Polyphenol and Phytic Acid Contents of Cereal Grains As Affected by Insect Infestation

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Polyphenol and phytic acid contents of wheat, maize and sorghum varied significantly and increased progressively with the levels of grain infestation (25, 50 and 75%) caused by *Trogoderma granarium* Everts and *Rhizopertha dominica* Fabricius separately and mixed population. Higher insect infestation (50 and 75%) of wheat and sorghum led to substantial increase (by weight) in polyphenol and phytic acid due to the selective feeding activities of the beetles. Feeding of both insect species on maize caused proportional increase in polyphenol but decrease in phytic acid due to variation in the distribution of these compounds in seed components and also selective feeding habit of insects. Storage of uninfested cereal grains for up to 4 months did not cause appreciable change in the levels of these antinutrients.

Keywords: *Antinutrients; cereals; insect infestation; storage*

INTRODUCTION

Cereal grains including wheat, maize, and sorghum are less expensive sources of carbohydrates, proteins, β-vitamins, and certain minerals, but insect pests cause substantial reduction in the levels of nutrients during storage in Asia and Africa (Salunkhe *et al.*, 1985). Among insect pests, *Trogoderma granarium* (a germ eater) and *Rhizopertha dominica* (an endosperm eater) are most serious under tropical and subtropical storage conditions (Atwal, 1976; Salunkhe *et al.*, 1985). In earlier studies, these two insect species have been found to cause significant reductions in available carbohydrates (Jood *et al.*, 1993) and bioavailability of proteins in wheat and maize grains (Jood and Kapoor, 1992; Jood *et al.*, 1992). Infested grains also contained higher amounts of nonprotein nitrogen and uric acid (Jood and Kapoor, 1993).

Information on the effect of insect infestation on the antinutrients of the cereal grains is scanty. Among these, polyphenol and phytic acid are potential antinutrients in cereals affecting bioavailability of major nutrients (Salunkhe *et al.*, 1981; Reddy *et al.*, 1982; Thompson and Yoon, 1984). This paper reports the effect of various grain infestation levels (25, 50, and 75%) caused by *T. granarium* and *R. dominica* separate and mixed populations on the levels of polyphenol and phytic acid in wheat, maize, and sorghum grains.

MATERIALS AND METHODS

Mass cultures of two insect species, *T. granarium* and *R. dominica*, were maintained in the ambient laboratory temperature (28–39 °C) and humidity (60–90%) conditions. The grains of commonly consumed cultivars of three cereals, WH-147 (wheat), Compositive Vijay (maize), and JS-20 (sorghum), apparently free from insect infestation, were procured and further subjected to aluminum phosphide fumigation to eliminate any untraced insect population. After fumigation, such grains were put in 108 glass jars (20 × 15 cm), each containing 1.5 kg of grains. The jars were covered with muslin cloth with the help of elastic bands and placed in the laboratory for 10 days for conditioning of grains. On the 10th day, the moisture level of grains ranged from 10 to 11%, which is congenial for multiplication of both insect species (Pingale and Girish, 1967). The jars of each food grain were grouped into three sets.

In the first set of each food grain, 60 larvae of *T. granarium* per jar were released to obtain three levels of infestation (25, 50, and 75%) in three replicates. Larvae were used because of their high grain-damaging potential; the adults of *T. granarium* do not feed on grains and as such are harmless. In the second set, 60 adults of *R. dominica* were released, and in the third set a mixed population of both species (30 larvae of *T. granarium* plus 30 adults of *R. dominica*) was released to achieve three infestation levels in three replications. Adults of *R. dominica* are more harmful than larvae. Hence, adults were preferred over larvae to create infestations on grains. In addition, in each set, controls (jars without insects) were also kept simultaneously to study the effect of storage periods. To achieve desired infestation levels, grain samples (500 grains/jar) were inspected twice a week after the release of insects, and the grains that showed signs of insect damage were considered to be infested and the same were used to calculate infestation percentage. The observation frequency was increased at later stages to ensure 25, 50, and 75% grain infestation, respectively, at ambient laboratory conditions. On the day that the desired level of infestation was actually achieved, grains were immediately disinfested with aluminum phosphide fumigation to prevent further damage and also to kill the insect population. At the end of each experimental storage period, control grains were not subjected to fumigation as they were free from insect infestation due to initial exposure to aluminum phosphide. After fumigation at each infestation level, grains were cleaned by passage through a 4 mesh sieve to separate insects and frass (dust). Control grains were free from frass and insect fragments during sieving. Grains after cleaning were powdered in a Cyclotec mill to pass through a 60 mesh sieve and then stored in airtight polyethylene bottles for further chemical analysis.

Polyphenol and Phytic Acid Estimation. Polyphenolic compounds were extracted from the defatted samples by refluxing in 1% HCl in methanol (Singh and Jambunathan, 1981) and estimated as tannic acid equivalents according to the Folin–Denis procedure (Swain and Hills, 1959). Phytic acid was determined according to the procedure of Hang and Lantzsch (1983).

Statistical Analysis. The data were subjected to analysis of variance (ANOVA) in a completely randomized design to

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Table 1. Effect of Storage on Polyphenol and Phytic Acid Contents (Milligrams per 100 g, on Dry Matter Basis)[a]

cereal	storage period (months)	polyphenol	phytic acid
wheat	0	482 ± 4.2	889 ± 4.2
	1	482 ± 3.8	889 ± 3.6
	2	484 ± 6.2	892 ± 3.5
	4	487 ± 5.4	896 ± 4.5
	mean	484	892
maize	0	635 ± 4.6	698 ± 6.0
	1	635 ± 4.2	698 ± 4.5
	2	638 ± 5.1	699 ± 4.2
	4	642 ± 6.6	699 ± 4.8
	mean	638	699
sorghum	0	829 ± 5.5	912 ± 5.2
	1	829 ± 5.3	912 ± 6.1
	2	830 ± 6.8	914 ± 4.2
	4	833 ± 4.5	916 ± 4.8
	mean	830	914
cereal	SE(m)	60	74
	CD ($P < 0.05$)	178	212
storage period	SE(m)	80	75
	CD ($P < 0.05$)	NS	NS
cereal × storage period	SE(M)	150	145
	CD ($P < 0.05$)	NS	NS

[a] Values are means ± SD of six independent determinations. CD denotes critical difference. Differences of two means between the storage period/cereal exceeding this level are significant.

determine the significant differences among treatments (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

Effect of Storage on Polyphenol and Phytic Acid Contents. Amounts of polyphenol in fresh samples of wheat, maize, and sorghum grains were 482, 635, and 829 mg/100 g, respectively, whereas those of phytic acid were 889, 698, and 912 mg/100 g, respectively (Table 1). Sorghum had significantly ($P > 0.05$) higher amounts of these antinutrients as compared to wheat and maize. Storage of the three cereal grains did not cause any noticeable change in the levels of antinutrients for up to 4 months. Polyphenol varied from 482 to 487, from 635 to 642, and from 829 to 833 mg/100 g in wheat, maize, and soghum, respectively. During the same period (0–4 months of storage), corresponding changes in phytic acid were 889 to 896, 698 to 699, and 912 to 916 mg/100 g. This indicated that there is no adverse effect of storage on polyphenol and phytic acid contents, unlike vitamins and starch, which decreased significantly during the same period (Jood, 1990; Jood *et al.*, 1993).

Effect of Insect Infestation on Polyphenol. Insect infestation caused a substantial increase in proportion (by weight) of polyphenol content in all three cereals (Table 2). *R. dominica* caused a significant ($P < 0.05$) increase in polyphenol content of wheat (8–22%), maize (7–17%), and sorghum (9–18%) at 50 and 75% infestation levels, while *T. granarium* caused a significant increase in wheat (8%), maize (6%), and sorghum (8%) only at the 75% infestation level. This variation may be attributed to selective feeding activities of the two insect species: *R. dominica* is primarily an endosperm feeder (internal feeder) and avoids feeding on the testa layer of seed lying between pericarp and aleurone layers. The testa layer has been reported to contain polyphenol in sorghum, which imparts resistance against the attack of birds, molds, and pests (Salunke *et al.*, 1985). As *R. dominica* feeds exclusively on endosperm and spares the testa, this selective feeding habit of this insect has led to a proportional increase in polyphenol content in the infested grains. On the other hand, *T. granarium* is primarily a germ feeder (external feeder) but also causes slight damage to the bran (consisting of pericarp and testa layer) portion of the seed (Atwal, 1976). This nature of feeding might have hindered the faster rate of accumulation of polyphenol in the three cereals as compared to *R. dominica*. Distribution of polyphenol in wheat and maize seeds is poorly understood, but in the present study both cereals responded to insect attack in a mannar similar to that of sorghum. The mixed feeding of both insect species produced

Table 2. Effect of Insect Infestation on Polyphenol Contents (Milligrams per 100 g, on Dry Matter Basis)[a] of Wheat, Maize, and Sorghum Grains

insect species	infestation level (%)	wheat	maize	sorghum
T. granarium	25	490 ± 5.2 (2)[b]	645 ± 3.1 (2)	839 ± 3.0 (1)
	50	500 ± 4.3 (4)	663 ± 3.3 (3)	869 ± 4.2 (5)
	75	522 ± 4.6 (8)	695 ± 3.6 (6)	894 ± 5.0 (8)
	mean	504	668	867
R. dominica	25	499 ± 3.8 (4)	663 ± 4.0 (4)	862 ± 5.2 (4)
	50	519 ± 3.9 (8)	678 ± 3.2 (7)	900 ± 6.2 (9)
	75	590 ± 4.5 (22)	740 ± 2.9 (17)	974 ± 6.6 (18)
	mean	536	694	912
T. granarium + *R. dominica*	25	495 ± 3.6 (3)	655 ± 2.5 (3)	862 ± 5.2 (4)
	50	510 ± 3.4 (8)	678 ± 2.0 (7)	900 ± 6.8 (9)
	75	575 ± 5.0 (19)	730 ± 4.5 (15)	970 ± 4.8 (17)
	mean	530	689	911
control	0	482 ± 3.2	635 ± 5.0	829 ± 4.2
insect species	SE(m)	8.50	12.20	15.25
	CD ($P < 0.05$)	25.25	NS	45.25
infestation level	SE(m)	10.25	15.30	16.50
	CD ($P < 0.05$)	30.75	45.82	49.50
insect species × infestation level	SE(m)	19.75	29.50	30.50
	CD ($P < 0.05$)	59.20	NS	91.25

[a] Values are means ± SD of six independent determinations. CD denotes critical difference. Differences of two means between the insect species/infestation level exceeding this level are significant. [b] Figures in parentheses are percent increase over control.

Table 3. Effect of Insect Infestation on Phytic Acid Contents (Milligrams per 100 g, on Dry Matter Basis)[a]

insect species	infestation level (%)	wheat	maize	sorghum
T. granarium	25	916 ± 3.5(+3)[b]	626 ± 6.2(−10)	925 ± 5.0(+1)
	50	925 ± 9.2(+4)	510 ± 6.0(−27)	934 ± 4.2(+2)
	75	968 ± 4.6(+9)	400 ± 4.2(−43)	954 ± 5.6(+5)
	mean	936	512	934
R. dominica	25	925 ± 5.0(+4)	683 ± 3.9(−2)	954 ± 6.6(+5)
	50	986 ± 6.2(+11)	672 ± 3.8(−4)	994 ± 3.2(+9)
	75	1120 ± 7.2(+26)	646 ± 4.2(−7)	1094 ± 5.2(+20)
	mean	1010	667	1014
T. granarium + *R. dominica*	25	910 ± 7.0(+2)	672 ± 5.0(−4)	933 ± 6.2(+2)
	50	925 ± 4.2(+4)	626 ± 4.2(−10)	954 ± 5.3(+5)
	75	986 ± 5.6(+11)	500 ± 5.3(−28)	994 ± 4.9(+9)
	mean	940	599	960
control	0	889 ± 5.9	698 ± 4.0	912 ± 6.5
insect species	SE(m)	30.50	35.22	22.45
	CD ($P < 0.05$)	91.25	105.62	66.32
infestation level	SE(m)	31.25	32.12	25.36
	CD ($P < 0.05$)	93.75	96.35	76.00
insect species × infestation level	SE(m)	55.23	60.25	50.12
	CD ($P < 0.05$)	165.00	180.50	NS

[a] Values are mean ± SD of six independent determinations. CD denotes critical difference. Differences of two means between the insect species/infestation level exceeding this level are significant. [b] Figures in parentheses are percent change over control.

intermediate results. However, *R. dominica* appeared to have a greater effect on the increase in the proportion of polyphenol in the three cereals than *T. granarium* in mixed feeding because the levels were closer to those produced by this species when allowed to feed alone.

Effect of Insect Infestation of Phytic Acid. Infestation of *T. granarium* caused a slight increase (3–9%) in the proportion of phytic acid of wheat grains (Table 3). On the other hand, *R. dominica* and the mixture of *T. granarium* and *R. dominica* feeding resulted in a significant increase of phytic acid. In wheat, about 70% of the phophorus is present in the form of phytic acid, which is mainly concentrated in the bran/aleurone layer (4.12%) and germ portion (3.91%) of the seed, whereas the endosperm contains only 0.004% phytic acid (Reddy *et al.*, 1982). This distribution pattern clearly exposed phytic acid to the attack of the germ feeder *T. granarium*. This mode of feeding is responsible for a marginal increase in the amount of phytic acid in wheat grains. *R. dominica* feeds extensively on the major internal component (endosperm) of the seed, thereby leading to a significant increase (11–26%) in the proportion of phytic acid at higher infestation levels (50 and 75%).

It is interesting to note that both species of insects caused reductions in phytic acid content of maize grains, though significant ($P < 0.05$) losses were caused by *T. granarium* (10–43%) and the mixture of *T. granarium* and *R. dominica* (4–28%). In maize, the distribution of phytic acid has been reported as 0.04, 6.39, and 0.07% in endosperm, germ, and bran, respectively (Reddy *et al.*, 1982). The higher level of phytic acid accumulated in the germ portion is likely to be reduced by the attack of germ feeders. *T. granarium*, being a germ feeder, caused marked reduction in the level of phytic acid. The marginal decrease in phytic acid content owing to *R. dominica* feeding might be due to loss of endosperm, having some amount of phytic acid. The distribution of phytic acid in sorghum grains is poorly understood. *T. granarium* and the mixture of *T. granarium* plus *R. dominica* did not cause any appreciable change in phytic acid content. However, *R. dominica* alone caused a significant proportional increase (9–20%) in phytic acid at 50 and 75% levels of infestation.

There was significant interaction between insect species and infestation levels with regard to polyphenol in wheat and sorghum and phytic acid in wheat and maize. It may be inferred from the present study that insect infestation in cereals leads to higher levels of antinutrients, thereby further affecting the bioavailability of nutrients adversely.

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