Phosphine resistance in stored grain insect pests in India

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Abstract

Resistance to phosphine fumigant in stored grain insect pests such as *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis* and *Cryptolestes* spp was surveyed in India. The insects have been collected from wheat, rice, paddy and maize stacks in central storages located in representative places in the country Resistance screening tests by FAO method on 110 populations of *T castaneum*, 38 of *R. dominica*, 36 of *S oryzae*, 25 of *O surinamensis* and 8 of *Cryptolestes* spp revealed that phosphine resistance was common in the field populations in all the species. The frequency of the resistance was highest among *T. castaneum* (100%), *R dominica* (95%) and *O surinamensis* (92%) and was relatively less in *S oryzae* (72%)

Probit regression lines for the most resistant and the most susceptible strains have been calculated. Among the field collected insects the highest level of resistance was shown by *R. dominica* (LD_{99} 9mg/L, 40 hr exposure) followed by *S oryzae* (LD_{99} 10mg/L, 20 hr exposure). High level resistance was also shown in the eggs of a resistant strain of *T castaneum* Factors involved in the development of resistance in field strains have been discussed.

The study revealed the seriousness of problem and the urgent need 1) to revise the dosage schedules, 2) to improve the standard of fumigations and 3) to conduct trials on suitable substitutes.

Introduction

India produces about 82 million tonnes of rice, 67 million tonnes of wheat, 9 million tonnes of maize and about 23 million tonnes of millets and other coarse grains. The grains are handled, transported and stored in gunny sacks About 70% of the total production is reported to be retained by the farmers for consumption, seed purposes and for sale later on The remaining 30% goes to the central pool which is maintained for public distribution and for export (Semple et al 1992). The Food Corporation of India, the Central Warehousing Corporation, State Warehousing Corporations and State Civil Supplies Corporations are involved in the storage of food grains in India. Grain bags are stacked, 150 tonnes per stack, 12 stacks in a unit and 3 units each in conventional masonry godowns About 5% of the central pool is stored in metal or concrete silos located in a few centres. Average storage loss in central storages due to insect infestation has been reported to be about 3%. The major grain pests include the red flour beetle *Tribolium castaneum*, the lesser grain borer *Rhyzopertha dominica* and the rice weevil *Sitophilus oryzae* among beetles and the tropical warehouse moth, *Ephestia cautella* among moth pests (Table 1) In addition, psocids predominantly *Liposcelis* spp are commonly encountered and the tiny insects pose serious problems in the godowns in the coastal belt (Rajendran 1994)

Table 1. Important insect pests of stored grains in India

Species	Remarks
Beetles	
Tribolium castaneum	Ubiquitous
Rhyzopertha dominica	Common in indoor and outdoor stacks, paddy rice in particular
Sitophilus oryzae	Occur in wheat especially soft varieties and maize
Trogoderma granarum	Confined to extreme climatic zones
Oryzaephilus surinamensis	Common in grains having more dockage
Cryptolestes spp	Common in milled rice having more dockage
Moths	
Ephestia cautella	Major moth pest in many godowns
Corcyra cephalonica	Occur sporadically on milled rice
Sitotroga cerealella	Noticed in new arrivals especially in paddy rice
Others	
Liposcelis spp.	Ubiquitous; serious problem in coastal areas; in other places outbreaks are frequent during monsoon

As in most other parts of the world phosphine is the major

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fumigant used for disinfestation of stored cereal grains at the farm level as well as in the central storages in India (Rajendran and Narasimhan, 1994; Thakre et al 1988) Aluminium phosphide tablet preparations liberating phosphine are used for fumigation of grain stacks, stored indoor or outdoor (Table 2) The fumigations are repeated as and when necessary On an average the stacks are fumigated every three months. Whole godown or shed fumigation is carried out occasionally

There have been occasional reports on the occurrence of phosphine resistance in stored grain insects and control failures under field conditions following phosphine treatments in India (Champ and Dyte 1976; Rajendran, 1989; Rajendran and Narasimhan, 1994; Udeaan, 1992) The status of insect resistance to contact insecticides and fumigants in India has been reviewed earlier by Rajendran (1992) There has been, yet, no systematic study on the incidence of phosphine resistance in different parts of the country to indicate the extent of the problem and the need to tackle it Therefore the present investigation was undertaken to investigate the current situation with regard to phosphine resistance among the insect pests of stored grains in India

Table 2. Current pest control practices in central storages.

Control method	Chemicals, dosage	Frequency of application
A Fumigation		
Stacks	Aluminium phosphide 3 tablets per tonne of grains, 5 – 7 days exposure	As situation warrants
Whole godowns	1 5 tablets per m^3	Occasional
B *Residual sprays*		
Stacks, alleyways	Malathion, 50% EC at 150 mg a i /m^2	Fortnightly
	Deltamethrin[a], 2.5% WP at 30 mg a i /m^2	Every 3 months
C Space sprays		
Inside godowns	Dichlorvos, 76 % EC at 17 mg/ m^3	Fortnightly

[a] Introduced in 1997, application restricted to 20 % of stocks in some organisations

Materials and Methods

Collection of insects

Adults of *S oryzae*, *T castaneum*, *O surinamensis* and *Cryptolestes* spp. were collected from 36 grain storage depots/godowns belonging to the Food Corporation of India, Central Warehousing Corporation, State Warehousing Corporations and State Civil Supply Corporations, located in fifteen states and an union territory (Fig 1) Atleast two storage centres have been visited in each state The insects have been collected from grain stacks of paddy rice, milled rice, wheat and maize, preferably from the oldest stack present in the godown at the time of visit In two cases i e Karnal in Harayana state and Samalkot in Andhra Pradesh, insects were collected, also from paddy stored outdoor The insects were brought to the laboratory and cultures raised on diets mentioned in FAO (1975) at 30℃ for *R. dominica* and 25° ± 2℃ in other cases and at 65 ± 10% rh

Fig. 1. Location of grain storage centres where insects were collected for resistance tests

Screening for resistance

When sufficient number of adults were available, they were sieved out from the cultures and counted into 2 cm × 10 cm size glass tubes, 50 per tube and the tubes were placed in individual desiccators of 0.85 l or 2 8 l capacity In each tube Whatman filter paper strip of 1cm × 5cm size was put to absorb the para-benzo quinones secreted by *T castaneum* and in other cases to give a rough surface for the insects to hold/rest The tubes were covered with muslin cloth held by elastic bands The desiccators which served as fumigation chambers were provided with rubber septa for dosing the fumigant as well as for gas sampling The fumigation proceedure as described in FAO (1975) was followed The gas concentrations in the fumigation chambers were checked with a Bedfont Phosphine Monitor having a built in pump for gas circulation If required, phosphine was added again and the concentration was rechecked The insects were tested against phosphine at three concentrations- 1) discriminating dose, 2) a lower and 3) a higher dose (× 4 discriminating dose) For example, *T castaneum* adults were tested at 0.04 (discriminating dose), 0.03 (lower dose) and 0.16 mg/L (× 4 of 0.04 mg/L) *Cryptolestes* spp were tested at the discriminating concentration of 0 06mg/L as developed by Mills (1983) There were atleast 4 replicates for each concentration along with an equal number of controls. Insect mortality was assessed at the end of 14 days

Further tests

Based on the mortality data arrived for lower and x4 discriminating doses some of the popuations of *T castaneum*, *R dominica* and *S oryzae* were identified as the most susceptible or the most resistant ones Their mortality response to a range of six to nine phosphine concentrations was studied In these experiments, *R dominica* were exposed for 40 hr since they were tolerant to high phosphine concentrations in 20 hr exposures The final corrected mortality data arrived were analysed by probit analysis (Finney 1971) The insects were also tested against methyl bromide following the FAO (1975) method to check whether they have developed any cross-resistance to methyl bromide

In one the most resistant strains of *T castaneum* 0 − 1 day old eggs were collected and were tested in 0 85 L desiccators at 96 eggs per replicate, 7 replicates per concentration Their mortality response (egg mortality) was compared with that of a susceptible strain QTC 4 from Queensland, also tested under the same conditions but with 6 replicates per concentration

Results

Screening tests revealed that phosphine resistance was common in all the insect species studied (Table 3) *T. castaneum* was exceptional that all the populations were resistant to the fumigant More than 90% of the populations of *R dominica* as well as *O surinamensis* and about 72% of the populations of *S. oryzae* were noted resistant. In the majority of the resistant populations of *R dominica* and *T castaneum*, less than 10% mortality was recorded at the discriminating and at higher dosages (Table 4) Among the most resistant strains identified, the highest level of resistance was shown by *R dominica* (Table 5). In *T castaneum* the most susceptible strain among the field populations was still highly resistant, LD_{50} being 0.14 mg/L whilst for a normal susceptible strain the reported value is 0.009 mg/L only (FAO 1975)

The most susceptible as well as the most resistant strains did not show any cross-resistance to methyl bromide fumigant The eggs of a resistant strain of *T castaneum* were also markedly resistant to phosphine (Table 6)

Table 3. Occurrence of phosphine resistance among field-collected stored grain insect pests

Species	Number of populations		Frequency of resistance occurrence (%)
	Screened[a]	Found Resistant	
Tribolium castaneum	110	110	100
Rhyzopertha dominica	38	36	95
Sitophilus oryzae	36	26	72
Oryzaephilus surinamensis	25	23	92
Cryptolestes spp	8	7	88

[a] Discriminating concentrations recommended by FAO (1975) and Mills (1983) (for *Cryptolestes*) used

Table 4. Data on populations showing less than 10% mortality response at discriminating and higher (× 4 of discriminating) doses of phosphine

Insect	No of populations showing	less than 10% kill at doses
	discriminating	higher
T castaneum	95(86%)	59(54%)
R dominica	23(61%)	22(58%)
S oryzae	13(36%)	2(6%)

[*] Figures in parantheses are the percentages out of total populations tested

Table 5. Lethal doses (mg/L) of phosphine against the most susceptible and the most resistant strains of field collected grain insect pests.

Insect (code)	LD_{50} (fiducial limits)[a]	LD_{95} (fiducial limits)[a]
SUSCEPTIBLE		
S oryzae (F52)	0.004(0 002, 0.005)	0 010(0.007, 0 0225)
R.dominica (TN6)	0.006(0 002, 0.012)	0.074(0 034, 0.913)
T. castaneum (F18)	0 138(0.098, 0.185)	1.054(0 698, 1.953)
RESISTANT		
T.castaneum (F43)	1.33(1.04, 1.56)	3.41(2 83, 4.66)
S.oryzae (F32)	1.70(1.07, 2 23)	6.11(4 44, 11.51)
R.dominica (F10)	3.61(2.35, 4 38)	6.84(5.54, 11.97)

[a] Exposure period for *R dominica* (F10) was 40 hrs and in all other cases 20 hrs only

Table 6. Mortality of 0 – 1 day old eggs of susceptible and resistant strains of *T. castaneum* exposed to phosphine for 72 hrs at 26 – 28℃.

Susceptible strain		Resistant strain	
Phosphine dose(mg/L)	% kill (mean ± SE)[a]	Phosphine dose(mg/L)	% kill (mean ± SE)[a]
Control	18.8 ± 3.5	Control	23.5 ± 7.1
0.04	63.7 ± 36.1	0.14	29.5 ± 12.6
0.06	86.8 ± 16.3	0.28	71.1 ± 20.1
0.07	95.0 ± 7.1	0.42	89.9 ± 13.6

[a] Mean of 6 replicates in susceptible strain and 7 in resistant one with 96 eggs per replicate

Discussion

Screening tests on field collected insects exposed the prevalence of occurrence of resistant strains in grain storages. Phosphine has the inherent drawback to allow insects exposed at sublethal doses to develop resistance to the chemical rapidly and this has been proved by laboratory selections (Winks 1986). Under field conditions there are various factors, technical and non-technical responsible for the development of resistance and the type of these factors may vary between countries. Based on the observations and discussions with the technical staff during insect collections, some of the drawbacks in the current system of storage and pest management practices have been identified. In most of the godowns there were a few grain stacks which were remaining since more than a year due to management or technical problems. Such stacks undergo repetitive fumigations as per the standing instructions. The insect population in these stacks are invariably resistant to phosphine

One of the means of predicting the success of fumigation is to check before termination, if the target gas concentration has been achieved or not (Graver and Annis, 1994). Gas monitoring to check the levels of phosphine gas in grain stacks or in whole godowns under fumigations is yet to be practised in the storage centres This is one of the essential requirements for a scientific fumigation operation (Taylor et al 1994). The fumigation covers used at present are made of low density polyethylene, 250 micron thickness and impregnated with carbon to withstand UV radiation during outdoor storage of grains. Thus the specification of the covers are intended mainly for open storage. In most of the godowns the same black polyethylene covers are used for fumigation of grain stacks inside the godowns. These covers when used for outdoor storage, get damaged and when they are used for fumigations without rectifying the damages, gas leakage occurs and the insects survive the treatments. Sand-snakes are generally recommended by the concerned authorities for sealing the cover to the floor during cover fumigation. However, mud-sealing has often been noted as the common method in most parts of the country. Differences in the method of sealing with mud have also been observed in different regions. In some places, very thin layer of mud has been used. During the exposure period of 5 – 7 days, the mud dries up and develops cracks facilitating the ingress of air into the stackand leakage of phosphine This results in exposure of insects to sublethal concentrations of phosphine. Due to practical difficulties, the stacks in a godown unit are not fumigated all at a time. Fumigated as well as infested fresh lots remain close leading to reinfestation and requiring refumigation (Fig. 3).

Open storage of paddy and wheat stocks are common in grain production/ procurement regions (Fig. 2). The stacks are normally built on smoothly finished plinths with proper wooden dunnage as in cover and plinth (CAP) storage. However, when there is a demand for storage space, the stacks are built on unfinished grounds or in field sites. Control failures will be inevitable in fumigation of stacks built on unfinished floor on the field itself as gas loss through sorptions by soil will be very high. The hot conditions outside will again lead to escape of the gas through cover. Moreover cross infestation and quick re-infestation of the outdoor stacks occur warranting repeated fumigations Similarly, during whole godown fumigations 100% mortality may not be achieved as most of the godowns are gable (asbestos) roofed and cannot be made totally gas-tight Furthermore, windows and ventilators and the rolling shutters in doorways in the godowns are sealed by mud or newspaper strips with starch paste, which are poor barriers for phosphine.

Fig. 2. Outdoor storage of paddy rice.

Fig. 3. A stack under fumigation and new lots awaiting for fumigation . All the stacks are not fumigated simultaneously.

Toxicity data for the most resistant strains in the present study have shown the occurrence of high level resistance in *S. oryzae* and *R. dominica* (Table 5). High level resistance in *R. dominica* has been recorded in other countries also. The maximum levels of resistance recorded worldover are: × 100 in *R. dominica*, × 40 in *O. surinamensis*, > × 16 in *T. castaneum* and × 14 in *Cryptolestes ferrugineus* (Champ 1985). Nationwide surveys on the incidence of phosphine resistance in stored grain insects have been conducted in some countries. In USA a relatively low order of phosphine resistance has ben encountered and control failures due to the occurrence of resistant populations has not been established (Zettler 1990). Similarly in Australia occurrence of resistance at low level has been reported (White and Lambkin 1990; Emery 1994).

Comparatively a larger percentage of populations have shown the incidence of phosphine resistance in India (Table

3). The extent of the infestation problem in the grain storages in different places are likely to vary and accordingly the frequency of pest control operations also changes. The intensity of pest infestation is determined by the ambient temperature and humidity, the degree of housekeeping within the storage premises and the number of transactions (issues and receipts) of grain bags in the godown Depending on the selection pressure the degree of resistance will vary In the present investigation most of the populations of R. dominica were highly resistant that less than 10% mortality was recorded at x4 discriminatory concentrations (Table 4)

The options to tackle insect resistance to a particular pesticide include use of pesticides in rotation or in sequence or use of alternatives. The industrialized countries have already found some promising, new fumigants such as carbonyl sulphide and methyl phosphine and also reinvestigated the suitability of old fumigants such as carbon disulphide and ethyl formate (Taylor 1997; SGA 1997) Changes in application techniques using cylinder-based phosphine formulations have also been made in Australia and UK (Taylor 1997). In India, at present, no other alternative fumigant which is economical and equally effective is readily available for fumigation of large number of grain stacks. Earlier Muthu et al. (1984) have tested ethyl formate as a grain fumigant. Unlike phosphine or methyl bromide, the effective dosage for ethyl formate is very high ie , > 300g/m^3 and 72 hr exposure. Muthu et al (1978) established that methyl iodide is effective against stored grain insects like methyl bromide and has excellent microbial action too The use of such alternatives, is necessary to eliminate the resistant population in the grain storages in India.

Under the current situation the following changes will help to check further development and/or the spread of phosphine resistance. The practice of first-in-first-out should be strictly followed for the grain stocks More number of godowns should be constructed preferably in dry areas. Open storage should be discouraged and if at all necessary, the stacks should be built on well-finished plinths. Gas monitors should be provided to all district storage centres They have to be used to ascertain the retention of effective concentrations of phosphine There should be separate covers (other than the black polyethylene covers used for outdoor storages) preferably nylon reinforced PVC sheets of lighter shades for fumigating the stacks inside the godowns. Treatment of all the 12 grain stacks in an unit at a time should be encouraged Residual spray treatment of grain stacks should also cover the top layer of bags to avoid the flying adults settling on the top to start the infestation

Conclusion

The study has established the widespread occurrence of phosphine resistance among the stored grain insect pests in India. Evidently the resistance levels were very high in some of the populations of *R dominica* and *S. oryzae* In view of the acute problem, it is necessary to revise the current dosage schedules based on field experiments. The sealing method need to be improved and gas monitoring during fumigations should be practised. Field trials need to be conducted on suitable substitutes such as ethyl formate, methyl phosphine, carbonyl sulphide and CO_2. Research on some of these aspects are under progress in this laboratory.

Acknowledgments

The author thanks the Food Corporation of India, Central Warehousing Corporation, Punjab State Civil Supplies Corporation, Tamilnadu State Civil Supplies Corporation and State Warehousing Corporations of Punjab, Rajasthan, Haryana and Uttar Pradesh for extending cooperation during the collection of insects in their storage centres. Thanks are also due to Mr M. Jayaram, Mr. N Muralidharan, Ms. Malini Bharadwaj, Rekha Savanth, Shrilatha B and Kirti . R. Nayak for their technical assistance and Dr N. G. K. Karanth, Project leader and the Director, CFTRI for encouragement. This project was funded by the Australian Centre for International Agricultural Research (ACIAR), Australia.

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