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Current status of phosphine resistance in stored grain insects in Malaysia

(Status semasa kerintangan serangga bijirin terhadap gas fosfin di Malaysia)

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Abstract

A survey was conducted in 2001 to determine the current status of phosphine resistance among insects infesting grain mills and stores in all states of Malaysia. The objective was to compare resistance levels with those of earlier surveys and to evaluate weaknesses in the conduct of fumigations. Four species of grain beetles were collected from 13 states and were tested with discriminating doses according to the standard FAO method. Resistance was recorded in 9 of the 13 states surveyed.

All samples of the important primary species *Sitophilus oryzae* and *Sitophilus zeamais* were susceptible as in earlier surveys. Resistance is prevalent among the two most common secondary species, *Tribolium castaneum* and *Oryzaephilus surinamensis*. The 57% premises found to contain resistant populations of either or both species did not show an upward trend in the spread of resistance. Although *Rhyzopertha dominica* was frequently found, the surviving individuals from collected samples were insufficient for testing. Due to substandard fumigation of wheat and maize in silos, samples from these imported grain contained higher frequencies of resistant insects (87.5%) than samples from rice/paddy (44%). Resistant populations could be returned to a susceptible state by maintaining high standard of gastightness, by not overdosing, and using alternative pest control techniques.

Introduction

Fumigation is an effective method for controlling insects and rodents in grain or other dried agricultural commodities stored either in bulk (e.g. in silos) or as packed produce (e.g. in plastic or gunny bags stacked on pallets). This technique is considered residue-free by most markets and is particularly important in international trade in which zero tolerance is generally applied to all live pests.

Worldwide, the most commonly used gases as fumigant for pest control in food storage are methyl bromide and phosphine. Methyl bromide is the main gas used for quarantine treatment for agricultural and timber products, but with the impending phasing out of methyl bromide (an ozone depleter) (Desmarchelier 1998), there will be heavy reliance on phosphine.

Phosphine has been used widely in the last three decades in Malaysia as a fumigant for controlling stored product pests. Its ease

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of application has contributed to its wide acceptance, even though the gas exposure period is considerably longer (5–7 days) than that required for methyl bromide (24 h). Usually the gas (hydrogen phosphide, PH_3) is produced from commercially formulated solid forms of aluminium phosphide upon exposure to moisture in the air.

In Malaysia improper fumigation practices, especially fumigation of enclosures with poor gastightness and overdosing, are the major reasons for the occurrence and increase of resistance among storage insects to fumigants (Rahim and Sulaiman 1999). Phosphine fumigation is important in Malaysia, and periodic resistance surveys are needed because they show the prevalence of resistant insects and whether resistance is becoming more common. This information can serve as a warning that fumigation practices need to be improved.

Materials and methods

Collection of beetles

Stored grain insects were collected from grain terminals at ports, imported grain (e.g. wheat and maize) mills, milled rice storage depots, and paddy milling complexes in Peninsular Malaysia, Sabah and Sarawak. Insect samples were taken from 33 premises *(Table 1)*, 10 of which (including 6 in Sabah and Sarawak) were new locations, not sampled previously in 1991 and 1995.

At each location, all populations of a specific species collected from grain samples were pooled in 500 mL glass jars containing pre-prepared media of either mixture of brown rice plus kibbled wheat (for *Rhyzopertha dominica* L., *Sitophilus oryzae* L. and *Sitophilus zeamais* Motsch.) or milled oat plus rice bran (*Tribolium castaneum* Herbst and *Oryzaephilus surinamensis* L.). The insects were sifted in situ from spear samples from bagged grain (ca. 100 g per bag: sampling ratio of 1 in every 100 bags), floor sweepings (5 kg), and 2–3 kg grain taken from the bottom of each silo. The insect samples were taken to MARDI's stored grain laboratory. The insects (500 individuals per 500 mL jar from each location) were reared using the same media type in a rearing room at 30 ± 3 °C and 75 ± 5% relative humidity (RH).

Resistance testing was done on several species of major importance in grain in Malaysia, namely, *T. castaneum* (red flour beetle), *O. surinamensis* (sawtoothed beetle) and *Sitophilus* spp. *Sitophilus* spp. was not identified to species but *S. oryzae* is typically found in paddy and *S. zeamais* in maize. Due to low survival in laboratory cultures, *R. dominica* (lesser grain borer) was not tested. These insects constitute the predominant species in stored grain (paddy or rice, wheat, maize, and other cereals) in Malaysia. *Sitophilus* spp. and *R. dominica* are considered primary species due to their ability to bore whole grain thus capable of incurring vast damage. The other two species are secondary insects, feeding only on already damaged and powdered grain.

The collected insects (parent) were reared in the laboratory to obtain the F_1 adult generation, which were then screened for resistance. During sampling, relevant personals were interviewed with respect to fumigation practices, stocking and pest control management.

Resistance screening

Testing was done by the FAO Method No. 16 (Anon. 1975), in which adult insects were exposed for 20 h to discriminating concentrations of phosphine: 0.04 mg/L for *Sitophilus* spp. and *T. castaneum*, and 0.05 mg/L for *O. surinamensis*.

Phosphine was generated from aluminium phosphide, according to the FAO methodology (Anon. 1975). The volume of gas necessary to obtain the desired concentration was calculated, and applied to the desiccators with a gastight syringe. After the exposure period, the insects were transferred to glass flasks (2 cm diameter x 7.5 cm) containing a small quantity of rice bran and closed with a screen top. They were kept for 14 days at 30 ± 3 °C and 70%

Table 1. Status of insect resistance to phosphine gas in Malaysia based on discriminating dose conducted in the year 2001

State	Location	Grain	% Mortality			Remark*
			TC	SO/SZ	OS	
Perlis	G101	Rice	–	100	97.1	R
	K104	Paddy	100	100	–	S
Kedah	G201	Rice	100	100	98.3	R
	G205	Rice	100	100	98.2	R
Penang	G301	Rice	100	–	–	S
	K303	Paddy	100	–	99 & 100	S**
	FM307	Maize	79.8	–	39.5	R
Perak	K402	Paddy	100	100	96.7 & 96.8	R**
	FLM407	Wheat	49.4	–	32.5	R
	K409	Paddy	36.3	100	100	R
Selangor	G506	Rice	100	–	–	S
	G507	Rice	100	–	100	S
Negeri Sembilan	G606	Rice	100	100	–	S
	FM603	Maize	100	–	–	S
Malacca	G701	Rice	–	–	99.2 & 90.8	R**
	G703	Rice	100	–	100	S
	FM706	Maize	95.7 & 80.8	–	–	R**
Johore	G801	Rice	99.2	–	–	R
	G802	Rice	100	100	84.7	R
	FLM805	Wheat	88.2 & 91.8	–	–	R**
Pahang	G901	Rice	99.2	100	97.7	R
	K902	Paddy	100	100	–	S
	FLM903	Wheat	9.1 & 7.8	–	–	R**
Terengganu	K1001	Paddy	100	–	–	S
	G1002	Rice	–	100	100	S
Kelantan	K1103	Paddy	100	100	–	S
	K1104	Paddy	100	–	–	S
Sabah	G1502	Rice	–	–	81.0	R
	G1503	Rice	–	–	92.6	R
	G1513	Rice	–	–	100	S
	FM1514	Maize	12.0 & 10.2	–	–	R
Sarawak	G1601	Rice	–	100	–	S
	FM1610	Maize	94 & 78.2	–	–	R**

*Location with at least one species recorded resistant (R) or all susceptible (S)
**Insect strain tested twice to verify the percent mortality obtained in the first test
Resistant (R) defined as <100% mortality
G = Rice warehouse, K = Paddy mill, FM = Feed mill, FLM = Flour mill
TC = *Tribolium castaneum*, SO = *Sitophilus oryzae*, SZ = *Sitophilus zeamais*, OS = *Oryzaephilus surinamensis*

Table 2. Change in the status of phosphine resistance in stored product insects over 11 years (1991–2001)

		1991	1995	2001
States		45.4% (5/11)	60.0% (6/10)	69.2% (9/13)
Premises		38.0% (8/21)	66.0% (15/25)	57.5% (19/33)
Serious resistance	No. premises <50% mortality	9.5% (2/21)	8.0% (2/25)	15.1% (5/33)
Insects	*Sitophilus* spp.	0.0% (0/12)	0.0% (0/19)	0.0% (0/13)
	T. castaneum,	20.0% (4/20)	40.0% (8/20)	38.4% (10/26)
	O. surinamensis	Not tested	44.4% (4/9)	76.4% (13/17)
	R. dominica	100% (6/6)	75% (6/8)	Not tested

Table 3. Serious phosphine resistance in stored grain insects (1991–2001)

Year	Detected resistance	Reference
1991	FM306 (*T. castaneum*, 4.2% mortality)	Rahim (1991)
	K1104 (*R. dominica*, 7.5%)	
1995	FM805 (*R. dominica*, 15.2%)	Rahim and Sulaiman (1999)
	FM407 (*T. castaneum*, 28.8–39.3%)	
2001	FM1514 (*T. castaneum*, 10.2–12.0%)	This study
	FLM903 (*T. castaneum*, 7.8–9.1%)	
	K409 (*T. castaneum*, 36.3%)	
	FLM407 (*T. castaneum*, 49.4%, *O. surinamensis*, 32.5%)	
	FM307 (*O. surinamensis*, 39.5%)	

K = Paddy mill, FM = Feed mill, FLM = Flour mill

RH, after which the number of dead insects were counted. The test was repeated when the mortality obtained was too low (<10%) or a susceptible strain (from previous survey) showing resistance (mortality from both tests is presented in *Table 1*).

Results and discussion

Resistance to phosphine was detected in stored grain insects from nine of the 13 states (69.2%) in Malaysia *(Table 1)*. The survey included the states of Sabah and Sarawak that were not surveyed previously, and resistance was found in both of these states. Since 1991 there has been a gradual increase in the number of states and premises with resistance *(Table 2)*, but the percentage increment is not strictly comparable since the studies included different numbers of states/premises.

From the four states that did not have resistant populations in the present survey, only Negeri Sembilan has been free of resistance since 1991. Resistant *R. dominica* samples were previously detected in three other states: one location each in Selangor (Port Kelang) in 1991, and in 1995 in Terengganu (Bukit Kenak) and Kelantan (Tumpat). The current status of these three states is uncertain since *R. dominica* was not screened for resistance due to low numbers of progeny developing from the sample populations.

All *Sitophilus* species from premises collected were susceptible to phosphine *(Table 1)*. The *S. oryzae* and *S. zeamais* strains have been collected in surveys since 1991 but have yet to show resistance to phosphine *(Table 2)*. In the present study, 13 samples were collected from BERNAS's premises: 8 from rice depots (infestation solely from *S. zeamais*) and 5 from paddy complexes *(S. oryzae)*. Infested milled rice is fumigated only once or twice a year, but no phosphine use was recorded on stored paddy. *Sitophilus* species have a naturally high tolerance of phosphine hence the development of resistance is less likely (Li and Li 1994). Whereas *R. dominica* has a naturally low tolerance, hence the high record of resistance from previous surveys in 1991 and 1995 *(Table 2)*. High resistance records for this species are particularly likely, hence the need to be tested in future study.

Resistance was only detected in *T. castaneum* and *O. surinamensis* populations. From the 33 premises sampled, 57.5% contained resistant populations from either or both species *(Table 1)*. The number of premises infested with resistant insects showed a slight decrease since the last survey (66% in 1995) after a drastic increase from 38% in 1991. Insects detected as resistant in 1991 and 1995 were from the species *T. castaneum*, *O. surinamensis* and *R. dominica* (*Table 2*). Since the last survey, the number of resistance samples increased strongly for *O. surinamensis* but have only seen a slight decrease for *T. castaneum*.

On the basis of information collected during interviews, there appears to be no increase in fumigation frequency that otherwise could have explained the higher number of test samples with <50% mortality. Mortalities in most (85%) of the premises were >80% for *T. castaneum* and *O. surinamensis*. Nevertheless the FAO discriminating concentrations should kill all susceptible individuals. They will probably kill almost all heterozygous as well (Li and Li 1994; Bengston et al. 1999; Collins et al. 2002). Therefore a population with <100% mortality must be considered to be resistant to the gas.

The number of survivors in tests indicates the number of resistant individuals but not the strength of resistance or likelihood of fumigation success. Strength of resistance can only be determined by exposing resistant individuals to a range of concentrations and comparing the results with susceptible strains and other resistant strains. The frequency of resistant individuals in samples is, however, a useful indicator of selection pressure.

The incomplete control is largely attributed to gas leakage due to improper sealing, in pelletised commodity the plastic sheet enclosure is not well sealed around the floor perimeter, or in the case of bulk grain fumigation poor sealing at the joint of connecting elevators and ducting, and lack of aeration system within the silo to circulate the gas through the grain mass.

Rather than the frequency of fumigation, it is substandard fumigation, inter-state movement of infested grain, and use of recycled bags that contribute to the increase in the numbers of premise showing resistant strains in stored grain. That conclusion is also based on the occurrence of resistant insects in paddy, where records showed that this grain was not fumigated or very rarely on the freshly milled rice within the mill. The increase in the number of premises with resistant *O. surinamensis* could have been due to the spread of the resistant strain to new locations through grain trade.

Populations with the highest frequency of resistant individuals (<50% mortality) were found in animal feed and flourmills that handle imported maize and wheat *(Table 3)*. At these premises fumigations are substandard and are not supervised by licensed fumigators. The softer grains (as compared to paddy) are conducive to fast insect population proliferation whenever primary pests such as *Sitophilus* spp. and *R. dominica* are present. The heavy infestation would require frequent fumigation. Since 1991 serious phosphine resistance was detected at 8–15% of the surveyed premises *(Table 3)*.

Premises with high frequencies of resistant individuals require urgent attention by the appropriate authorities (e.g. Ministry of Agriculture and Health) in terms of training on fumigation practices (technique and frequency) and supervising implementation of resistance management strategies. Resistance management options include adjusting dose rates and frequency of application, limiting the area of application, using alternative chemicals (e.g. admixture of grain insecticides), systematic grain stocking management (including stock rotation), and reintroduction of susceptible pest insects. Most of these tactics are essentially various ways of minimising the relative fitness of resistant genotypes, either by preserving susceptible homozygotes or destroying resistant genotypes (Roush 1989).

Conclusion

Potential replacement fumigants lack the versatility possessed by phosphine. If the present phosphine fumigation continues to prevail, it is possible that in the future all mills and grain storage depots in Malaysia will contain phosphine resistant populations of one or more insect species. This will increase the risk of control failures. Consequently, it is important to ensure the effective life span of phosphine is prolonged.

The five cases of low mortalities as documented here nonetheless indicate urgent need to address the problem of insect resistance to the gas. Rigorous enforcement of laws related to fumigation practices, and ensuring adequate training for personnel responsible for quality maintenance in every mill and storage must be given the due priority. Training must also emphasise the role of sanitation and good store management practices to reduce infestation pressure. Since resistance comes about by selection with a toxicant of the more tolerant individuals in a population of a pest, it follows that the rate of selection is dependent on the number of insects available for selection. Any reduction in the number of insect available, e.g. by hygiene and good housekeeping reduces variability and the probability of resistance developing (Champ 1985).

Fumigation should achieve 100% kill to prevent selection occurring. Hence, awareness of the necessity to ensure the very basic requirements for effective fumigation must be emphasised, i.e. gastightness of the enclosures, minimum effective dosage, and recommended exposure period.

As a guide, the acceptable dose and gastightness of the phosphine fumigated enclosure is one that gives a final concentration at, or above, 80 ppm on completion of a 7-day exposure period (Annis et al. 1990). Ideally, every fumigation must therefore include the measurement of the residual gas level, at least prior to process termination, as a criterion for the success of pest control process.

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Abstrak

Satu bancian telah dibuat pada tahun 2001 untuk mengemas kini maklumat status kerintangan (kebal) serangga perosak terhadap gas fosfin. Serangga perosak diambil dari gudang penyimpanan dan kilang bijirin di semua negeri di Malaysia. Objektif kajian adalah untuk mengesan perkembangan kerintangan dan mengenal pasti kelemahan pelaksanaan amalan pengawalan perosak secara rawatan gas racun. Empat spesies kumbang bijirin yang diambil dari 13 negeri telah diuji dengan kaedah yang diunjurkan oleh FAO.

Populasi kumbang yang rintang dikenal pasti di 9 daripada 13 negeri. Rawatan fosfin masih lagi berkesan untuk mengawal spesies primer *Sitophilus oryzae* dan *Sitophilus zeamais*. Dua spesies sekunder yang paling kerap terdapat di dalam gudang iaitu *Tribolium castaneum* dan *Oryzaephilus surinamensis*, lebih kerap menunjukkan kerintangan. Sebanyak 57% premis yang mengandungi populasi yang rintang, sama ada dari salah satu atau kedua-dua spesies ini, tidak menunjukkan peningkatan bagi kerintangan. Walaupun sampel *Rhyzopertha dominica* kerap dikutip tetapi jumlahnya tidak mencukupi untuk diuji.

Di kilang yang mengandungi bijirin import (gandum dan jagung) dengan amalan rawatan gas racun yang kurang sempurna, kerintangan perosak adalah lebih kerap (87.5%) berbanding dengan di gudang/kilang beras dan padi (44%). Premis yang mengandungi serangga yang rintang boleh dipulih melalui amalan rawatan yang kedap udara, tidak menggunakan dos yang tinggi, serta menggunakan kaedah kawalan alternatif.

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