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EFFECT OF PHOSPHINE AND DURATION OF STORAGE ON FUNGAL POPULATION AND SOME CHEMICAL COMPONENTS OF SOYBEAN MEAL

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ABSTRACT

The effects of phosphine and duration of storage were investigated on the fungal population, and on protein, total lipid and ash contents, urease activity, and changes in moisture content (m.c.) of soybean meal. The soybean meal was stored in 8 stacks for 190 days in a BULOG warehouse. Each stack consisted of 20 bags (70 kg/bag). Four stacks were treated twice with phosphine (2.1 g/tonne), namely at the beginning, and after 95 days of storage. Fumigation exposure time was 5 days. Four untreated stacks served as control. Seventeen species of fungi were isolated from the stored soybean meal using the dilution method. There was a variation in the total population of fungi during storage, both in stacks treated with phosphine and untreated ones. The total population decreased after treatment, but then increased during storage. The concentration of phosphine applied also reduced the population of certain species of fungi during storage, particularly *Eurotium chevalieri* and *Wallemia sebi*, but its effect was not enduring. During storage there was a decrease in protein, total lipid and ash contents, and urease activity both in stacks treated with phosphine and untreated ones. The decrease in protein content of stacks treated with phosphine was significantly different from that in untreated ones at 35 and 100 days of storage. The decrease in lipid content in stacks treated with phosphine was significantly different from that of untreated ones after 190 days of storage. The decrease in ash content of stacks treated with phosphine was significantly different from that of untreated ones after 100 days of storage. Urease activity was not affected by phosphine treatment.

INTRODUCTION

In Indonesia, soybeans are an important secondary food crop after maize, while soybean meal is an important component of feed, because of its

relatively high protein content (42 - 50%). As Indonesia has a humid tropical climate, soybean meal is easily infected by fungi during storage.

Aspergillus and *Penicillium* are common fungi invading stored-products. They can cause a loss in weight, reduced nutritional content, heating and mustiness, and can produce mycotoxins.

Phosphine is a widely known fumigant used usually for insect control. However, little research has been done on the effects of this fumigant on the development of fungi and their reciprocal effects on protein, total lipid and ash contents, as well as urease activity of stored soybean meal.

The objective of this study was to investigate the effects of phosphine and duration of storage on the development of fungi in stored soybean meal. In addition, the impact of phosphine on protein content, total lipid and ash contents, urease activity and changes in moisture contents (m.c.) was also observed.

MATERIALS AND METHODS

Stacks of soybean meal and fumigation

Soybean meal was stored in 8 stacks for 190 days in a BULOG (National Logistics Agency of Indonesia) warehouse. Each stack consisted of 20 bags (70 kg/bag). Four stacks (4 replications) were treated twice with phosphine at a dosage rate of 2.1 g/tonne of soybean meal, once at the beginning of storage and again after 95 days of storage. Fumigation exposure time was 5 days. Four untreated stacks served as control. Each stack was arranged randomly.

Methods of sampling

Initial samples were taken from each stack (5 bags/stack) before fumigation, after the first fumigation (5 days of storage), after 35, 65 and 95 days of storage, 5 days after the second fumigation (100 days of storage), and after 130, 160 and 190 days of storage. Initial samples were also taken from the control. The samples were drawn from three points of each bag using a spear sampler. These samples were mixed thoroughly to obtain a pooled representative sample (approximately 1 kg/stack). Representative samples were divided using a sample divider into 4 working samples for the analysis of 1) fungi, 2) protein and lipid contents, 3) ash content and urease activity, and 4) m.c.

Fungal, protein, lipid, ash, urease activity, and m.c. analysis

Fungi were isolated using a dilution method on Dichloran 18% Glycerol Agar (DG 18) (Pitt and Hocking, 1985). The protein, total lipid and ash contents were determined using the Kjeldahl, Soxhlet Extraction, and Furnace Muffle methods, respectively, (Horowitz, 1984). Urease activity was

determined using visible spectrophotometry (Smith *et al.*, 1956), whereas m.c. was measured using an oven method (BSI, 1980).

Identification of the fungi

Fungal identification was determined according to Samson *et al.* (1984), and Pitt and Hocking (1985).

The experimental data were analyzed statistically according to a completely randomized design.

RESULTS AND DISCUSSION

Effects of phosphine and duration of storage on the development of fungi

Seventeen species of fungi were isolated from stored soybean meal during storage. These were: *Aspergillus candidus, A. flavus, A. niger, A. penicilloides, A. sydowii, A. tamarii, A. wentii, Cladosporium cladosporioides, C. sphaerospermum, Endomyces fibuliger, Eurotium chevalieri, E. repens, E. rubrum, Mucor circinelloides, M. racemosus, Penicillium citrinum* and *Wallemia sebi.* The predominant species were *A. sydowii, E. chevalieri* and *W. sebi.*

Analysis of covariance showed that between the treated and untreated stacks there were no significant differences in the total population of fungi during storage (Table 1). There was variation in the total population of fungi during storage, both in stacks treated with phosphine and untreated ones. It was assumed that changes in total population of fungi during storage were related to the stages of development of the various species of fungi. According to Hocking (1991), phosphine appeared to affect growing fungi, but had little effect on dormant spores and mycelium.

The total population decreased after treatment, but increased again during storage. After the first treatment it decreased from 576 colonies/g to 88 colonies/g, and then increased from 88 colonies/g to 634 colonies/g. After the second treatment, it decreased from 1,084 colonies/g to 818 colonies/g, and later increased from 818 colonies/g to 6,988 colonies/g (Table 1).

Development of the fungal population in treated stacks was relatively stable as compared with the untreated ones (Table 1). According to Hocking (1991), phosphine, even at low levels, (0.1 g/m^3), could retard the development of storage fungi in grains even if the m.c. was slightly above the m.c. levels accepted normally for safe storage.

Populations of *A. sydowii* were relatively stable both in treated and untreated stacks from the beginning until 100 days of storage (Fig. 1). Populations of *E. chevalieri* and *W. sebi* decreased after treatment, but the effect of phosphine was only temporary (Figs. 2 and 3). The development of the two species of fungi in stacks treated with phosphine was relatively stable in comparison with development in the untreated ones.

Table 1: Total population of fungi in treated and untreated stacks during storage.

Storage time in days	Total fungal population (Colonies/g) control stack	fumigated stack	F-value[c]
0	1610	576[a]	
5	8977	88	0.07
35	2473	634	0.09
65	4322	844	0.08
95	9430	1084[b]	0.03
100	2733	818	0.32
130	10878	6988	0.26
160	4233	3185	0.18
190	2799	627	0.77

(a) First phosphine fumigation.
(b) Second phosphine fumigation.
(c) Difference not significant according to analysis of covariance.

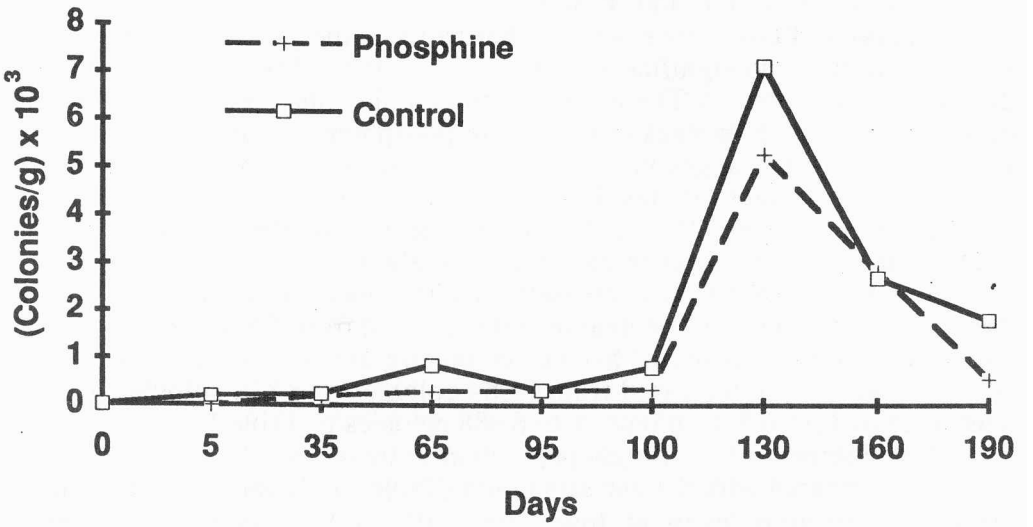

Fig. 1: Population of *Aspergillus sydowii* in treated and untreaed stacks during storage.

Effect of phosphine and duration of storage on protein content

Protein content decreased with duration of storage (Table 2). Presumably the reduction was due to both insects and fungi utilizing nitrogen from the proteins of soybean meal. Lysine is the largest component of protein next to leucine in soybean meal. It was assumed that the reduction in

protein content was due to degradation of amino acids, and also there was a decrease in nitrogen of non protein components (Sinha and Muir, 1973; Fennema, 1976).

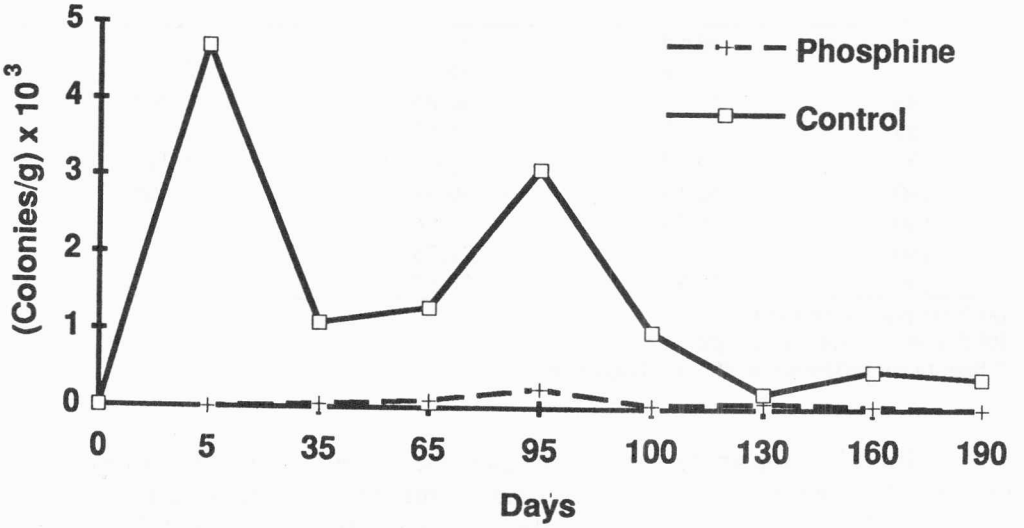

Fig. 2: Population of *Eurotium chevalieri* in treated and untreated stacks during storage.

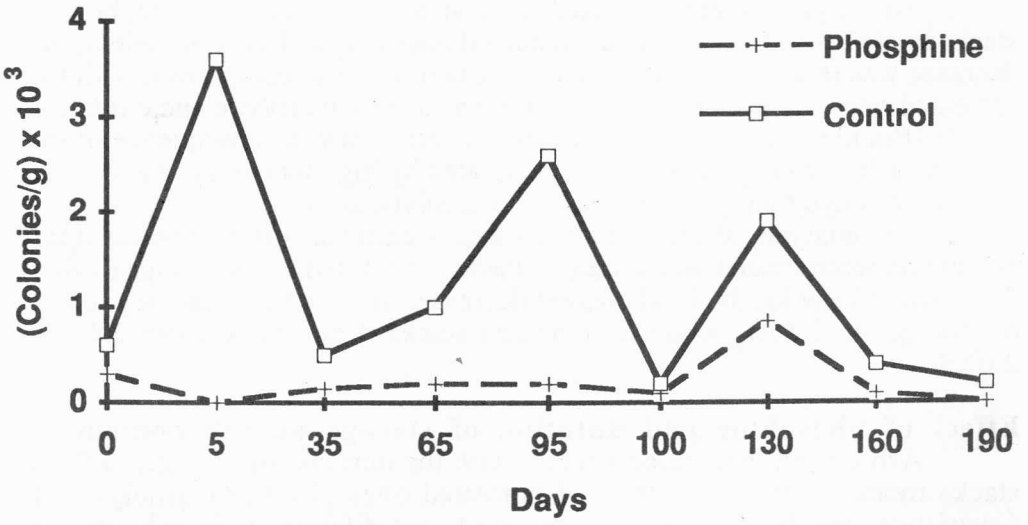

Fig. 3: Population of *Wallemia sebi* in treated and untreated stacks during storage.

Table 2: Protein content in treated and untreated stacks during storage.

Storage time in days	Protein content (%)		F-value
	control stack	fumigated stack	
0	44.09	40.95(a)	
5	42.18	39.70	0.07
35	40.59	36.05	7.00*
65	38.42	33.87	0.05
95	35.84	32.65(b)	4.78
100	33.65	30.33	10.50*
130	30.28	28.86	2.14
160	26.37	27.76	0.15
190	22.95	26.67	6.07

(a) First phosphine fumigation.
(b) Second phosphine fumigation.
* Significant difference at 95% confidence level.

Based on the analysis of covariance, there were significant differences in terms of protein content between treated and untreated stacks after 35 and 100 days of storage (Table 2). In treated stacks, the protein content decreased from 41% (at the beginning of storage) to 36.1 and 30.3%, while in untreated stacks it decreased from 44.1% to 40.6 and 33.7%.

Effect of phosphine and duration of storage on total lipid content

Total lipid content decreased as duration of storage increased, both in stacks treated with phosphine and untreated ones (Table 3). Nevertheless, the decrease was relatively moderate in the treated stacks in comparison with the untreated ones. Presumably the decrease was due to hydrolytic and oxidative effects (Buckle *et al.*, 1987). The hydrolytic effect was a consequence of the activity of lipase enzyme that was accelerated by high temperature and m.c., and by the fungi, owing to the latter's high lipolytic activity.

The analysis of covariance showed significant differences in lipid content between treated and untreated stacks after 190 days of storage (Table 3). In treated stacks, the lipid content decreased from 2.52 (at the beginning of storage) to 2.10%, while in untreated stacks it decreased from 2.53 to 2.05%.

Effect of phosphine and duration of storage on ash content

Ash content decreased slightly with the duration of storage, both in stacks treated with phosphine and untreated ones (Table 4). Analysis of covariance revealed that there were significant differences in ash content between treated and untreated stacks after 100 days of storage (Table 4). In

treated stacks the ash content decreased from 7.30 (at the beginning of storage) to 7.23%, while in untreated stacks it decreased from 7.30 to 7.10%. The decrease in ash content in untreated stacks was greater than that in treated ones after 100 days of storage. It was assumed that the decrease in ash content was due to the release of phosphorus from phytic acid in soybean meal by enzyme phytase, and that phosphine could inhibit the activity of this enzyme, and therefore the release of phosphorus from phytic acid was reduced (Smith, 1978; Snyder, 1987).

Table 3: Total lipid content in treated and untreated stacks during storage.

Storage time in days	Lipid content (%)		F-value
	control stack	fumigated stack	
0	2.527	2.522(a)	
95	2.197	2.217(b)	1.39
190	2.046	2.095	8.28*

(a) First phosphine fumigation.
(b) Second phosphine fumigation.
* Significant difference at 95% confidence level.

Effect of phosphine and duration of storage on urease activity

Urease activity decreased with the duration of storage, both in stacks treated with phosphine and untreated ones (Table 5). It was assumed that the reduction in urease activity was attributed to denaturation of the enzyme urease by heat generated from fungal metabolism and growth (Smith, 1978; Snyder, 1987). Based on analysis of covariance, there were no significant differences in urease activity between treated and untreated stacks during storage (Table 5).

Effect of phosphine and duration of storage on moisture content

Moisture content is the most important factor in the determination of development of storage fungi (Christensen and Kaufmann, 1969, 1974). There was a variation in m.c. both of stacks treated with phosphine and untreated stacks during storage (Table 6). It was assumed that the variation in m.c. was affected by environmental conditions, especially the temperature and relative humidity (r.h.) of the warehouse during storage. In stacks treated with phosphine, the m.c. ranged from 11 - 14%, while in the untreated stacks it ranged from 10.6 - 14.5% (Table 6). BULOG has established the standard for m.c. of stored soybean meal at 14%.

Analysis of covariance for stacks treated with phosphine and untreated ones showed very significant difference in m.c. after 100 days of storage with values of 11.9 and 10.6%, respectively (Table 6). It seems likely that the m.c.

changes were primarily due to environmental fluctuations in the warehouse (temperature and relative humidity). Therefore the effect of phosphine on m.c. was negligible.

Table 4: Ash content in treated and untreated stacks during storage.

Storage time in days	Ash content (%) control stack	Ash content (%) fumigated stack	F-value
0	7.30	7.30(a)	
5	7.35	7.29	1.14
35	7.27	7.19	1.41
65	7.18	7.23	0.04
95	7.18	7.26(b)	1.29
100	7.10	7.23	15.15*
130	7.16	7.15	0.04
160	7.10	7.13	1.86
190	7.14	7.12	0.80

(a) First phosphine fumigation.
(b) Second phosphine fumigation.
* Significant difference at 95% confidence level.

Table 5: Urease activity in treated and untreated stacks during storage.

Storage time in days	Urease activity ($\%NH_3$) control stack	Urease activity ($\%NH_3$) fumigated stack	F-value(c)
0	4.97	2.48(a)	
5	3.68	2.42	0.00
35	3.72	2.16	1.89
65	3.44	2.56	0.21
95	2.84	1.40(b)	2.97
100	2.35	1.40	0.23
130	1.38	1.10	0.73
160	0.92	0.43	1.53
190	0.70	0.42	1.47

(a) First phosphine fumigation.
(b) Second phosphine fumigation.
(c) Difference not significant according to analysis of covariance.

Correlation between moisture content and total population of fungi

Based on statistical analysis, there was a negative correlation (r^2= 0.77) between m.c. and total population of fungi in stacks treated with

phosphine (Fig. 4). The total fungal population decreased with an increase in m.c.. Normally, the increase in m.c. up to a certain level may induce the development of storage fungi. In this experiment, it was assumed that the negative correlation was affected by the inhibition of the development of certain species of fungi by phosphine. The lack of correlation between m.c. and total population of fungi on untreated stacks ($r^2 = 0.09$) could be attributed to the low m.c.s of the soybean meal.

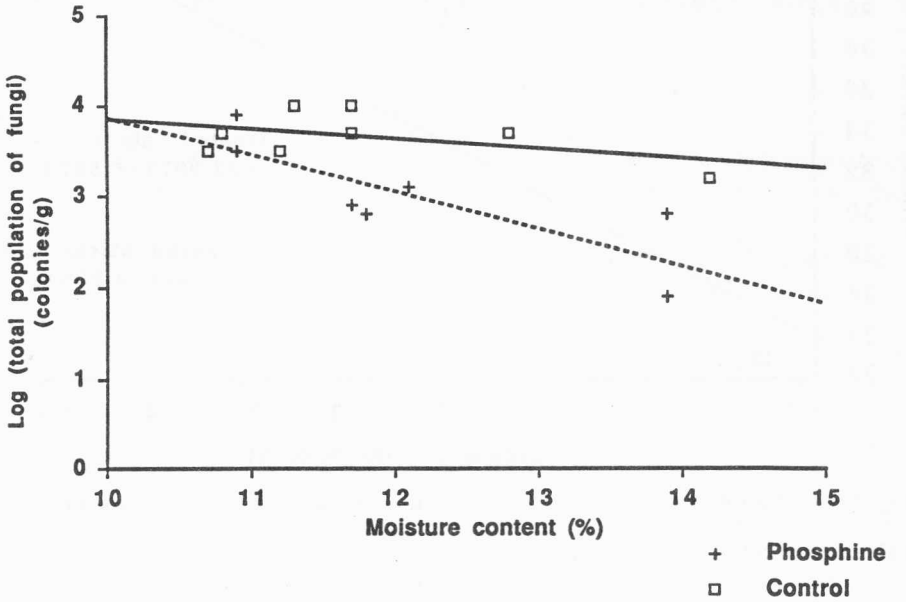

Fig. 4: Correlation between moisture content and total population of fungi in treated and untreated stacks.

CONCLUSIONS

- Stacks treated with phosphine and untreated ones did not reveal significant differences in the total populations of fungi during storage.
- There was a variation in the total population of fungi both in treated and untreated stacks during storage. The total population decreased after treatment, but then increased throughout the storage period.
- Phosphine at the applied concentration could also reduce the population of certain fungal species during storage, but its effect was not lasting. The affected species were *E. chevalieri* and *W. sebi.*
- Duration of storage influenced protein, total lipid, ash contents, and urease activity. These decreased as storage was prolonged both in treated and untreated stacks.
- The decrease in protein content in stacks treated with phosphine was slightly but significantly different from that in untreated ones after 35 and 100 days of storage.

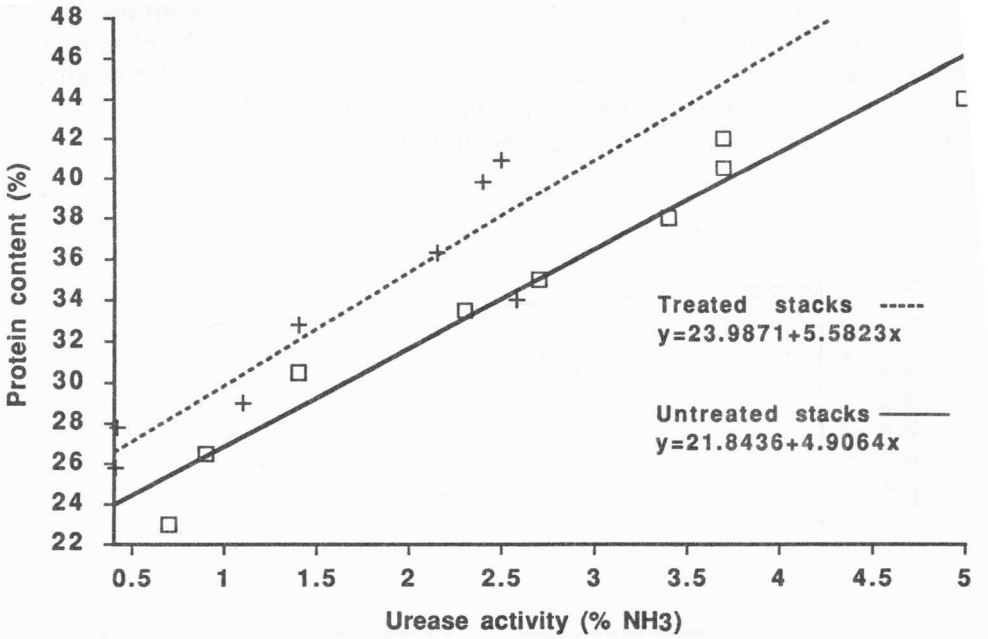

Fig. 5: Correlation between protein content and urease activity, in treated and untreated stacks.

- Decrease in lipid content in stacks treated with phosphine was slightly but significantly different from that in untreated ones after 190 days of storage.
- Decrease in ash content in stacks treated with phosphine was significantly different from that in untreated ones after 100 days of storage.- Urease activity was not affected by phosphine treatment.
- There was a variation in m.c. both in treated and untreated stacks during storage. In stacks treated with phosphine, the m.c. ranged from 11 - 14%, while in the untreated stacks it ranged from 10.6 - 14.5%.
- There was a negative correlation between m.c. and total population of fungi on stacks treated with phosphine, and no correlation between the two parameters on untreated stacks. On treated stacks the total population of fungi decreased with the increase in m.c.
- There was no correlation between total population of fungi and protein content neither in stacks treated with phosphine nor untreated ones.
- There was a positive correlation between protein content and urease activity both in stacks treated with phosphine and untreated ones. Protein content decreased with increase in urease activity.

ACKNOWLEDGEMENT

The authors are grateful to the National Logistics Agency (BULOG), Jakarta, Indonesia for its financial support. The authors also thank Mr. Sunjaya and Ms. Ina Retnowati, scientists of the Tropical Agricultural Pest Biology (TAgPB) Programme, SEAMEO BIOTROP; Ms. Betty, a student from Pakuan University, Bogor, Indonesia; the technicians of the Laboratory of Plant Pathology and Laboratory of Chemistry, TAgPB Programme, SEAMEO BIOTROP; and Dr. A.D. Hocking, CSIRO, North Ryde, Australia, for confirmation of fungal identification.

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