The effect of *Sitophilus zeamais* on fungal infection, aflatoxin production, moisture content and damage to kernels of stored maize

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Abstract

The effect of *Sitophilus zeamais* on fungal infection, aflatoxin production and moisture content was investigated.

Samples (250g) of commonly cultivated maize (*Zea mays*) var. Arjuna, moisture content ±14%, were stored in glass jars at ambient temperature (28±4C) and relative humidity of 65±5% for 3 months. Ten pairs of insects (1–7 days old) were placed in each jar at the beginning of storage. Control jars contained only maize and three replications were used for each treatment. Fungi were isolated and enumerated using dilution plating on Dichloran 18% Glycerol Agar (DG18). Fourteen species were isolated with *Aspergillus flavus*, *Eurotium repens* and *P. citrinum* being dominant.

The total fungal population in maize treated with the insect increased significantly after 2 and 3 months of storage to 3.0×10^6 and 5.9×10^7 colonies/g respectively compared with the comparable controls (2.3×10^5 and 2.4×10^5 colonies/g). There was no significant difference in aflatoxin B_1 content among the treatments with both insect treated and control maize showing an increase in aflatoxin content over time. Aflatoxin content of insect treated maize before storage, and after 1,2 and 3 months was 32, 45, 83 and 134 µg/kg respectively compared with 23, 41, 69 and 160 µg/kg for the comparable controls.

The moisture content of maize treated with insects was significantly higher after 2 and 3 months storage (17.3 and 36.3%) than that of the control samples (13.6 and 13.5% respectively). The percentage of insect-damaged kernels increased significantly during storage, being 0, 10, 69 and 98% respectively after 0, 1, 2 and 3 months.

Introduction

Maize is an important secondary crop in Indonesia. It is also an important component of both human and animal diets. During storage maize can be infested by insects, microorganisms and rodents. Insects and fungi are, respectively, the first and the second-most important organisms causing the deterioration of stored products.

Sitophilus zeamais is an important insect that causes deterioration of stored maize (Pedersen 1983). The insect can infest the inner part of the grain and transmit fungal spores.

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Maize stored under humid tropical climates such as that of Indonesia can readily by infected with fungi. They cause dry matter loss, decrease nutritional content and produce heat, musty flavour and mycotoxins.

Among the known mycotoxins, aflatoxins have received the greatest attention because they are carcinogenic and hazardous both to humans and domestic animals. Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* (Butler 1974).

The objective of this study was to investigate the effect of *S. zeamais* on fungal infection of stored maize together with aflatoxin production, moisture content and insect-damaged kernels.

Materials and methods

Storing of maize and insect infestation

Samples (250g) of normally cultivated maize (*Zea mays*) var. Arjuna were stored in glass jars at ambient temperature (28 ±4°C) and relative humidity of 65 ± 5% for 3 months. The initial moisture content of the maize was ± 14%.

Before storage, the maize was fumigated with phosphine (2 g/t of maize) for 5 days, in order to be free from other insects during storage.

Ten pairs of insects (1–7 days old) were placed in each jar at the beginning of storage. Three replications were used for each treatment. As a control, jars containing only maize were also used.

Methods of sampling

Initial samples were derived from each jar before storage, and then after 1, 2 and 3 months of storage. Each sample was divided twice to obtain four working samples for moisture content, fungal, and aflatoxin analysis; and a reserve sample.

Moisture content, fungal, aflatoxin, and insect-damaged kernel analyses

Moisture content (based on wet weight) was determined using the oven method (BSI 1980). Fungal isolation was carried out using the dilution method on Dichloran 18% Glycerol Agar (DG18) (Pitt and Hocking 1985). Aflatoxin content was determined using thin layer chromatography (Blaney et al. 1984).

The number of insect-damaged kernels derived from each jar was determined using the formula:

Identification of the fungi

Fungi were identified following the methods described by Samson et al. (1984) and Pitt and Hocking (1985).

Experimental design

All experimental data collected were analysed statistically using a completely factorial randomised design.

Results and Discussion

Population of *S. zeamais*

The insect population increased with increasing length of storage.

According to Grist and Lever (1969) *S. zeamais* is an insect able to reproduce rapidly, i.e. it produces 5–7 generations in one year. The insect can also lay 300–500 eggs during its life (4–5 months).

Dobie et al. (1991) reported that at 27°C and relative humidity of 70% the life cycle of *S. zeamais* varied from 31 and 37 days.

Based on analyses of variance the length of storage gave a very significant difference in the insect population (Table 1). Using Duncan's Multiple Range Test at 95% confidence level, the insect population at the beginning of storage was not significantly from the population after 1 month of storage, but was significantly different after 2 and 3 months of storage (Table 2). The insect population after 2 months of storage was very significantly different from the population after 3 months of storage. The insect populations before storage, and after 1, 2 and 3 months, were 40.0, 40.0, 683.0 and 1783.7 insects/jar, respectively.

The effect of *S. zeamais* and length of storage on moisture content

Moisture content of the substrate is the important factor in storage, because it determines the development of insects and microorganisms that cause the deterioration of stored products (Sinha and Muir 1973).

Based on analyses of covariance there was a very significant difference between control and insect infestation on moisture content of maize after 2 and 3 months of storage (Table 3). Moisture contents in the control and treatment after 2 months storage were 13.6 and 17.3%, respectively, while after 3 months storage they were 13.5 and 36.5%.

The increase in moisture content resulted from the respiratory activity of an increasing *S. zeamais* population. Moisture content of the substrate was also affected by the temperature and relative humidity of the storage. The temperature range during storage was 24-29.2°C and the relative humidity 45–75%.

The effect of *S. zeamais* and length of storage on fungal populations

Fourteen species of fungi were isolated from the stored maize: *Acremonium strictum, Aspergillus flavus, A. niger, A. versicolor, A. wentii, Cladosporium cladosporioides, Endomyces fibuliger, Eurotium chevalieri, E. repens, E. rubrum, Fusarium semitectum, Penicillium citrinum, P. islandicum* and *Wallemia sebi*. The predominant species were *A. flavus, E. repens* and *P. citrinum*.

Total population of fungi increased with increasing length of storage, in both control and treated samples (Table 4). However, the increase was greater in the treatment compared with that in the control, particularly after 2 months of storage, when moisture content increased, creating conditions conducive to fungal growth and reproduction (Christensen and Kaufmann 1969).

Based on analyses of covariance the total fungal population on maize with insects was very significantly different from that of the control after 2 and 3 month storage. The populations on the control and on maize with insects after 2 months of storage were 227778.0 and 2977778.3 colonies/g, while after 3 months storage they were 236111.3 and 59277556.0 colonies/g, respectively (Table 4).

Populations of fungal species on the control and treated samples, before and after 1, 2 and 3 months of storage, are presented in Table 5. The predominant species were *Aspergillus flavus, Eurotium repens* and *P. citrinum*.

Based on analyses of covariance, populations of *A. flavus* on maize treated with insects were not significantly different from those on the control during storage.

Populations of *A. flavus* on the control before and after 1, 2 and 3 months of storage were 388, 3833, 5000 and 18889 colonies/g, respectively; while on the treated maize they were 136, 21278, 11111 and 4833333 colonies/g, respectively.

Populations of *E. repens* on the control before and after 1, 2 and 3 months of storage were 28, 53722, 219445 and 210000 colonies/g, while on treatment they were 1189, 75167, 2583333 and 2833333 colonies/g, respectively.

Table 1. Analyses of variance of *Sitophilus zeamais* population on maize during storage

Source of var.	Df	SS	MS	F-value
Treatment	3	6090527.583	2030175.861	97.77**
Error	8	166117.333	20764.667	

** Very significant differences based on 99% of confidence level.

Table 2. Population of *Sitophilus zeamais* on maize during storage

Length of storage (months)	Population of *S. zeamais* (insects/jar)
0	40.0c
1	40.0c
2	683.0b
3	1783.7a

Numbers followed by the same letter do not significantly according to DMRT at 95% confidence level.

Table 3. Moisture content of maize in control and in treatment during storage

	Moisture content (%)			
Length of storage (months)	0	1	2	3
Control	14.9	13.5	13.6	13.5
Treatment	14.6	14.3	17.3	36.3
F-value		10.98	282.91**	157.36**

**Significantly different at 99% confidence level according to analysis of covariance.

Table 4. Total population of fungi on maize during storage

	Total population of fungi (colonies/g) Length of storage (months)			
	0	1	2	3
Control	2351.0	65611.0	227778.0	236111.3
Treatment	5360.0	158667.0	2977778.3	59277556.0
F- value		6.85	35.46**	76.55**

**Significantly different at 99% confidence level according to analysis of covariance.

Table 5. Population of the fungal species on the control and on maize treated with *Sitophilus zeamais* before storage, and after 1, 2 and 3 months of storage

Fungi	Control				Treatment			
	Fungal population (colonies/g)				Fungal population (colonies/g)			
Length of storage (months)	0	1	2	3	0	1	2	3
Acremonium strictum	461	167	0	0	303	56	0	888889
Aspergillus flavus	388	3833	5000	18889	136	21278	11111	4833333
A. niger	23	0	0	0	8	0	0	0
A. versicolor	23	0	0	1667	62	0	0	1166667
A. wentii	6	0	0	0	0	568	5556	0
Cladosporium cladosporioides	6	167	0	1111	56	30556	0	166667
Endomyces fibuliger	0	0	0	1111	0	0	238889	10555556
Eurotium chevalieri	0	2278	0	0	0	21111	0	0
E. repens	28	53722	219445	210000	1189	76167	2583333	2833333
E. rubrum	13	0	0	0	0	3333	0	0
Fusarium semitectum	0	0	0	0	0	0	5556	55556
Penicillium citrinum	1400	2278	3333	1111	3583	5944	133333	0
P. islandicum	0	0	0	2222	0	0	0	38777778
Wallemia sebi	1	3167	0	0	10	667	0	0

Table 6. Analyses of variance of percentage of insect-damaged kernels during storage

Source of var.	Df	SS	MS	F-value
Treatment	3	20035.20176	6678.40059	4463.00**
Error	8	11.97113	1.49639	

**Very significant difference based on 99% of confidence level.

Populations of *P. citrinum* on the control before and after 1, 2 and 3 months of storage were 1400, 2278, 333 and 1111 colonies/g, while on the treated maize they were 3583, 5944, 133333 and 0 colonies/g, respectively. In general, for all lengths of storage the populations of each fungal species on maize treated with *S. zeamais* were higher than on the control.

The effect of *S. zeamais* and length of storage on aflatoxin production

Aflatoxin B1 content increased with increasing length of storage, both on control and treated maize samples.

Based on analyses of covariance, aflatoxin B_1 content on maize with insects was not significantly different from that on the control during storage. According to Butler (1974) aflatoxin production is affected among other things by the strain of *A. flavus* and moisture content of the grain. Concentrations of aflatoxin B_1 on maize with insects, before and after 1, 2 and 3 months of storage were 32.07, 45.32, 83.40 and 134.43 ppb, while on the control the amounts were 23.32, 41.45, 69.26 and 159.92 ppb, respectively.

The effect of *S. zeamais* and length of storage on damaged kernels

The percentage of insect-damaged kernels increased with increasing length of storage, paralleling increasing insect populations.

Based on analyses of variance length of storage gave very significant differences in insect-damaged kernels (Table 6). According to DMRT at the 95% confidence level, percentages of insect-damaged kernels before and after 1, 2 and 3 months of storage differed significantly from each other (Table 7); they were 0.0, 9.6, 68.8 and 98.3%, respectively. According to Hall (1970) insect activity was affected by moisture content of substrate, temperature and relative humidity in the storage.

Table 7. The percentage of insect-damaged kernels of maize during storage

Length of storage (months)	Damaged Kernels (%)
0	0,0a
1	9.6b
2	68.8c
3	98.3d

Numbers followed by the same letter do not differ significant according to DMRT at 95% confidence level.

Figure 1 shows maize kernels following 3 months storage with or without *S. zeamais*.

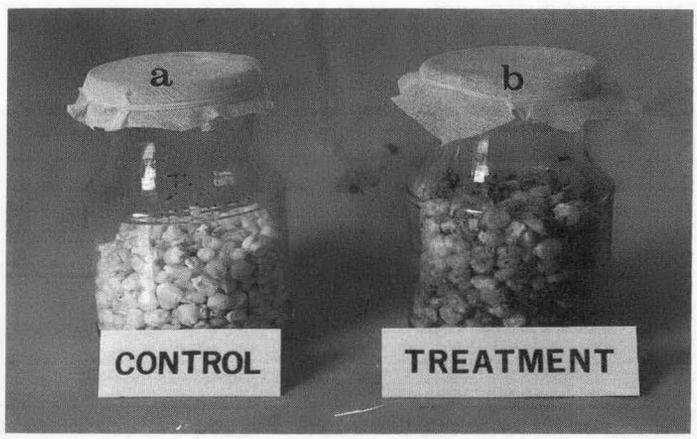

Fig. 1. Maize not treated (a) and treated (b) with *Sitophilus zeamais* after 3 months of storage.

Conclusion

During storage, 14 species of fungi were isolated. The predominant species were *Aspergillus flavus*, *Eurotium repens* and *Penicillium citrinum*.

Increases in moisture content, total fungal population and percentage of insect-damaged kernels were effected by increasing *S. zeamais* population.

This study highlights the importance of controlling *S. zeamais* on maize, because the insect not only causes damage of kernels, but can also stimulate fungal growth.

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