Effect of Wet Harvesting on Biodeterioration of Rice

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ABSTRACT

Two varieties of rice, Ratna and Annapurna, were subjected to four processing conditions after high moisture harvest. Leaving the harvested crop in windrows on the bund (embankment of the plot) and then stacking it in the open increased exposure to rain and resulted in deterioration of grain. Delayed and improper processing associated with rain resulted in decreased head rice yield, discoloration of grains, infection by fungi, and production of mycotoxin. Maximum grain deterioration resulted when the crop was piled into a stack; this lowered head rice yield and led to production of mycotoxin. Under identical processing conditions, Ratna tended to perform better than Annapurna in milling, head rice yield, and resistance to infection by fungi. Delay in drying a wet-season crop resulted in mold formation, increase in temperature, and even germination leading to biodeterioration. Immediate oven or sun drying seems to be the best way to prevent deterioration of the rice crop.

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The postproduction system is a vital component of the rice industry, and proper harvesting, threshing, and drying are integral parts of the system. A wet-season crop, harvested with approximately 18–25% grain moisture, is difficult to handle. Delay or improper drying of grain causes heat burns or heat discoloration (yellowing of kernels), and fermentation results in brownish discoloration. Improper drying, heavy dew, or drizzle induces fissuring; this results in low milling recoveries, losses caused by infestation by fungi, and contamination with mycotoxin (de Padua 1979).

Boller and Schroeder (1973) detected small quantities of aflatoxin B_1 in stored rice caused by growth of *Aspergillus parasiticus*. Karki et al (1979) obtained aflatoxin B_1 (15 ppb) from 12 rice samples of Nepal. Such mycotoxins as aflatoxin caused by *A. flavus*, zearalenone caused by *Fusarium moniliforme*, and curvularin-like mycotoxin caused by *Curvularia lunata* have been reported in stored and freshly harvested rice grains in India (Gangopadhyay and Chakrabarti 1981, 1982).

We therefore investigated the effect of various postharvest practices (threshing and drying) on milling and nutritional qualities of rice to identify situations that cause infestation by fungi resulting in production of mycotoxins.

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MATERIALS AND METHODS

Two varieties of rice, Ratna and Annapurna, were harvested from the farm of the Central Rice Research Institute, in Cuttack, India, during June 1981. Harvesting, threshing, and drying were as follows: In treatment T_1, the crop was harvested wet and immediately threshed; the grain was dried in sun (13–14% moisture). In treatment T_2, the crop was harvested wet, left in rows on the bund (earthen embankment of the plot) for 7 days, and then threshed. Grains were dried as in T_1. In treatment T_3, the crop was harvested wet, left in rows on the bund for 7 days, bundled and stacked in the open for 21 days, and then threshed. Grain was dried as in T_1. In treatment T_4, the crop was harvested wet and immediately threshed. Grain was dried mechanically in an oven at $55 \pm 5°$ C.

The crop was harvested in three replicates from 0.02 ha (200 m^2) plots at a moisture level of $18.0 \pm 0.14\%$ for Ratna and $24.0 \pm 0.29\%$ for Annapurna. In treatment T_3 the harvested crop (rice stalk with grains attached) was piled into a stack (diameter 150 cm and height 80–85 cm). The stack was kept in the open on a cement threshing floor where the temperature ranged from 25.7 to 33.9° C. The temperature inside the stack was 30–38° C due to generation of heat. Rainfall ranged from 0 to 11.4 mm (average 2.68 mm) during the period of stacking. The stacks and grains were covered at night with tarpaulin, except in treatment T_4, to avoid contact with dew, and were milled six months from the the date of final drying.

A 200-g dried sample (13–14% moisture) was hulled in a laboratory Satake Rice Machine (dehusker) Type THU. After a 5.0–5.5% polish, head rice was prepared in a laboratory Satake

Grain Testing Mill Type TM-5. The head rice and brokens were separated in a Satake Test Rice Grader Type TRG 05A. Samples were weighed on an August Sauter balance to an accuracy of 50 mg.

Nitrogen was determined by micro-Kjeldahl method (AOAC 1950) on brown and polished rice; mean values were obtained from three replications and protein content was calculated (N × 5.95).

Fungal Colonies

Rice kernels from all treatments were surface sterilized in 1% NaOCl for 1 min. The grains were placed on three types of agar media: 1) coconut agar (200 ml of fresh coconut extract, 600 ml of demineralized water, and 16 g of agar), 2) yeast agar (15 g tryptone, 10 g yeast extract, 0.5 g ferric citrate and 15 g agar in 1 L demineralized water), and 3) malt-salt agar (20 g malt extract, 75 g NaCl and 18 g agar in 1 L demineralized water). From each treatment, 100 grains were plated (10 grains per plate in 10 replications). Fungal colonies for all samples, counted with a colony counter a week after incubation, were identified by the morphological characters of the taxons (Ainsworth et al 1971).

Aflatoxin Extraction

Polished rice (50 g) was powdered and added to 100 ml of a mixture of methanol, chloroform, and water (75:10:15, v/v). The mixture was blended at high speed for 2 min and then centrifuged at 1,500 rpm for 1–3 min. The supernatant was retained. The residue was extracted again with a mixture of methanol, chloroform, and water (75:10:15, v/v) shaken vigorously for 1 min. Eighty milliliters of 20% ammonium sulphate solution and 40 ml of hexane were added to 40 ml of the methanol extract, and the mixture was shaken thoroughly in a separating funnel. The lower proteinaceous gummy material was discarded, and 6 ml of methylene chloride was added to the aqueous phase. The lower methylene chloride layer was collected in a glass vial, and extraction with methylene chloride process was repeated three times. One-half milliliter of benzene and acetonitrile (98:2, v/v) was added to the glass vial, and this solution was passed through an activated charcoal column following the method of Seitz and Mohr (1977). The colorless extract was evaporated to dryness on a water bath at 40° C. The dried granular material was dissolved in 10 ml of demineralized water, and a 2-ml aliquot solution was examined in an ultraviolet spectrophotometer; two peaks were observed between 254 and 350 nm. Thin-layer chromatography was conducted on an activated silica gel plate using chloroform and acetone (88:12, v/v) as a solvent. R_f values were determined by observing the spots under an ultraviolet lamp (280–350 nm range), and an aflatoxin B_1 standard was used for comparison with unknowns. Bioassay followed the method of Gangopadhyay and Chakrabarti (1982) using Ratna grains from T_3.

RESULTS AND DISCUSSION

After harvest, rain before threshing wetted the grain in treatment T_2 and the stack in T_3. Discoloration (excessive browning or blackening) of the grains was especially prominent in treatment T_3 for both Ratna and Annapurna. Treatment T_3 rendered the grain of Annapurna unfit for milling but had less effect on grain from Ratna. In treatments T_2 and T_3, the grain of both varieties germinated on the bund and during stacking. Germination was greater in treatment T_3 than in T_2 for both varieties. Delay in handling resulted in mold formation, increase in temperature by 4–5° C, and germination.

Milling

Hulling, milling, and head rice yields, and brokens were calculated on three replications. The hulling yield of Ratna varied within a narrow range, from 76.4 ± 0.25% in T_1 to 75.1 ± 0.41% and 75.1 ± 1.47% in T_2 and T_4, respectively, but was comparatively low in T_3 at 71.3 ± 1.13% (Table I). The trend was similar in Annapurna with yields of 73.7 ± 0.37% in T_1, 72.3 ± 0.76%, and 73.3 ± 0.39% in T_2 and T_4, respectively, and complete spoilage in T_3.

The milling yield of Ratna was highest (71.9 ± 0.7%) in T_1 and lowest (66.3 ± 0.82%) in T_3, and treatments T_4 and T_2 yielded 71.1 ± 0.35% and 70.1 ± 0.17%, respectively. A similar trend was observed in Annapurna, which resulted in milling yields of 70.3 ± 0.36%, 70.0 ± 0.29%, and 67.3 ± 0.22% for treatments T_1, T_4, and T_2, respectively, and complete spoilage in T_3. Thus, for both varieties treatment T_3 had a detrimental effect, and treatment T_2 resulted in lower milling yield of Annapurna.

The head rice yield of Ratna was highest (62.0 ± 0.93%) in treatment T_1 and lowest (38.2 ± 0.2%) in T_3. Treatments T_4 and T_2 gave 60.9 ± 0.38% and 49.8 ± 0.64% head rice yield, respectively. A similar trend was observed in Annapurna with 47.2 ± 0.44% head rice yield in T_1 and complete spoilage of grain in T_3. Treatments T_4 and T_2 resulted in 44.8 ± 1.27% and 37.1 ± 0.66% head rice yield, respectively. For both varieties, the head rice yield was lower after oven drying (T_4) (60.9 ± 0.38% and 44.8 ± 1.27%) than after sun drying in T_1 (62.0 ± 0.93% and 47.2 ± 0.44%). Lower head rice yield may be caused by a fast-heating process in an ordinary oven. Lower head rice yield from sun drying than from shade drying (Govindaswami and Ghosh 1969) indicates the importance of slow drying.

Head rice yield from all treatments was higher for Ratna than Annapurna. Ratna was also superior to Annapurna in total milling yield. The higher milling and head rice yield of Ratna may be a genetic character. Breakage showed a reverse trend to head rice yield in each treatment. In Ratna breakage was maximum (28.1 ± 0.15%) in treatment T_3 followed by 20.3 ± 0.33%, 10.2 ± 0.06%, and 9.9 ± 0.55% in treatments T_2, T_4, and T_1, respectively. The trend was similar in Annapurna. Head rice yield was lower and breakage was higher in treatments T_3 and T_2 than in T_4 and T_1 for both varieties. Improper drying causes fissuring that results in broken kernels and lower milling yield (de Padua 1979), and treatments T_3 and T_2 may have reflected improper drying.

The results indicate that the usual practice of farmers in India—harvesting at high moisture content, leaving the crop on the bund, stacking, and then threshing—can lead to severe loss in milling and head rice yield, and increased breakage.

Protein

Protein content of brown and polished rice did not vary among

TABLE I
Effect of Processing Conditions on Rice Yield

Treatment	Yield[a] (% of Rough)							
	Ratna				Annapurna			
	Hulled	Milled	Head rice	Broken	Hulled	Milled	Head rice	Broken
T_1	76.4 ± 0.25	71.9 ± 0.70	62.0 ± 0.93	9.9 ± 0.55	73.7 ± 0.37	70.3 ± 0.36	47.2 ± 0.44	23.1 ± 0.79
T_2	75.1 ± 0.41	70.1 ± 0.17	49.8 ± 0.64	20.3 ± 0.33	72.3 ± 0.76	67.3 ± 0.22	37.1 ± 0.66	30.2 ± 0.52
T_3	71.3 ± 1.13	66.3 ± 0.82	38.2 ± 0.20	28.1 ± 0.15	...[b]	...	...	...
T_4	75.1 ± 1.47	71.1 ± 0.35	60.9 ± 0.38	10.2 ± 0.06	73.3 ± 0.39	70.0 ± 0.29	44.8 ± 1.27	25.2 ± 1.22

[a] Mean and standard deviation of three replicates.
[b] Not determined.

TABLE II
Influence of Processing on Mycotoxin-Producing Fungi and Mycotoxin in Rice

Treatment/Fungi	Number of Colonies/100 Polished Grains								Mycotoxin Content (ppb)	
	Coconut		Yeast Extract Tryptone		Malt Salt		Total			
	Ratna	Annapurna	Ratna	Annapurna	Ratna	Annapurna	Ratna	Annapurna	Ratna	Annapurna
T_1										
Aspergillus parasiticus	20	35	15	20	30	36	65	91		
Penicillium islandicum	10	12	12	8	0	0	22	20	2	3
A. flavus	5	0	5	9	3	3	13	12		
Total	35	47	32	37	33	39	100	123		
T_2										
A. parasitcus	45	40	40	45	56	39	141	124		
P. islandicum	15	16	12	18	10	21	37	55	15	16
A. flavus	9	20	15	13	12	20	36	53		
Total	69	76	67	76	78	80	214	232		
T_3										
A. parasiticus	65	...[b]	64	...	75	...	204	...		
P. islandicum	20	...	20	...	12	...	52	...	28	...
A. flavus	15	...	10	...	12	...	37	...		
Total	100	...	94	...	99	...	293	...		
T_4										
A. parasiticus	3	5	5	0	3	6	11	11		
P. islandicum	0	0	0	5	0	0	0	5	nd[c]	nd
A. flavus	2	0	5	3	3	3	10	6		
Total	5	5	10	8	6	9	21	22		

[a] Aflatoxin B_1 equivalent.
[b] Not determined.
[c] Not detectable.

the treatments except that in treatment T_3 Ratna grain showed a slightly higher protein value of 8.8 ± 0.03% compared with 7.8 ± 0.03%, 7.9 ± 0.12%, and 7.9 ± 0.06% in treatments T_1, T_2, and T_4, respectively. This may be associated with infection of the grain by fungus. The protein content of Annapurna brown rice was 6.7 ± 0.03%, 6.8 ± 0.06%, and 6.8 ± 0.03% in T_1, T_2, and T_4, respectively; the corresponding values for polished rice were 5.8 ± 0.03%, 6.1 ± 0.06%, and 6.0 ± 0.06%.

The protein content of brown and polished rice of the two varieties differed considerably in all treatments and was higher in Ratna than in Annapurna. Protein data indicate a possibility of positive association between protein content and fungus infestation leading to mycotoxin production. The protein content of brown and polished rice from treatments T_2, T_3, and T_4 is higher than from treatment T_1. This is expected, as protein content of rice is governed by various factors. Protein content of polished rice is less than that of brown rice for both varieties in all treatments, confirming earlier findings of Sahay and Hota (*unpublished*) and indicating that the protein status is not affected by harvesting and threshing practices.

Fungal Growth and Aflatoxin Content

Growth of mycotoxin-producing fungi and aflatoxin content as influenced by the treatments demonstrated maximum fungal colonies (293) in T_3 followed by 214 in T_2, 100 in T_1, and 21 in T_4 in Ratna (Table II). The trend in Annapurna was similar, with 232, 123, and 22 colonies in T_2, T_1, and T_4, respectively. Because Annapurna grains were completely spoiled in T_3, fungal counts could not be obtained, but the number of colonies in T_3 must have been maximum. Higher infection rates in Annapurna than in Ratna support the presumption that the trend in T_3 of Annapurna could have been similar to that in T_3 in Ratna. After soaking (before extraction of aflatoxin) Ratna grain from T_3 showed splitting of husk and characteristic fluorescence of grains infested by aflatoxin-producing fungi.

Presence of *A. parasiticus*, *Penicillium islandicum*, and *A. flavus* showed similar trends in total number of colonies, with maxima in T_3 and T_2 and minimum in T_4. Least development was to be expected in T_4 because grains were dried in the oven. In all treatments growth of *A. parasiticus* was greater than that of *P. islandicum* or *A. flavus*. *A. parasiticus* may play a dominant role in the production of aflatoxin in rice; this is further supported by the fact that the growth of *A. parasiticus* was greater in Annapurna than in Ratna in all treatments except T_4.

The aflatoxin contents were 2, 15, and 28 ppb in T_1, T_2, and T_3 of Ratna and 3 and 16 ppb in T_1 and T_2 of Annapurna. These results show greatest infection in T_3 grains and higher susceptibility of Annapurna. The toxin was not detectable in grain of T_4 from either variety. Presence of aflatoxin in the grains of T_3 (Ratna) was confirmed by bioassay.

CONCLUSION

Our results suggest that freshly harvested wet-season crops should be immediately threshed and dried in the sun or dried mechanically. Crops can be left in rows on the bund for a few days only, as there is a danger of infection by fungi, leading to lower head rice yield and health hazards caused by aflatoxin contamination.

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