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International Journal of Insect Science

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RAPID COMMUNICATION

Effect of Iron Fortified Wheat Flour on the Biology and Physiology of Red Flour Beetle, *Tribolium castaneum* (Herbst)

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Abstract: Iron overload in the fortified flour can influence the life stages and physiology of the insects. The present study was carried out to evaluate the effect of commercially available premix iron fortified flour as well as effect of different concentrations of post-mix iron fortified flour (30–5 ppm) on biology of red flour beetle, *Tribolium castaneum* (Hebrst.). Larval and pupal duration, total developmental time, fecundity and larval weights in two consecutive generations of beetle were compared with control treatment. Amylase and protease activities of gut of the beetle were also measured in premix and postmix flours.

Results showed that larval mortality increased in two sources of premix iron flour when compared with control. Larval weight was reduced in first generation only. The larval mortality was significantly higher in 30 ppm postmix iron fortified flour than in other postmix concentrations and control treatment. The larvae of *T. castaneum* fed on two sources of premix and in various concentrations of postmix iron fortified flour revealed an increase in amylases and decrease in protease activities.

Keywords: fortified flour, wheat, *Tribolium castaneum*, amylases, proteases

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Introduction

The fortification of wheat flour is being practiced in many countries including Pakistan to overcome nutritional deficiencies. Human beings as well as other animals have shown biochemical adaptations when fed on fortified wheat flour. Lysine fortification of wheat flour has attained positive nutritional status of cereal eating families of Pakistan.[1] Experiments on rats showed higher absorption of calcium, iron and zinc when fed on fortified flour than on unfortified flour.[2] There is currently no convincing evidence that demonstrate a clear relationship between iron stores and clinical disease. Attempts by others to confirm the putative relationship between sizes of iron stores (based of serum ferritin concentrations) observed in Finland have yielded conflicting results.[3] Data from Chile showed that there has been an increase in the rate of colorectal cancer since 2000, when the government introduced a mandatory program of fortification of wheat flour with folic acid. A similar increase was reported in the United States and Canada in the late 1990s, after the introduction of folic-acid fortification there.[4] Iron deficiency and anemia may impair athletic performance, and iron supplements are commonly consumed by athletes. However, iron overload should be avoided because of the possible long-term adverse health effects.[5]

The red flour beetle, *Tribolium castaneum* (Hebrst) (Tenebrionidae: Coleoptera) is among those insects which deteriorates the quality and quantity of stored wheat throughout Pakistan.[6] *T. castaneum*, being primary pest of flour, has highest rate of population increase recorded for any stored product pest.[7] Different wheat varieties have shown resistance to *T. castaneum*, while using flours of these varieties in the experiments.[8] These varieties have been found biochemically different as well, and these biochemical variations can affect insects in many ways. Wheat enriched with vitamins and cations resulted in the change in development and population build up of *T. castaneum*,[9,10] however, no information on overload of iron on these biological parameters of the beetle is known, though insects need iron in trace amount.[11]

In order to evaluate short/long term effect of iron fortification for wheat flour, the present studies were conducted to determine change in the biology and enzymes systems of *T. castaneum* fed on two types of flours, i.e. (i) Commercially available Iron fortified flour (pre-mix) (ii) post-mix unfortified flour (post-mix).

Materials and Methods

The mass rearing and experiment on the biology of *T. castaneum* in fortified and unfortified flour were performed in the Laboratory of Insect Toxicology, Dept. of Agric. Entomology, and enzyme assays were done in Dept. of Chemistry and Biochemistry, University of Agriculture, Faisalabad.

Stock culture of *T. castaneum*

Eggs of *T. castaneum* for studying its biology were collected from stock culture which has been maintained in the laboratory for many years. Adults of *T. castaneum* were released in the glass jars (450 g) in equal number of males and females, these jars containing sterilized wheat and yeast were kept in an incubator at 28 ± 5 °C and 70% ± 2 r. h. After 4 days interval, the adults were removed from this flour and then added to another sterilized amount of wheat flour for egg laying. The old flour, containing eggs of *T. castaneum*, was sieved and eggs were thus collected and put in iron fortified flour to get adults which were further used in subsequent experiments.

Studies of biology of *T. castaneum* in premix commercially available flour

Commercially available iron fortified flour from two different local flour mills in Faisalabad, Pakistan, was obtained having concentration of Iron (sodium ferrous sulfate + EDTA) at 10 ppm in each. Savaira and Millat are brand names of the iron fortified flours from above sources. Sodium ferrous sulfate + EDTA was provided to the flour mills by Government of Pakistan under "Wheat Flour Fortification Project", Ministry of Health, Islamabad.

Eggs from *T. castaneum* culture were kept in Petri dishes over moist tissue paper of size of Petri dishes to obtain homogeneous population of the 1st instar larvae. These larvae were added in equal number to the four replicates of both fortified flours as well as to that of control (unfortified flour). Larval duration started from releasing of 1st instar up to pupal formation and pupal duration was up to emergence of adult. Total developmental time was larval + pupal duration.

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Studies of biology of *T. castaneum* in postmix flour

Wheat grains were ground to obtain fresh flour up to the particle size to be passed through mesh 60 sieve so that the eggs can be collected easily. Five jars measuring 450 g each were half filled with this ground flour. 12.813 mg, 25.625 mg, 51.25 mg and 76.875 mg of iron source (Sodium ferrous sulfate + EDTA) was weighed on an electronic balance and were mixed thoroughly with the help of a mixer to the appropriate quantity of flours in the jars to get 5 ppm, 10 ppm, 20 ppm and 30 ppm Iron concentrations. Jars with only flour (without sodium ferrous sulfate + EDTA) served as control. Larvae were added to the fortified and control flour as stated in the studies in premix flour.

Data were obtained on larval duration, pupal duration, total developmental time, fecundity, larval mortality and weight of fully grown larvae in successive two generations. First generation means development of adult from the eggs which were collected from iron fortified and unfortified flour. Second generation was adult stage obtained from eggs of first generation.

Enzyme assays

Final instar larvae (2 g) of *T. castaneum* from each treatment were immobilized on a cold tray and dissected to remove the whole midgut in cold insect saline (1.2 M NaCl, 0.5 M KCl, and 1 mM $CaCl_2$). Pooled midguts were homogenized in respective buffer (10 mM potassium phosphate buffer), used for enzyme estimation. The homogenate was centrifuged for 15 minutes at 14,000 rpm at 4 °C. The supernatant was separated and used for enzymes estimation. Proteases activity was determined by the casein digestion assay described by Drapeau et al.[12] Modified method reported by Varavinit et al[13] with soluble potato starch solution as substrate was used for amylase analysis. Protein in enzyme solution was determined by Bradford.[14]

Statistical analysis

Data on larval mortality and weight and enzyme assays were subjected to one way analysis of variance (ANOVA) using Statistica Software (Statistica, 1997). Means in each case were compared using the Tukey test at a significance level of 0.05 for all statistical tests. Standard error of means was calculated from Minitab (ver. 11).

Results and Discussion

The larval and pupal duration, total development time, fecundity (number of eggs laid per female) had non-significant difference ($P > 0.05$) in premix and postmix experiments in first and second generations (data not shown). Larval mortality between two generations had non-significant difference and, hence, were pooled and compared with control.

The larval mortality increased significantly in two sources of premix flours when compared with control. Larval weights of first generation in two sources of premix flours was significantly less than control, however, this difference was non-significant in second generation. Larval weight between two sources of premix flour had non significant difference between one another in 1st generation (Table 1).

Post mix flour showed different trend on larval mortality. The mortality in highest concentration (30 ppm) was significantly different from other concentrations and control. Larval weights in first generation were reduced in different concentrations of iron in post mix flour but non significant difference was found between control and the lowest concentration (5 ppm). The weights of second generation larvae in various concentrations had non significant difference among them (Table 2).

Tables 3 and 4 shows the gut enzymes, amylase and proteases, of larvae fed on different sources of premix flours and concentrations of postmix flour.

Table 1. Comparison of mean larval mortality (%) and weight (μg) in premix flour.

Treatments	% larval mortality	Larval weight (mg)	
		1st generation	2nd generation
Savaira	30.20 ± 2.32 a	1.92 ± 0.01 b	1.70 ± 0.02 ns
Millat	22.80 ± 0.97 b	2.12 ± 0.05 b	1.96 ± 0.01
Control	17.00 ± 1.22 c	2.48 ± 0.01 a	1.84 ± 0.01

Values are Means ± SE. Means sharing same letters in a column are not significantly different at $P < 0.05$.

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Table 2. Comparison of mean larval weight and mortality (%) in postmix flour.

Treatments	% larval mortality	Larval weight (mg)	
		1st generation	2nd generation
30 ppm	37.65 ± 0.70 a	1.13 ± 0.00 d	2.07 ± 0.18 ns
20 ppm	29.33 ± 0.18 b	1.00 ± 0.00 c	2.00 ± 0.20
10 ppm	30.34 ± 0.45 b	1.37 ± 0.00 b	2.33 ± 0.07
5 ppm	30.07 ± 0.24 b	1.44 ± 0.30 a	2.30 ± 0.15
Control	29.42 ± 0.48 b	1.44 ± 0.34 a	2.22 ± 0.10

Values are Means ± SE. Means sharing same letters in a column are not significantly different at $P < 0.05$.

Amylase activity increased on premix flours from two different sources and protease activities decreased correspondingly than control treatments (Table 3).

Amylases activity increased in 30 ppm post mix flour and had non significant difference with 20 and 10 ppm concentrations. Control had the lowest amylases activity and had non significant difference with 5 ppm concentration of post mix flour. Proteases activity was less in all concentrations than control which in turn had significant difference among them. Lowest proteases activity was found in 10 ppm concentration of post mix flour (Table 4).

The effect of iron enriched wheat flour on biology and enzymes has not been studied earlier, however, adult fecundity of the flour beetles, *T. confusum* and *T. castaneum*, measured on twelve different diets: top patent flour; top patent flour enriched with riboflavin, thiamine and niacin, singly or in combinations; top patent flour mixed with bran, germ or both; and whole wheat flour was improved on these diets but effect on larval development time was non-significant.[9] Iron fortified wheat flour in the present study had no effect on fecundity, nevertheless, resembles the above results as non-significant difference in larval duration was found. The results of larval weights match with Medici and Taylor[15] in which high iron contents in the diet resulted in decrease of larval weight of *T. confusum* as compared to low iron content diet.

In another study addition of sodium chloride, sodium bicarbonate, magnesium sulphate, iron phosphate, sodium bromide, potassium bromide, and citric acid reduced insect population growth even at low concentrations of 10 ppm, or 0.01 per cent. However, magnesium chloride exerted beneficial effects and resulted in enhanced population growth of test insects. In contrast to a non treated control group, the population of *T. castaneum* increased in proportion to the concentration of magnesium chloride in the diet.[10]

Inhibition of insect growth and delayed breeding brought about by the addition of an excess of some mineral ions to their diet might have been due to an ionic imbalance in the insect system and consequent physiological disturbances. Replacement of mineral ions in another form of same ion in the diet has resulted in asynchronous development of gypsy moth *Lymantria dispar* (L.).[16] Insects have the potential for an iron overload, and thus metal ions that might be toxic need to be sequestered. Ferritin is one example of iron sequestration protein.[17] Proteases in *T. castaneum*

Table 3. Comparison of mean amylases and proteases activity between premix flours and control.

Treatments	Enzyme activities	
	Amylase (ng mg protein^{-1})	Proteases (IU mg protein^{-1})
Savaira	0.58 ± 0.02 a	0.62 ± 0.06 b
Millat	0.54 ± 0.05 a	0.67 ± 0.00 b
Control	0.42 ± 0.04 b	0.92 ± 0.00 a

Values sharing same letters in a column are non significant at $P < 0.05$.

Table 4. Comparison of mean Amylases and proteases activity between postmix flours and control.

Treatments	Enzyme activities	
	Amylase (ng mg protein^{-1})	Proteases (IU mg protein^{-1})
30 ppm	0.66 ± 0.06 a	0.34 ± 0.03 e
20 ppm	0.58 ± 0.03 ab	0.52 ± 0.01 d
10 ppm	0.57 ± 0.05 ab	0.71 ± 0.04 c
5 ppm	0.48 ± 0.06 bc	0.91 ± 0.03 b
Control	0.41 ± 0.08 c	0.99 ± 0.02 a

Values sharing same letters in a column are non significant at $P < 0.05$.

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might be involved in this process and were thus found in low activity levels in iron fortified flours as compared to control. Further studies on these aspects will help to improve the knowledge of effect of fortified flour on insect physiology.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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