observed. Increase in the content of crude protein apparently seems illogical as larvae of *T. granarium* preferably eat on grain germ, which contains protein. Hence, there should have been an inverse relationship between the infestation level and protein content of the grain but we found a positive correlation between the two parameters against the hypothesis. A survey of literature revealed apparently contradicting results. Some showed increase in protein contents under the influence of *T. granarium* infestation whereas other showed reduction in the protein contents. For example, Jood & Kapoor (1992a,b; 1993) carried out investigations on protein and starch digestibility of wheat, maize and sorghum grains affected by *T. granarium* and *R. dominica*. They observed that *T. granarium*, primarily a germ feeder, reduced protein digestibility of wheat and maize more than did *R. dominica* or a mixed population of both insect species. In the same year they evaluated protein quality of wheat grains having 25, 50 and 75% infestation caused by mixed populations of *T. granarium* and *R. dominica* by rat growth and nitrogen balance studies. They observed that feeding of a diet containing insect-infested wheat grain (50 and 75%) resulted in marked decreases of food intake, body weight gain, food efficiency ratio, protein efficiency ratio, nitrogen absorption, biological value, net protein utilization and dry matter digestibility. These parameters showed negative association with infestation levels (Jood & Kapoor, 1992a). Further experiments carried out by Jood & Kapoor (1993) exhibited contradicting results wherein they recorded a significant increase in the levels of total nitrogen, total protein, non-protein nitrogen and uric acid contents of wheat, maize and sorghum grains having 25, 50 and 75% infestation caused by *T. granarium* and *R. dominica* separately and in mixed population. They also observed a significant reduction in protein nitrogen and true protein contents of three cereal grains at 75% infestation level. The picture became clearer by the findings of Samuels & Modgil (2003) who stated that an increase in insect infestation and storage period though significantly increase crude proteins, moisture, ash, crude fiber, non-protein nitrogen and uric acid contents; yet, true proteins, crude fat, calorific value, weight and density of wheat grains are significantly reduced at ($p<0.05$). That is why Aja *et al*., (2004) suggested that while determining the effect of insect infestation on protein contents of stored grains gluten index should be measured as an index of protein quality. Later studies have revealed that increase in crude protein contents in infested grains is attributed to the production of non beneficial rather harmful proteins such as cast skins, exuviates, dead insects, wings, legs and other body parts of the insects that come along with the infested grain samples. On the other hand, true protein or beneficial proteins are factually reduced (Jood *et al*., 1995; Prabhakumary & Sini, 2008). Besides, the increase in protein percentage may be due to significant depletion of carbohydrate percentage.

Carbohydrates: Findings of the present investigations enunciated a negative effect of *T. granarium* infestation and the carbohydrate contents of wheat grains. Maximum reduction of 6.64% carbohydrate was observed in BWP-97 having maximum progeny development and weight loss followed by GA-2002 (5.26), BWP-2000 (4.88), Manthar

Table 2. Biochemical changes induced in wheat varsities by *T. granarium* infestation.

Wheat varieties	Crude protein		Crude fat		Crude fiber		Ash content		Carbohydrate	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
BWP-97	9.20 g	12.03 d	1.36 e	2.76 a	2.14 b	3.45 b	0.80 g	1.53 bcd	81.04 a	74.4 b
Manthar	9.20 g	12.03 d	1.51 d	2.87 a	2.36 a	3.79 a	0.86 f	1.41e	77.69 c	73.3 bc
Bhakkar-2000	9.84 f	10.94 e	1.66 bc	2.50 b	2.14 b	3.43 b	0.98 d	1.39 e	75.79 d	72.20 c
BWP-2000	10.93 e	10.94 e	1.59 cd	2.15 c	1.99 c	3.20 c	1.00 d	1.49 d	79.85 b	74.97 b
GA-2002	11.48 d	13.13 c	1.50 d	2.18 c	2.15 b	3.46 b	0.91 e	1.50 cd	73.76 e	68.50 d
Inquilab-91	12.03 c	13.14 c	1.947 a	2.87 a	2.24 ab	2.94 d	1.53 ab	1.66 a	80.7 ab	78.05 a
DWR-97	12.03 c	12.03 d	1.713 b	2.30 bc	1.74 d	2.63 e	1.44 c	1.56bcd	68.07 g	65.93 e
Panjnad	12.58 b	14.22 a	1.983 a	2.19 c	1.58 e	2.23 f	1.50 b	1.58 bc	69.97 f	67.3 de
Wafaq-2001	13.10 a	13.67 b	1.953 a	2.21 c	2.11 bc	2.27 f	1.55 a	1.59 ab	69.97 f	67.2 de
Means	**11.15**	**12.45**	**1.69**	**2.44**	**2.05**	**3.04**	**1.17**	**1.52**	**75.21**	**71.32**

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

(4.39), Bhakkar-2000 (3.59), Wafaq-2001 (2.77), Inquilab-91 (2.65), Panjnad (2.67) and DWR-97 (2.14%), respectively (Table 2). These results showed a positive value of the correlation coefficient between progeny development and carbohydrate contents to the tune of 0.678 with the infested grains. It is obvious from the values of correlation co-efficient that reduction in carbohydrate was not solely dependent upon the progeny development. There are some other factors, which may have contributed toward reduction in carbohydrate contents. Such factors are varietals resistance as well as insect preference. The results are in conformity with the findings of Jood & Kapoor (1992c) who carried out investigations on the effect of storage and insect infestation on starch and protein digestibility of cereal grains. They found that *T. granarium* significantly reduced starch digestibility of wheat and maize. Further studies carried out by them also revealed a significant reduction in carbohydrate contents of wheat, maize and sorghum when artificially infested with *T. granarium* and *R. dominica*. The results are also in line with findings of previous workers such as Hameed *et al.*, (1984) who observed a significant decrease in carbohydrate contents of wheat grains due to the attack of *T. granarium* larvae. These results were later on confirmed by Jood *et al.*, (1996b), Daniel *et al.*, (1977) Prabhakumary & Sini (2008).

Crude fat contents: It is evident from the Table 2 that maximum increase in crude fat was observed in case of BWP-97 whereas minimum was observed in case of Panjnad. There was found a higher positive correlation (0.85) between increase in fat percentage and weight of frass after the infestation. The phenomenon is contradictory to the facts that *T. granarium* primarily feeds on germ of the wheat grain, which is storehouse for protein and lipids. Under these circumstances higher infestation should have resulted in fat content reduction of the wheat grains but present findings have revealed opposite results, which are in conformity with those observed by Hameed *et al.*, (1984). Later studies revealed opposite results indicating significant decrease in crude fat contents with increase in the infestation level. The results are in conformity with the previous findings, which showed a significant decrease in fat contents with the increase in infestation level (Jood & Kapoor, 1993, Jood *et al.*, 1993; 1996a). Samuels & Modgil (2003) also reported significant decreases in crude fat, weight, density, calorific value and true proteins with increase in insect infestation and storage period. In case of present investigations increase in fat is simply a percent increase resulting from depletion of carbohydrates etc., due to infestation and hence is not an actual increase in fat content.

Ash contents: The results have revealed significant increase in ash contents of the infested grains. A maximum of 0.73% increase was observed in wheat variety BWP-97 followed by GA-2002, Manthar, BWP-2000, Bhakkar-2000, Inquilab-91, DWR-97, Panjnad and Wafaq-2001 with 0.59, 0.55, 0.49, 0.41, 0.13, 0.12, 0.08 and 0.04%, respectively. Increase in fat content is commonsensical because of feeding preference of *T. granarium* larvae, which bore into germ and prefer to eat germ as compared to the bran portion which is chief store house of minerals in case of wheat grains (Salunkhe *et al*., 1985). Our findings are in line with those of Hameed *et al.,* (1984) who recorded significant increase in ash contents in wheat grains attacked by the *T. granarium* larvae. Later on, Jood *et al*., (1992) also concluded that *T. granarium* is primarily a germ feeder appeared to eat a portion of bran, thus offsetting the increase in mineral composition of wheat grains which stores minerals mostly in its bran portion. Our results are also in conformity with those of Samuels & Modgil (2003) who found a significant increase in crude fiber with increase in infestation and storage period.

Conclusions

Results of the present investigations have revealed highly significant changes in nutritional composition of wheat kernel of different wheat varieties when subjected to artificial infestation of Khapra beetle. Apparently, there was found a varying quantity of exuviates, flour dust, live and dead adults and cast skins on different wheat samples indicating different levels of susceptibility or resistance. Color change as well as foul odor was also observed in severely infested samples. There was found a positive correlation between infestation levels and the protein, fat, ash and fiber contents of wheat grains. However, relationship between carbohydrate contents and the infestation level was found significantly negative. Maximum depletion and changes in nutritional composition were observed in case of BWP-97; whereas Wafaq-2001 proved as the most resistant wheat variety against the attack of Khapra beetle showing minimum changes in Kernel composition. Literature has revealed that the insect resistance mechanisms of cereal grains are complex and depend on physico-chemical and biochemical properties of the grain and on the subsequent biochemical and physical adaptation of post-harvest insects to these properties (Baker, 1986; 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. Nutrional composition of such varities is least affected by the attck of insect pests.

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