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A Survey of Resistance to Phosphine in Some Coleopterous Pests of Stored Wheat and Rice Grain in Pakistan

M. Sardar Alam, S. Shahid Shaukat*, Mubarik Ahmed, Shamim Iqbal and Akhlaq Ahmad
Tropical Agricultural Research Centre, Pakistan Agricultural Research Council,
*Department of Botany, University of Karachi, Karachi-75270, Pakistan

Abstract
Wheat and rice godowns in Pakistan have three to twelve years history of phosphine fumigation. Coleopterous adults and larvae from these godowns were collected, cultured and tested for phosphine resistance. Strains of *Tribolium castaneum* Hbst., *Rhizopertha dominica* F. and *Trogoderma granarium* Everts., exhibited varied levels of resistance, whereas all four strains of *Sitophilus oryzae* L. showed marginal resistance over the susceptible strain. Certain strains of *R. dominica* and *T. castaneum* were found to have 80-fold resistance. The possible causes of resistance and the methods of their control are discussed.

Introduction
The disintestation of grains by phosphine fumigation is most widely used in tropical countries for preventing damage by insect pests during grain storage. In some of these countries substandard techniques of phosphine fumigation and its application in leaky godowns (where optimal concentrations cannot be maintained) have led to the development of phosphine resistance in major insect pests of stored grains (Mills, 1983; Taylor, 1989; Mills *et al*., 1990). The development of resistance in these insects against phosphine is of concern because even low level of resistance could be of practical significance for the control of insects in stored products (Monro *et al*., 1972; Price and Mills, 1988; Taylor, 1989). The problem is not circumvented by increasing the fumigant concentration because such application is not only uneconomical but high dosage may also cause necrosis which results in increased survival of insects (Nakakita *et al*., 1974).

The phenomenon of insect pest resistance to phosphine has often been reported. During the FAO global survey of pesticide susceptibility to stored grains pests, phosphine resistance was recorded in 82 of the 849 strains tested (Champ and Dyte, 1976). Appreciably high resistance was recorded in *Rhyzopertha dominica* strains collected from Ghana and India. Borah and Chahal (1979) reported the failure of phosphine to control khapra beetle, *Trogoderma granarium* in warehouses in India. Tyler *et al.* (1983) documented the development of resistance in stored grain insect pests against phosphine in food warehouses in Bangladesh. The major tolerant species was *R. dominica* though other species including *Tribolium castaneum, Oryzaephilus surinamensis* and *Cryptolestes* spp. also exhibited reduced degree of susceptibility to phosphine. Accounts of laboratory, assessment of phosphine resistance to insect pests from various tropical countries have been given by Attia and Greening (1981), Mills (1983), Taylor (1986) and Taylor and Halliday (1986). Taylor (1986) reported in Pakistan 3 out of 4 strains of *R. dominica* and 7 and out of 8 strains of *T. castaneum* tested were found to be resistant.

Materials and Method
Insect Material: Live adults and larvae of *R. dominica, T. castaneum, S. oryzae* and *T. granarium* were collected from various wheat and rice godowns located in different parts of Pakistan. The godowns were known to be infested with insects and had a history of annual phosphine fumigation for at least 3 years. The collected insects were brought in laboratory and each strain was reared separately at optimal conditions of development. Reference strains were also cultured in the similar manner, which, with the exception of *T. granarium* were obtained from ODNRI Slough, U. K., while that of *T. granarium* was chosen from the local cultures at Grain Storage Research Laboratory, Karachi, after a series of tests. In case of *T. granarium,* fully grown larvae were used for experimentation. Whereas, for other species, adults of same age were tested.

Test for phosphine resistance: All insect strains collected were first exposed to a discriminating dose of phosphine in accordance with the method recommended by FAO (Anonymous, 1975). Phosphine was generated from aluminum phosphide tablets, collected over acidified water. Desiccators (10.8 1 volume) served as fumigation chamber. Within the desiccator, insects were confined in 50 cc beakers closed with nylon mesh. Aliquots of phosphine gas were injected into desiccators with micro syringe through a rubber septum fitted to a socket in the desiccator lid. The desiccators were kept in the environment chamber at 25°C and 65% R.H. for 20 h after which observations on mortality were made. Where 99-100 percent mortality was recorded in the first screening, the strains were assumed to be phosphine susceptible. Resistant strains were tested at a series of doses. Mortality data was subjected to probit analysis (Finney, 1964).

Results

Tribolium castaneum: A high degree of variability in response to phosphine among different strains of *T. castaneum* is obvious from Table 1. The most highly resistance strain was Tc-FMN2 with a resistance factor (RF) of 81.20. Two other strains Tc-SGG3 and Tc-S1305 were found to be highly resistant with RF of 67.11 and resistance strain was Tc-FMN2 with a resistance factor (RR of 81.20. Two other strains Tc-SGG3 and Tc-SBQ5 were found to be highly resistant with RF of 67.11 and 62.00 respectively. Three strains had RF between 30 and 40 while another three strains had RF ranging between 20 and 30 eight yielded RF values between 10 and 20. Four strains with relatively lesser degree of resistance had RF ranging between 2 and 8.11.

Rhizopertha dominica: Like *T. castaneum, R. dominica* strains also showed high order of variability to phosphine resistance (Table 2). Four strains viz. Rd-SGG3, Rd-SHD2, Rd-PSRG1 and Rd-FMN1 were found highly resistance with RF of 86.5, 78.6, 80.0 and 72.0 respectively. Two strains had RF values between 10 and 20 while the rest of the eleven strains had less than 10 fold resistance compared to the susceptible.

Trogoderma granarium: *T. granarium* (final stage larvae) strains also showed varied levels of resistance to phosphine (Table 3). Strain Tg-SGG3 was found to be most susceptible (LD_{50} = 0.917) and served as the reference. The most resistant strain was Tg-PMZ (J) with a RF 35.25 per cent. The rest of the four strains tested were 1 to 18 fold resistant over the susceptible (reference).

Sitophilus oryzae: All four *S. oryzae* strains were found be only marginally resistant to phosphine as compared the susceptible with RF values ranging from 1.09 to 1.2 (Table 4).

Discussion

A wide variation in the response to phosphine amor different strains of *T. castaneum, R. dominica* adults and *T. granarium* larvae has been demonstrated in this stud Strains of *T. castaneum* and *R. dominica* were recorded that had higher than 50-fold resistance to phosphine compared to the reference (susceptible) strains. Whereas four strains of *S. royzae* were found almost susceptible phosphine. Borah and Chahal (1979) and Mills (1983) have shown good correlation between laboratory assessment phosphine resistance and the results of fumigation in the field. Furthermore, the resistance level in *R. dominica* has been shown to correspond with resistance in egg stage (Bell *et al*., 1977) while in *T. castaneum* greater resistance phosphine has been observed in early and mid pupal than adults (Nakakita and Winks, 1980). Thus demonstration phosphine resistance in adult insects clearly implies this there could be problems in controlling both adult and immature stages of insect pests in commodities infested

Table 1: Resistance to phosphine in red flour beetle, *Tribolium castaneum* HBST (adult)

Locality	Strains	Slope	LD_{50}	95 % fiducial limit	R.F.
Karachi	Tc-SBQ1	2.39977	0.162	0.141-0.186	18.00
(Sindh)	Tc-SBQ2	2.04348	0.203	0.171 0.242	22.55
	Tc-SBQ3	2.43894	0.259	0.211-0.317	28.70
	Tc-SBQ4	1.93288	0.317	0.241-0.417	35.22
	Tc-SBQ5	11.10670	0.558	0.522-0.597	62.00
	Tc-SREL1	1.81183	0.197	0.160-0.243	21.88
	Tc-SERL2	1.45325	0.295	0.220-0.397	32.77
	Tc-SGGI(1)	2.47825	0.081	0.073-0.089	2.00
	Tc-SGGI(2)	1.63867	0.055	0.043-0.071	6.00
	Tc-SGGI(3)	1.14377	0.063	0.049-0.080	7.00
	Tc-SGGIII(1)	1.82217	0.094	0.070-0.124	10.44
	Tc-SGGIII(2)	8.17050	0.604	0.586-0.23	67.11
Lahore (Punjab)	Tc-PLH 1	1.46792	0.093	0.082-0.105	10.33
	Tc-PLH2	1.20439	0.099	0.064-0.153	11.00
	Tc-PLH3	1.35242	0.130	0.105-0.160	14.44
	Tc-MNG1	1.44298	0.095	0.073-0.123	10.55
	Tc-MNG2	2.41357	0.199	0.107-0.132	13.22
Multan (Punjab)	Tc-PMUL	1.84391	0.073	0.064-0.084	8.11
Mansehra	Tc-FM N1	1.80708	0.310	0.207-0.464	34.44
(N.W.F.P.)	Tc-FMN2	1.19482	0.731	0.259-0.209	81.20
Durgai (NWFP)	Tc-FDUR	1.71121	0.144	0.199-0.180	16.00

Tc = *Tribolium castaneum;* S = Sindh Province; BQ = Bin Qasim Rice Complex; SGG = Sindh Govt. Godown, Karachi; P = Punjab Province; LH = Lahore; MNG = Mange Mandi (Lahore); MUL = Multan; F = North West Frontier Provinc REL = Rice Export Corporation, Landhi, Karachi; OUR = Durgai; MN = Mansehra; R.F. = Resistance factor = LD_{50} resistant strain/LD_{50} of susceptible strain.

Table 2: Resistance to phosphine in lesser grain borer, *Rhyzopertha dominica* F. (adult)

Locality	Strains	Slope	LD_{50}	95 % fiducial limit	R.F.
Karachi	Rd-SGGI(1)	3.42524	0.086	0.079-0.094	10.75
(Sindh)	Rd-SGGI(2)	4.26029	0.025	0.021-0.028	3.13
	Rd-SGGIII	10.26210	0.644	0.611-0.678	80.50
Hyderabad	Rd-SHD1	2.50971	0.069	0.062-0.077	8.63
(Sindh)	Rd-SHD2	3.90716	0.855	0.665-1.098	78.62
Lahore	Rd-PLH 1	1.64083	0.035	0.028-0.045	4.37
(Punjab)	Rd-PLH2	3.40975	0.050	0.046-0.055	6.25
	Rd-PLH3	2.05130	0.026	0.016-0.044	3.25
Sargodha	Rd-PSRG 1	3.78280	0.640	0.583-0.703	80.00
(Punjab)					
Peshawar	Rd-FPW1	1.60620	0.036	0.028-0.046	4.50
(N.W.F.P.)	Rd-FPW2	1.73849	0.035	0.022-0.055	4.37
Mansehra	Rd-FMN1	7.65424	0.576	0.548-0.604	72.00
(N. W. F. P.)	Rd-FMN3	2.95149	0.038	0.034-0.044	4.75
	Rd-FMN5	2.30570	0.039	0.033-0.045	4.88
Durgai	Rd-FDUR1	2.24203	0.151	0.110-0.206	18.87
	Rd-FDUR2	1.56790	0.043	0.035-0.053	5.37
	Rd-FDUR4	2.18480	0.014	0.008-0.023	1.75

Rd = *Rhyzopertha dominica;* S = Sindh; SGG = Sindh Govt. Godown, Karachi; HD = Hyderabad; P = Punjab
Lh: Lahore; SRG = Sargodha; PW = Peshawar; MN = Mansehra; DUR = Durgai; F = North West Frontier Province

Table 3: Resistance to phosphine in khapra beetle, *Trogoderma granarium* Everts (final stage larvae)

Locality	Strains	Slope	LD_{50}	95 % fiducial limit	R.F.
Karachi	Tg-SGGIII	2.48947	0.917	0.807-1.043	Reference
(Sindh)	Tg-SGGII	3.22686	16.731	8.972-31.198	18.24
	Tg-SGGI	1.82383	9.678	7.623-12.287	10.55
Multan (Punjab)	Tg-PMUL	4.2408	13.966	7.932-24.592	15.23
Kizaffargarh	Tg-PMZ (R)	3.37783	14.249	7.838-25.903	15.54
(Punjab)	Tg-PMZ (J)	1.68532	32.333	3.942-265.213	35.25

Tg = *Trogoderma granarium*; SGG: = Sindh Govt. Godown, Karachi; P- Punjab Province; MUL = Multan,
MZ = Muzaffargarh; J = Jhalri; R = Rak-Harpalloo

Table 4: Resistance to phosphine in rice weevil, *Sitophilus oryzae* L. (adult)

Locality	Strains	Slope	LD_{50}	95 % fiducial limit	R.F.
Karachi	So-SGG II	2.20746	0.0123	0.0103-0.0147	1.12
(Sindh)	So-SGG I	2.86256	0.012	0.0080-0.0160	1.09
	So-SGG III	2.49460	0.013	0.0110-0.0150	1.18
	So-SBQ	2.35386	0.014	0.0120-0.0170	1.27

So = *Sitophilus oryzae;* SGG = Sindh Govt. Godown, Karachi; BQ = Bin Qasim Rice Godown, Landhi, Karachi

phosphine resistant strains. Furthermore, it has been shown by Rajendran (1992) that high level of resistance can result from selection with phosphine at any stage of the life cycle. Failure of phosphine fumigation attributable to resistance in *T. granarium* and *R. dominica* has been recorded from Multan, Punjab province. The warehouses in Multan have a history at least 12 years of phosphine fumigation. Though sufficient information is not available such situation may be prevalent in some other localities. The primary cause of the development of resistance is the use of substandard fumigation techniques, in particular the leakiness of the warehouses that leads to sublethal doses. Gas leaks not only for the mud plastered doors and ventilators but also through the porous walls. Current fumigation practices require high standards of sealing and dosing. The problem can largely be circumvented by enclosing the grain stacks in polyethylene sheets (Tyler *et al*., 1983; Ahmed, 1989; Chaudhry *et al*., 1989). Tyler *et al*. (1983) and Taylor (1989) have suggested increased fumigation exposure periods to assure complete insect mortality. GASGA (1986) has suggested more than 5 days exposure periods. Since fumigation, under gas proof polyethylene sheets permits the retention of gas for longer period, this technique should be preferred over the usual practice of total warehouse fumigation. To ensure standard fumigation, the operators must be adequately trained. Where phosphine resistance has developed to very high levels, methyl bromide can be considered as an alternative means to control insects (Charkrabarti and Bell, 1993).

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