The impact of insect pests on aflatoxin contamination of stored wheat and maize

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Abstract

Insect pests, moisture content, *Aspergillus flavus* infection rate and aflatoxin contamination were investigated in insect-free samples (IFS) and insect-damaged samples (IDS) of wheat and maize collected from different localities of Bihar (India). The rice-weevil, *Sitophilus oryzae* L., was recorded as the most dominant pest of wheat as well as of maize under storage. Other common coleopterous pests of both the grains were the rust red flour beetle, *Tribolium castaneum* Herbst, the lesser grain borer, *Rhyzopertha dominica* F., and the khapra beetle, *Trogoderma granarium* Everts. Mean number of insects in infested samples of wheat was 315 for *S. oryzae* followed by *R. dominica* (230). In case of maize the mean incidence of *S. oryzae* was 359 followed by *Oryzaephilus surinamensis* (308), *S. cereallela* (231).

The moisture contents of IDS lots were higher than the IFS lots. Ten of 25 IDS lots had moisture content above 20% while all the 25 IFS lots contained below this level of moisture. In case of maize 20% IDS lots had moisture level above 25%. The mean incidence of *Aspergillus flavus* group fungi of wheat was 87% in IDS lots and 25% in IFS lots. In maize the infection rate of *A. flavus* was, however, 33 and 21% in above two lots, respectively. The species of *Penicillium* also dominated in IDS lots than IFS lots. Similarly the incidence and level of aflatoxins were considerably higher in IDS lots of wheat and maize than the undamaged ones. In insect-damaged groups 76% wheat samples and 77.5% maize samples were contaminated by aflatoxin, while only 8% of wheat and 4% of maize samples from insect-free group were aflatoxin positive. Toxigenic strains of *A. flavus* were also isolated from the insects *S. oryzae* and *T. castaneum*

Introduction

Wheat (*Triticum vulgare* L.) and maize (*Zea mays* L.) are among the important cereal crops of India and are consumed in various ways by almost the entire population of the country. Wheat is exclusively cultivated as a *rabi* (winter) crop, while maize is cultivated in all the three seasons viz., monsoon (*kharif*), winter (*rabi*) and summer. Harvested grains are stored by farmers for considerable periods in various types of storage structures, usually made of mud, bamboo strips, *Cajanus cajan* reeds, palm leaves or paddy straw, in hessian (gunny) or plastic sacks (Bilgrami and Sinha 1987; Sinha 1990; Ranjan et al. 1992). Earthenware containers (*kothi*) of different shapes and sizes are also used frequently to store grains. These traditional storage methods inevitably provide suitable conditions for the growth and metabolism of the insects, rodents, and microorganisms responsible for quality loss in stored grains.

A number of insect pests have been reported to be associated with stored wheat and maize and their byproducts, causing losses of food intended for human and animal consumption (Storey et al. 1982; Imura and Sinha 1984; Lal and Shrivastava 1985; Demianyk and Sinha 1987). The occurrence of stored grain insect pests is very much influenced by geographical and climatic conditions (Lal and Srivastava 1985; Sinha and Sinha 1990). Almost all species have remarkably high rates of multiplication and, within one season, may destroy 10-15% of the grain and contaminate the rest with undesirable odours and flavours.

In addition to direct damage to stored grains, insects also play an important role in the development and distribution of fungal inocula (Agrawal et al. 1957; Christensen and Hodson 1960). Interrelations among abiotic and biotic variables in stored grain ecosystems determine the rate of physical and biochemical deterioration of stored grains (van Bronswijk and Sinha 1971; Sinha 1973, 1984; Mills 1983).

The present work records the common insect pests associated with stored wheat and maize, and data on *Aspergillus flavus* infection and aflatoxin contamination in insect-damaged and insect-free samples of wheat and maize.

Materials and Methods

Grain

Wheat samples (500 g) were collected from different villages in three districts, Madhepura, Purnea and Saharsa, of North Bihar (India) during November 1987. Insect-damaged samples (IDS) and insect-free samples (IFS) were kept separately and 25 lots of each were taken out randomly. Maize samples (1 kg) stored for 4-12 months were collected from different localities of Bhagalpur district of Bihar during June-July 1988. Forty lots from IDS and 15 lots from IFS were taken out randomly.

Insects

The IDS collected during the survey were sieved using a standard set of sieves and the insects separated identified according to the key of Hinton (1945). The numbers of each species were recorded.

General methodology

Moisture contents (wet weight basis) were determined by oven drying 10 g subsamples of grain for 19 hours at 130°C in case of wheat (ASAE 1975) and for 72 hours at 103°C for maize samples (USDA 1971). To record fungal incidence, 100 surface-sterilised (2% NaOCl for 3 minutes) kernels from each sample were placed in Petri dishes containing moistened blotter and Potato Dextrose Agar (PDA) (1:1 ratio), and incubated at 30 ± 2°C for 4-7 days. The grains were then examined under a Nikon SMZ-10 stereoscopic microscope and the incidence of fungi, especially the *A. flavus* group (Raper and Fennell 1965), was recorded.

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The incidence of *A. flavus* was also recorded in *Sitophilus oryzae* and *Tribolium castaneum*. Adult insects of each species, collected from IDS, were washed in 70% alcohol for 1 minute followed by 2% NaOCl (2 minutes) and then thoroughly washed in sterile water (Sinha and Sinha 1990). The insects were then injured mechanically by sterilised forceps and placed aseptically in Petri dishes containing PDA. The plates were incubated at 30 ± 2^0C for further examination and isolation of the *A. flavus* group. The *A. flavus* group isolates obtained from grains and insects were tested in SMKY liquid culture (Diener and Davis 1966) for their ability to produce aflatoxins.

Aflatoxin assays

Aflatoxins were extracted from grain samples of wheat and maize following the method of Thomas et al. (1975) and assayed by thin layer chromatography using toluene: isoamyl alcohol: methanol (90:32:3, v/v/v) (Reddy et al. 1970), together with aflatoxin standards (Sigma Chemical Co.). Aflatoxin B_1 was chemically confirmed by aqueous sulphuric acid (25%) spray (Przybylski 1975), and or by treatment with trifluroacetic acid (Stack and Pohland 1975). Positive extracts were redeveloped on high performance thin layer chromatography plates. Aflatoxin B_1 was quantified spectrophotometrically (Nabney and Nesbitt 1975) and densitometrically (CAMAG TLC Scanner-II) using UV-lamp and filter K-400.

Results and Discussion

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), was dominant in the insect-damaged samples of wheat and maize (Table 1). Other coleopterous pests included the red flour beetle, *Tribolium castaneum* (Tenebrionidae), the lesser grain borer, *Rhyzopertha dominica* F. (Bostrichidae), and the khapra beetle *Trogoderma granarium* Everts (Dermestidae). In maize, the saw toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), and the grain moth, *Sitotroga cerealella* L. were also recorded as common pests. These insect species were found in various combinations. Of the 25 infested samples of wheat *S. oryzae* was present alone in 9 and *R. dominica* in 1. *S. oryzae* was found together with *T. castaneum* in 7 samples, and with *R. dominica* or *T. granarium* in 2 samples each. *R. dominica* together with *T. granarium* or *T. castaneum* was recorded in 2 and 1 sample, respectively. *S. oryzae* with other two pests, *T. castaneum* and *T. granarium*, was present in only one sample.

Table 1 also shows the distribution and numbers of different insect pests in the 25 and 15 IDS lots of wheat and maize, respectively. In the case of wheat, *S. oryzae* was recorded in 21 IDS lots, followed by *T. castaneum* (9), *R. dominica* (6), and *T. granarium* (5). The number of insects per sample also varied (Table 1). The mean insect numbers in the infested samples were highest for *S. oryzae* (315), followed by *R. dominica* (230), *T. granarium* (121), and *T. castaneum* (107) per 500 g sample. In the case of maize also, *S. oryzae* was the most prevalent insect, and was recorded in 31 of 40 IDS lots, followed by *T. castaneum* (22), *O. surinamensis* (15) and *S. cerealella* (12). Seven IDS lots had only one insect species, of which *S. oryzae* was present in 4, while *T. castaneum*, *T. granarium* and *O. surinamensis* were present in one sample each. The presence of 4 insect species together was recorded in 6 IDS lots, while 2 and 3 species together were recorded in 15 and 12 IDS lots, respectively. The mean insect numbers in the infested samples were highest for *S. oryzae* (359), followed by *O. surinamensis* (308), *S. cerealella* (231), *T. castaneum* (212), *T. granarium* (80), and *R. dominica* (74). The occurrence of different insect species and the intensity of damage depend upon the agro-climatic conditions, including the storage methods and the ambient temperature and humidity (Sharma and Bhalla 1964; Bains et al. 1976; Sinha and Sinha 1990).

At the time of harvest, grains are practically free from infestations by stored grain insect species. In Bihar, where wheat and maize grains are stored mainly in gunny sacks, kothi and other traditional storage structures (Prasad et al. 1986; Ranjan et al. 1992), insects reach the grain either through infested gunny sacks or storage containers already containing infested grain. The monsoon climate also favours the multiplication of these pests.

The moisture content of the wheat and maize grain was comparatively higher in IDS lots than the IFS lots (Table 2). None of the IDS lots had a moisture level below 14%, either in wheat or in maize. In 3 and 8 IDS lots of wheat and maize the

Table 1. Number of insects in stored wheat and maize samples

Insect species	No. of samples infested	Insect numbers by species			
		Total	Mean of positive samples ± SDM	% of total insects	Range
		Wheat			
S. oryzae	21 (3,14,4)[a]	6609	315 ± 197	69.2	58-746
T. castaneum	9 (6,3,0)	966	107 ± 74	10.1	32-262
R. dominica	6 (2,4,0)	1380	230 ± 153	14.4	32-456
T. granarium	5 (2,3,0)	603	121 ± 69	6.3	34-225
Total insect recorded (mean):		9558 (382)			
		Maize			
S. oryzae	31 (2,22,7)[a]	11133	359 ± 213 4	5.5	54-863
T. castaneum	22 (6,16,0)	4654	212 ± 126	19.0	88-460
O. surinamensis	15 (1,12,2)	4623	308 ± 162	18.9	47-609
R. dominica	8 (5,3,0)	589	74 ± 24	2.4	46-102
T. granarium	9 (7,2,0)	722	80 ± 23	3.0	54-109
S. cerealella	12 (2,10,0)	2767	231 ± 102	11.3	79-432
Total insect recorded (mean):		24488 (612)			

[a]Numbers in parentheses are the numbers of samples containing insects in the population ranges <100, 101 - 500, > 500, respectively.

levels recorded were above 25%. Higher moisture levels in the insect damaged lots might be due to the respiratory activity of grain and insects (Howe 1972; Mills 1983; Sinha 1984).

The mean per cent incidence of *A. flavus* in IDS lots of wheat was much higher (87%) than in IFS lots (25%). Table 3 shows the occurrence of fungi which had above 10% incidence in the maize. The *A. flavus* group and species of *Aspergillus* other than *A. flavus* were associated with all IDS and IFS lots. Species of *Penicillium* were observed in 38/40 IDS lots and 11/15 IFS lots. Species of *Alternaria, Curvularia, Cladosporium, Drechslera,* and *Fusarium,* and members of the order Mucorales were recorded in IFS lots only. The incidence of the *A. flavus* group was nearly 33% in IDS lots and 21% in IFS lots. In the stored grains, *Aspergillus* spp. and *Penicillium* spp. were dominant.

Fungal growth was observed over the whole body surface of insects, but particularly on legs, antennae and at joints. The incidence of the *A. flavus* group was 56 and 49% on *S. oryzae* obtained from wheat and maize, respectively, and 37% on *T. castaneum* obtained from maize.

Of 286 isolates of *A. flavus*, 138 were found to be toxigenic in liquid culture (Table 4). Of these 56 produced aflatoxin B_1 alone and 74 produced both aflatoxin B_1 and B_2. Five isolates from maize and three from *S. oryzae* also produced aflatoxin G_1 in liquid SMKY medium. Aflatoxin B_1 above 5 µg/mL was produced by 121 isolates, but only 11 isolates elaborated aflatoxin B_2 above this level.

Of the 50 samples (25 IDS + 25 IFS) of wheat analysed for aflatoxins, 21 were contaminated (Table 5), 19 of them from IDS lots. The level of aflatoxin B_1 in the IDS lots varied from 113 to 1248 µg/kg, while the two IFS lots contained 85 and 113 µg/kg. In the case of maize, of 55 samples (40 IDS + 15 IFS), 35 were contaminated, of which 31 were from IDS lots (Table 5). The level of aflatoxin B_1 in IDS lots varied from 96 to 1476 µg/kg. The maximum level of aflatoxin B_1 in IFS lots was 512 µg/kg. The higher aflatoxin contamination in insect-damaged samples was due to the high incidence of *A. flavus* and high moisture content in the samples. We have shown previously that insect-damaged samples are more vulnerable to *A. flavus* infection and aflatoxin contamination both in wheat (Sinha and Sinha 1991) and in pre-harvest maize (Sinha and Ranjan 1989).

Conclusion

The findings of this study reflect the association and interactions between insects and fungi on stored wheat and maize (Fig. 1). Insects cause damage to the grains in various ways. By feeding on the germ and endosperm of kernels, insects reduce the germinability and also open the way for the entrance of postharvest fungi. Insects carry large numbers of *A. flavus* spores which may spread contamination. Insect-infested stored grain is more susceptible to *A. flavus* infection and thus to aflatoxin contamination than is clean grain.

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Table 2. Moisture content of wheat and maize kernels

Range of moisture content (%)	Insect-damaged samples		Insect-free samples	
	Wheat (n=25)	Maize (n=40)	Wheat (n=25)	Maize (n=15)
12.0–14.0	–	–	4	2
14.1–16.0	1	3	8	5
16.1–18.0	4	2	12	1
18.1–20.0	10	9	1	5
20.1–25.0	7	18	–	2
> 25.0	3	8	–	–

Table 3. Fungi recorded from surface sterilized maize kernels

Fungi[a]	Insect-damaged samples (n=40)		Insect-free samples (n=15)	
	No. of positive samples	Mean incidence of positive samples ± SDM	No. of positive samples	Mean incidence of positive samples ± SDM
Aspergillus flavus	40	33.3 ± 14.9	15	21.2 ± 8.6
other *Aspergilli*	40	22.3 ± 10.5	15	22.5 ± 7.6
Pencillium spp.	38	16.8 ± 4.2	11	23.0 ± 4.9
Alternaria spp.	–	–	6	18.3 ± 4.4
Curvularia spp.	–	–	8	16.1 ± 5.1
Cladosporium spp.	–	–	4	14.5 ± 2.1
Chaetomium spp.	2	13.5 ± 2.1	6	15.7 ± 3.4
Drechslera spp.	–	–	5	13.6 ± 2.7
Fusarium spp.	–	–	4	19.5 ± 6.0
Mucorales	–	–	10	17.1 ± 7.6

[a]Positive samples were considered when the incidence of fungi exceeded 10%/sample.

Table 4. Screening of *Aspergillus flavus* isolates for aflatoxin production

Source of isolates	No. of isolates screened	Total aflatoxins	No. of isolates produced aflatoxin		
			B_1	B_1B_2	$B_1B_2G_1$
Wheat kernels	80	30(37%)	11	19	–
Maize kernels	75	33(44%)	9	19	5
S. oryzae (wheat)	45	21(47%)	8	13	–
S. oryzae (maize)	49	26(53%)	11	12	3
T. castaneum	37	28(76%)	17	11	–
Total	286	138(48%)	56	74	8

Concentration of aflatoxins produced in SMKY liquid culture (μg/mL)	Aflatoxin		
	B_1 (n=138)	B_2 (n=82)	G_1 (n=8)
Below 1	17	32	–
1–5	19	39	1
>5–10	59	7	3
>10–15	35	4	2
>15	8	–	2

Table 5. Aflatoxin contamination in stored wheat and maize samples

Aflatoxin B_1 (μg/kg)	Insect-damaged samples		Insect-free samples	
	Wheat (n=25)	Maize (n=40)	Wheat (n=25)	Maize (n=15)
Up to 100	–	3	1	2
101–250	8	6	1	1
251–500	4	11	–	–
501–1000	5	8	–	1
>1000	2	3	–	–
Total	19(76%)	31(77.5%)	2(8%)	4(26.7%)

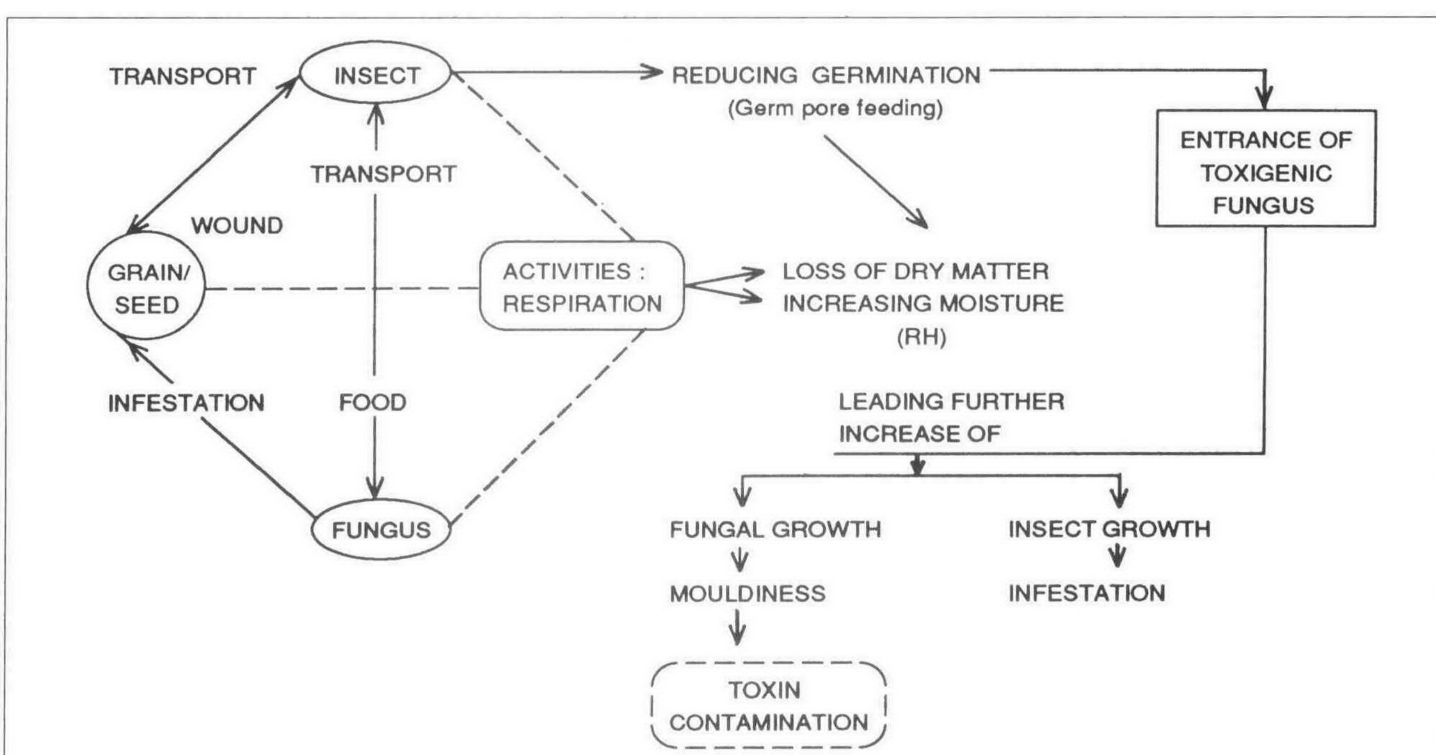

Fig. 1. Interactions of insect–fungus on stored grain/seed.

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