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Phosphine Resistance Status in Lesser Grain Borer *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) Strains Originating from the Tropical Countries

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Abstract

Stored product beetles that are resistant to the fumigant phosphine (hydrogen phosphide) have been reported for more than 50 years in many places worldwide. The high levels of phosphine resistance in lesser grain borer Rhyzopertha dominica (F.) have been noted from several countries including Bangladesh. This study was designed to evaluate the status of resistance to phosphine in Bangladeshi R. dominica and to verify the possible comparison among other phosphine resistant strains from tropical countries viz. Burkina Faso and Malaysia. The data reported and summarized here showed varied levels of resistance compared to the laboratory phosphine susceptible strain (RDLAB). Rhyzopertha dominica strains originating from Bangladesh (RDBGD) and Burkina Faso (RDBKF) exhibited higher levels of resistance to phosphine compared to the Malaysian strains (RDMAL). Analysis of dose-response data indicated that the RDBGD and RDBKF strains were the most resistant to phosphine under different exposure periods. At LC50, these two strains were more than 80-fold more resistant at all exposures compared to the susceptible strain. Results also revealed that RDBGD and RDBKF strains required a relatively high concentration of 334.94 and 240.081 mg L⁻¹ for 99% mortality. The mean survival time (MST) for the phosphine resistant and susceptible also varied significantly. The maximum MST was recorded for RDBGD and RDBKF strains. The present findings further confirmed that the Bangladeshi originated R. dominica strain contained higher resistance to phosphine compared to strains from other countries. This study could be useful in developing management strategies to prevent stored grain from being infested by resistant strains of R. dominica in tropical countries.

Keywords: phosphine, resistance, grain borer, tropical countries

Introduction

Tropical environments provide most favourable conditions for insect growth and multiplication in the ideal medium of food stored by humans. Some insects begin infestation in the field and continue attack in storage. In tropical developing countries, especially in humid zones, pest infestation in stored food is inevitable due to lack of proper management. Fumigation with phosphine (hydrogen phosphide, PH3) is the preferred means of controlling pests in many countries of the tropics.

The first global survey of phosphine resistance was conducted during the 1970s by Champ and Dyte (1976) who used a discriminating dose bioassay on adult insects (FAO 1975) and documented the occurrence of phosphine resistance in several key stored grain pest species across many countries. However, further studies indicate a substantial increase in phosphine resistance in stored product pests worldwide including Australia (Nayak et al. 2013), India (Kaur et al. 2015), Brazil (Lorini et al. 2007), and Malaysia (Rahim et al. 2004).

Phosphine resistance has been detected in most of the major species of stored product insects including *Rhyzopertha dominica*, *Tribolium spp., Sitophilus spp., Oryzaephilus surinamensis* and *Cryptolestes* spp. (Taylor, 1986). This appears to be due to the increased selection pressure in some countries (Halliday *et al*, 1983; Friendship *et al.*, 1986). The distribution of phosphine resistance is not known in detail but it is likely that resistance to phosphine is not an unusual phenomenon in most countries in which the fumigant has been used. The reported incidence of phosphine resistance is particularly high in the lesser grain borer *R.dominica* (F.) and two species of *Tribolium* (Champ and Dyte, 1976; Taylor, 1986). It has also been noticed that some of the strains of *R. dominica* collected from the field showed very high levels of resistance to phosphine (Mills, 1983). However,

fumigation failures have been reported from Bangladesh that were caused, in part, by high levels of phosphine resistance in *R.dominica* (Mills, 1983, Tylor *et al.* 1983). Potential replacement fumigants lack the versatility possessed by phosphine. The indiscriminate uses of phosphine fumigation may lead to the increase of risk in control failures. The aim of the present investigation was to examine the relative phosphine toxicity relationship between concentration and time exposure to phosphine resistant and susceptible strains of *R.dominica* originating from the tropical countries of Bangladesh, Malaysia and Burkina Faso.

Materials and Methods

Insects

Phosphine susceptible laboratory strain RDLAB and phosphine resistant strains RDBGD, RDBCF and RDMAL of the lesser grain borer *Rhyzopertha dominica*, originated from a population sampled in Bangladesh, Burkina Faso and Malaysia respectively, were used in this study. The frequency of phosphine-resistant adults in samples from *R. dominica* field populations was determined by earlier using the Food and Agriculture Organization Method No. 16 (FAO, 1975) (Reichmuth, 1983; Mills 1983; Rahim and Sulaiman, 1999). All these strains had been cultured for many decades at the Federal Research Centre for Cultivated Plants – Julius Kühn-Institut, Berlin, Germany (Table 1). They were reared on whole wheat and dried cassava in a controlled temperature room set at 25°C, 16L: 8D h photoperiod and 75% RH.

Fumigation Procedure

Production of phosphine

Pure (100%) phosphine was generated from magnesium phosphide granules (*Degesch Co.*, Frankfurt am Main) reacting to water ($Mg_3P_2 + 6H_2O = 3Mg (OH)_2 + 2PH_3$) (Hasan and Reichmuth, 2004). The method provides a convenient source of phosphine for dosing purposes over a period of time, depending on the rate of removal.

Table 1: Culturing details and specification of reference and phosphine-resistant strains of <i>R.dominia</i>	Table 1: Culturing	d specification of ref	rence and phosphine-resistant s	strains of R.dominica
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Strains	Origin of strains	Collection	&	# adults to seed	Adult wt. (mg)	Original Culture
		Test year		culture	Mean \pm SE*	maintained
RDLAB (S)	Germany	1978		150	1.45 ± 0.02	BBA Inst., Berlin
RDBGD(R)	Bangladesh	1982		150	1.42 ± 0.02	CSL,UK
RDBCF (R)	Burkina Faso	1993		150	1.25 ± 0.02	BBA Inst., Berlin
RDMAL (R)	Malaysia	1993		150	1.31 ± 0.01	BBA Inst., Berlin

S- Susceptible; R- Resistant; * mean of four replicates each having 50 insects.

Fumigation Test

Newly emerged (8-10 day-old) mixed-sex adults of R. dominica were selected for phosphine fumigation. The fumigation was carried out in cylindrical steel gauze cages (5.0 x 1.5 cm) containing a small quantity of diet (0.5 g). Treatments of phosphine consisted of three replicates containing 25 insects each. An untreated reference sample was kept for each type.

Glass Dressel flasks (2.5 l) were used as fumigation chambers. The flasks were connected to each other with a gas-tight PVC tube. A small electric pump was set up to recirculate the gas evenly throughout the apparatus. A silicon rubber septum fitted in the narrow tube, protruding from the gas reservoir, was used to inject and withdraw a phosphine sample with a gas-tight Hamilton syringe. An equal volume of air was withdrawn from the flask before injecting the respective concentration of phosphine. The sealed Dressel flasks contained saturated sodium chloride solution to achieve about 75% RH by the end of exposure period. The time of injection was recorded and the pump was operated to distribute the gas evenly. The concentration of phosphine in each of the flasks was assessed at the beginning and end of the fumigation period using quantitative gas chromatography (Hasan and Reichmuth, 2004). The glass flasks containing the experimental insects

were then disconnected without losing any gas and immediately transferred to the CT-room conditioned at 25°C, where they were kept throughout the exposure periods.

Post-fumigation

The adults *R. dominica* were fumigated at 25°C at a range concentrations of phosphine, 0 (control), 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg/l and exposure periods of 4.0, 5.0, 6.0 7.0 and 8h. The Exposure periods for each test was designed to encompass the dose-response range ensuring, where possible, that a 100% kill was achieved at the maximum dose. At the end of desired fumigation periods, the insect cages were removed from the fumigation chambers and kept in small petri dishes containing food media for assessment of mortality. The mortality of adult *R. dominica* was recorded each day after exposure to phosphine until complete mortality occurred.

Data processing

The LCs and LTs were estimated from a Probit regression analysis using PROC PROBIT from SAS version 9 (SAS 2002). Comparison among populations for differences in their level of resistance were made after computing resistant ratios based on the LC50 value for the population of interest divided by the LC50 of the laboratory-susceptible strain, referred to as the RR50.

Mean Survival Times (MST): The mean survival time (MST) for adult *R. dominica* strains exposed to phosphine was calculated following the model developed by Cheng and Ducoff (1989):

 $MST = 1/n \Sigma(t \times Yt)$

Where, n = number of beetles in the group, t = hour t, and Yt = number of beetles that died at hour t.

Results

The effect of phosphine on the mortality of resistant adult R. dominica originating from tropical countries varied significantly (P <0.001). Results of dose-mortality studies with three phosphine resistant populations of R. dominica and susceptible laboratory strain are reported in Tables 2 and 3. The results indicated clearly that the adults R. dominica originated from Burkino Faso and Bangladesh required higher concentration of phosphine at the mortality level of LC₅o when compared with resistant RDMAL strain from Malaysia. The strain RDBCF was 152-fold more resistant than the susceptible strain RDLAB when exposed for 5 h to phosphine (Fig. 5). However, the strains RDBCF and RDBGD showed more or less similar resistance as the RDLAB strain when exposed to 6, 7 and 8 h exposures at LC50 level (Table 2 & Fig. 5). The RR50 for the RDMAL strain gradually decreased as the exposure levels increased (Fig. 5). This pattern of relative resistance was also reflected in the LC₉₉ values (Table 2). At the LT₅₀, adult R. dominica exhibited several fold resistance to phosphine compared to the susceptible strain particularly at lower exposure levels (Table 2). The strains RDBGD and RDBCF showed more resistance to phosphine compared to RDMAL strain at the exposure up to 2h exposure at LT₅₀ level, but they exhibited more or less similar trends of resistance at higher exposures levels of 6 to 8 h (Table 2). However, this trend did not follow at the mortality level of LT99 at which the RDBCF and RDBGD strains showed higher resistance to phosphine compared to strain RDMAL. Table 3 shows, the higher level of resistance in RDBCF and RDBGD at lower concentration of 0.25 and 0.50 mg/l. Moreover, the strain RDBGD required 193.32 h for 99% mortality at concentration 3 mg/l. The present findings show that the estimated fiducial limits for LT₅₀ and LT₉₉ values were narrow and overlapping for all the phosphine resistant strains tested, indicating a good fit of the data in the linear regression model (Table3).

The mean survival time (MST) for the phosphine resistant and susceptible strains of R. dominica varied significantly (P < 0.001). The $F_{(df7,39)}F_{crit2.36)}$ values for phosphine concentration for the strains RDBGD, RDBCF, RDMAL and RDLAB were 43.72, 40.91, 10.17 and 5.12 respectively. The maximum MST was recorded for RDBGD and RDBKF strains in all concentrations (Figs. 1 & 2) while the minimum was in susceptible strains RDLAB (Fig. 4). Figure 4 shows that the MST for RDLAB strains was below

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to the range of 3 day in all concentrations and exposures except 0.50 mg/l at 4 h exposure. The strains RDBCF and RDBGD exhibited higher fold of resistance in terms lethal time at lower phosphine concentrations ranging from 0.25 to 2.0 mg/l compared to strain RDMAL (Fig. 6). However, the RDMAL showed higher fold of resistance compared to RDBGD strain at concentration ranging from 4.0 to 6.0 mg/l.

Table 2: Probit analysis for the mortality responses of phosphine susceptible and resistant adult *R. dominica* after different exposure periods to varying concentrations of phosphine at 25°C.

Expo -sure (hr)	Strains	n	LC₅₀ mg/l (95% FL*)	LC ₉₉ mg/l (95% FL*)	Slope ± SE	Intercept±S E	χ² values (df) P
	RDLAB	600	0.03 (0.01-0.10)	0.04 (0.01-0.10)	0.15±0.05	-0.45 ± 1.02	62.28 (22) P<.0.001
	RDBGD	600	3.54 (2.47-5.89)	452.23 (107.18 - 894.00)	0.28±0.04	0.02 ± 0.43	49.47 (22) P< 0.001
4	RDBCF	600	5.97 (4.32-9.62)	1704.00 (411.32-2453.00)	0.38 ±0.04	-0.29 ± 0.38	23.14 (22)
	RDMAL	600	3.25 (1.67-12.85)	1837.00 (120.08-22463.0)	0.16 ±0.05	0.90 ± 0.61	P< 0.393 105.70(22) P<0.001
	RDLAB	578	0.03 (0.01-0.09)	0.03 (0.01-0.09)	0.13 ±0.05	-0.43 ± 1.11	25.22 (22)
	RDBGD	259	2.57 (1.75- 4.01)	90.02 (29.89-109.50)	0.19±0.04	0.57 ± 0.46	P> 0.286 98.16 (22)
5	RDBCF	237	4.09 (2.62- 8.62)	3754.00 (400.59-4609.00)	0.29 ±0.05	-0.15 ± 0.54	P <0.001 38.35 (22)
	RDMAL	375	1.34 (0.68- 2.24)	601.81 (88.28-1178.23)	0.22±0.04	-0.19 ± 0.59	P<0.017 67.60 (22)
	RDLAB	568	0.02 (0.01-0.07)	0.03 (0.01-0.08)	0.13±0.05	-0.39 ± 1.11	P <0.001 35.37 (22) P<0.035
	RDBGD	299	1.82 (1.23 - 2.65)	69.97 (25.98- 524.45)	0.22±0.02	-0.07 ± 0.35	86.53 (22) P < 0.001
6	RDBCF	307	1.67 (1.18- 2.31)	312.29 (91.52- 430.13)	0.25±0.03	-0.46 ± 0.51	35.74 (22) P< 0.032
	RDMAL	381	0.75 (0.42-1.09)	143.49 (44.65- 284.24)	0.23± 0.04	-0.87 ± 0.57	42.97 (22) P<0.002
	RDLAB	593	0.02 (0.001-0.08)	0.02 (0.01-0.07)	0.12±0.05	-0.11 ± 1.16	21.60 (22) P> 0.484
	RDBGD	312	1.65 (1.08-2.42)	93.97 (31.66-910.81)	0.20±0.04	0.08 ± 0.49	77.41 (22) P<0.001
7	RDBCF	318	1.48 (0.97- 2.13)	308.83 (79.77- 474.68)	0.24±0.04	-0.45± 0.53	44.69 (22) P < 0.003
	RDMAL	408	0.61 (0.33- 0.90)	65.96 (25.10-412.12)	0.21±0.04	-0.79 ± 0.61	48.00 (22) P<0.001
	RDLAB	599	0.01 (0.002-0.06)	0.02 (0.01 – 0.08)	0.11±0.05	-0.10 ± 1.16	11.02 (22) P> 0.974
	RDBGD	338	1.17 (0.71- 1.71)	334.94 (79.68 – 682.00)	0.24±0.04	-0.54± 0.58	45.27 (22) P< 0.002
8	RDBCF	334	1.25 (0.85-1.72)	240.08 (73.91-421.03)	0.25±0.04	-0.61± 0.53	35.98 (22) P< 0.031
	RDMAL	437	0.32 (0.10 - 0.57)	133.11 (35.31- 262.26)	0.20±0.04	-0.81±0.74	44.58 (22) P<0.003

^{*}FL-fiducial limits

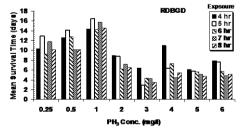


Fig 1: Mean survival time of phosphine resistant Bangladeshi strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

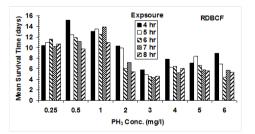


Fig 2: Mean survival time of phosphine resistant Burkina Faso strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

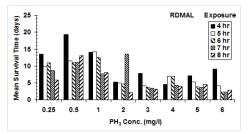


Fig 3: Mean survival time of phosphine resistant Malaysian strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

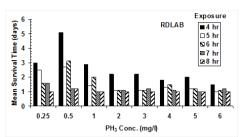


Fig 4: Mean survival time of phosphine susceptible laboratory strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

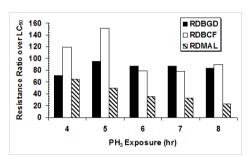


Fig 5: Resistance ratio in different strains of adult *R. dominica* over 50% lethal concentration fumigated at different exposures of phosphine.

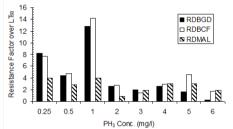


Fig 6: Resistance ratio in different strains of adult *R. dominica* over 50% lethal time fumigated at different concentrations of phosphine.

Table 3: Probit analysis for the mortality responses of phosphine susceptible and resistant adult *R. dominica* after five days exposures to phosphine at different concentrations at 25°C.

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PH₃ Conc mg/l	Strains	n	LT ₅₀ (hr) (95% FL)	LT ₉₉ (hr) (95% FL)	Slope ± SE	Intercept± SE	χ² values (df) P
	RDLAB	357	1.89 (0.36- 2.80)	8.16 (6.59-18.21)	0.27 ±0.03	0.35±0.69	13.79 (13) P< 0.388
	RDBGD	76	15.59 (9.43-37.70)	230.19(37.44-317.02)	0.59 ±0.14	2.44±0.75	21.37 (13) P<0.065
0.25	RDBCF	92	14.56 (9.66-65.84)	303.13(195.82- 491.01)	0.78± 0.10	1.03±0.60	7.19 (13) P<0.891
	RDMAL	138	7.55 (6.55- 10.81)	40.76 (20.27-77.47)	0.45±0.06	1.57±0.58	20.37 (13) P<0.086
	RDLAB	326	3.47 (2.29- 4.05)	8.52 (6.99-14.70)	0.28±0.03	-0.12±0.52	28.24 (13) P< 0.008
	RDBGD	375	15.29(10.27- 118.21)	200.248(47.94- 416.29)	0.74±0.15	1.90±0.73	12.59 (13) P< 0.479
0.50	RDBCF	84	16.43(10.26- 72.61)	374.15(59.41-543.16)	0.93± 0.09	0.64±0.48	4.05 (13) P< 0.990
	RDMAL	115	9.88 (7.98- 20.04)	104.58 (36.65-289.61)	0.62± 0.08	1.05±0.57	8.87 (13) P< 0.782
	RDLAB	359	1.64 (0.093- 2.68)	8.24 (6.52- 27.65)	0.26±0.03	0.31±0.71	14.30 (13) P<0.352
	RDBGD	375	21.10(17.26- 63.19)	344.55(116.92- 517.23)	0.59±0.19	3.13 ±0.77	24.10 (13) P<0.03
1.0	RDBCF	95	23.31(14.9-39.14)	125.10(68.15-201.73)	0.84±0.11	0.56±0.63	4.97 (13) P<0.975
	RDMAL	165	6.53 (5.96- 7.41)	33.69 (20.68-99.42)	0.43±0.05	1.07±0.49	14.17 (13) P<0.36
	RDLAB	344	2.09 (0.98- 5.21)	8.61 (4.61-13.28)	0.87±0.02	0.09±0.04	23.91 (13) P<0.020
	RDBGD	375	5.47 (4.79- 6.07)	34.96 (20.35-131.67)	0.39±0.04	0.69±0.55	18.53 (13) P< 0.138
2.0	RDBCF	188	5.83 (5.43 - 6.27)	19.56 (14.82- 32.17)	0.36±0.04	1.26±0.49	16.87 (13) P< 0.205
	RDMAL	301	1.65 (0.03-2.99)	49.41 (17.77-120.87)	0.31±0.03	-0.10±0.63	11.65 (13) P< 0.559
	RDLAB	362	2.37 (0.14 - 3.31)	6.40 (5.30-22.65)	0.26±0.03	-0.29±0.70	22.55 (13) P< 0.047
	RDBGD	302	4.73 (2.29-8.98)	193.32(95.17-319.16)	0.28±0.04	0.32±0.80	31.82 (13) P< 0.002
3.0	RDBCF	264	3.32 (1.60- 4.15)	35.93 (18.36-83.97)	0.34±0.03	0.01±0.54	12.75 (13) P< 0.466
	RDMAL	270	4.38 (3.46-4.94)	11.756 (9.14- 22.22)	0.27±0.03	0.86±0.53	33.23 (13) P< 0.002
	RDLAB	357	1.49 (0.04- 2.59)	9.17 (6.96- 49.90)	-0.33±0.70	0.27±0.03	14.58 (13) P< 0.331
	RDBGD	253	3.84 (0.04-4.98)	30.32 (13.39–79.12)	0.76±0.69	0.30±0.04	39.02 (13) P<0.001
4.0	RDBCF	237	4.34 (3.29-4.94)	30.87 (18.10-126.35)	-0.05±0.36	0.38±0.02	5.27 (13) P<0.968
	RDMAL	246	4.55 (3.97-4.97)	17.07 (12.97-28.78)	0.43±0.42	0.33±0.03	13.67 (13) P<0.397
	RDLAB	364	2.26 (0.67- 3.04)	6.21 (5.36-10.07)	-0.34±0.70	0.26±0.03	6.13 (13) P<0.941
	RDBGD	234	1.89 (0.15 – 4.26)	72.36 (31.65–97.25)	0.22±0.78	0.37±0.05	12.51 (13) P< 0.485
5.0	RDBCF	196	5.45 (4.29- 6.39)	81.25 (29.73-150.17)	0.36±0.54	0.42±0.04	11.93 (13) P< 0.533
	RDMAL	261	3.55 (0.27- 4.64)	31.07 (14.08- 102.51)	0.39±0.60	0.32±0.03	28.54 (13) P<0.007
	RDLAB	371	1.194 (0.21 – 2.43)	5.32 (3.92 - 7.89)	0.25±0.03	0.22±0.78	13.67 (13) P< 0.39
	RDBGD	247	4.50 (2.17 -6.83)	51.97 (30.51–74.98)	0.32±0.05	0.56±0.75	27.57 (13) P< 0.010
6.0	RDBCF	239	3.98 (2.39-4.75)	45.97 (21.57-70.88)	0.37±0.03	0.02±0.48	9.44 (13) P<0.738
	RDMAL	276	4.29 (3.17- 4.92)	11.42 (8.76-24.44)	0.86±0.55	0.27±0.03	42.67 (13) P<.0.001

Discussion

Our results revealed that the response of the adults of phosphine resistant and susceptible strains of *R. dominica* to phosphine fumigation varied significantly (P<0.001). All the phosphine resistant strains exhibited high levels of resistance compared to the susceptible strain. The strain RDBCF showed more resistance to phosphine compared to other resistant strains tested. Results also indicate that for resistant as well as susceptible strains, time of exposure to phosphine was more critical a factor for effective fumigation than concentration (Tables 1 & 2). The MST in all the resistance strains varied substantially. The MST values were found to be phosphine concentration dependent (Figs. 1-4) and it decreased as the concentration increased. The values of resistance ratio indicate that RDBGD and RDBCF strains were several folds higher resistant compared to the RDMAL

strain (Fig. 5). Phosphine resistance in lesser grain borer *R. domiica* has been reported from a number of countries and it is to be expected that this number will increase (Champ and Dyte, 1976; Mills, 1983; Taylor, 1986; Reichmuth, 1986; Price and Mills, 1988 Opit *et al.* 2012, Afful *et al*, 2018). Moreover, phosphine resistance has been detected in most of the other major species of stored product insects including *Tribolium* spp., *Sitophilus* spp., *Oryzaephilus surinamensis* and *Cryptolestes* spp. (Taylor, 1986). This appears to be due to the increased selection pressure in some countries (Halliday *et al*, 1983; Friendship *et al.*, 1986). Our results confirm the findings of others who reported the geographic variation in phosphine resistance in *R. dominica* (Collins *et al.* 2000, Benhalima *et al.* 2004, Lorini *et al.* 2007, Cato *et al.* 2017 Auful *et al.* 2018).

The distribution of phosphine resistance is not known in details but it is likely that resistance to phosphine is not an unusual phenomenon in most countries in which the fumigant has been used. The reported incidence of phosphine resistance is particularly high in the lesser grain borer *R.dominica* (F.) and two species of *Tribolium* (Champ and Dyte, 1976; Taylor, 1986). It has also been noticed that some of the strains of *R. dominica* collected from the field showed very high levels of resistance to phosphine (Mills, 1983; Afful et al., 2018). Fumigation failures have been reported from Bangladesh that were likely caused by high levels of phosphine resistance in those populations of *R.dominica* (Tylor *et al.* 1983). Mills (1983) also reported that the susceptible individuals of *R.dominica* could be killed at 0.03 mg/l phosphine dose at 20 h exposure while a field strain from Bangladesh could survive as high as 1.45 mg/l of phosphine for 20 h, and 72 hr exposure to this dose was require to produce a complete kill of these insects. Rahim et al. (1999) reported the presence of phosphine rresistance in stored grain insects including *R. dominica* from nine of the 13 states in Malaysia. The molecular mechanisms of phosphine resistance in *R. dominica* as well as other stored product pests are multifaceted and it is still under investigations (Chaudhry, 2000, Jagadeesan *et al.* 2012, Schlipalius *et al.* 2012, Chen *et al.* 2015).

Phosphine fumigation has the advantages of needing relatively low dosages when compared with other fumigants, being cost-effective, and least effects on the quality of fumigated grains. Consequently, despite the drawbacks in the existing storage and fumigation practices in the tropical countries, phosphine fumigant has helped preservation of food grains economically and at the same time meeting consumer demands with regard to the quality of the food grain. It is possible that these resistant strains could be controlled in tropical countries using phosphine fumigation, if conducted properly. This study also suggests that proper resistance assessment techniques can help to determine occurrence of phosphine resistance in populations of *R. dominica* in the field level of tropical as well as developing countries.

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References

- AFFUL E., ELLIOTT, B., NAYAK, M.K., AND T.W. PHILLIPS, 2018: Phosphine Resistance in North American Field Populations of the Lesser Grain Borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Journal of Economic Entomology **111**, 463-469.
- BENHALIMA H., CHAUDHRY M.Q., AND MILLS K.A. AND N.R. PRICE, 2004: Phosphine resistancein stored-product insects collected from various grain storage facilities in Morocco. Journal of Stored Product Research **40**, 241–249.
- CATO A. J., ELLIOTT B., NAYAK M.K. AND T.W. PHILLIPS, 2017: Geographic variation in phosphine resistance among North American populations of the red flour beetle. Journal of Economic Entomology **110**, 1359–1365.
- CHAMP B.R. AND C.E. DYTE, 1976: Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Production and Protection Series, 5, FAO, Rome, Italy.
- CHAUDHRY M.Q. 2000: Phosphine resistance. Pesticide Outlook 11, 88–91.
- CHEN Z., SCHLIPALIUS D., OPIT G., SUBRAMANYAM B. AND T.W. PHILLIPS, 2015: Diagnostic molecular markers for phosphine resistance in U.S. populations of *Tribolium castaneum* and *Rhyzopertha dominica*. PLoS One **10:** 121-143.

- CHENG C. C. AND H.S. DUCOF,F 1989: High-dose mode of death in *Tribolium*. Entomologia Experimentalis et Applicata **51**: 189–197. Collins P.J., Daglish G.J., Nayak M.K., Ebert P.R., Schlipalius D.I., Chen, W., Pavic J., Lambkin T.A., Kopittke R.A. and B.W. Bridgeman, 2000: Combating resistance to phosphine in Australia. In E. J. Donahaye, S. Navarro and J. G. Leesch (Eds.), Proceedings of the International Conference for Controlled Atmosphere and Fumigation in Stored Products, Fresno CA, pp 593–607.
- FAO, 1975: Recommended methods for detection and measurement of resistance of agricultural pests to pesticides tentative method for adults of some major pest species of stored cereals, with methyl-bromide and phosphine FAO Method No 16. FAO Plant Protection Bulletin 23: 12–25.
- FRIENDSHIP C.A.R., HALLIDAY D. AND A.H. HARRIS 1986: Factors causing resistance to phosphine in insect pests of stored produce V. Howe (Ed.), Proceedings of GASGA Seminar on Fumigation Technology in Developing Countries, Tropical Development and Research Institute, London, pp. 141-149
- HALLIDAY W.R., ARTHUR F.R AND J.L. ZETTLER 1983: Resistance status of red flour beetle (Coleoptera: Tenebrionidae) infesting stored peanuts in the Southeastern United states. Journal of Economic Entomology 81: 74-77.
- HASAN M.M. AND CH. REICHMUTH, 2004: http://onlinelibrary.wiley.com/advanced/search/results?searchRowCriteria%5B0%5D.fieldName=author&start=1&resultsPerPage=20&searchRowCriteria%5B0%5D.queryString=%22M.%20M.%20Hasan%22Relative toxicity of phosphine against the bean bruchid Acanthoscelides obtectus (Say) (Col., Bruchidae). Journal Applied Entomology 128: 332-336.
- JAGADEESAN R., COLLINS P.J., DAGLISH G.J., EBERT P.R., AND D.I. SCHLIPALIUS, 2012: Phosphine Resistance in the Rust Red Flour Beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): Inheritance, Gene Interactions and Fitness Costs. PLoS ONE **7(2):** 31582. doi:10.1371/journal.pone.0031582
- Kaur R., Subbarayalu M., Jagadeesan R., Daglish G.J., Nayak M.K., Naik H.R., Ramasamy S., Subramanian C., Ebert P.R. and D.I. Schlipalius, 2015: Phosphine resistance in India is characterised by a dihydrolipoamide dehydrogenase variant that is otherwise unobserved in eukaryotes. Heredity 115: 188–194.
- LORINI I., COLLINS P.J., DAGLISH G.J., NAYAK M.K. AND H. PAVIC, 2007: Detection and characterization of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science **3:** 358–364.
- MILLS K. A. 1983: Resistance to the fumigant hydrogen phosphide in some stored-product species associated with repeated inadequate treatments. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie **4(1/3):** 98-101
- NAYAK M.K., HOLLOWAY J.C., EMERY R.N., PAVIC H., BARTLET J. AND P.J. COLLINS, 2013. Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) Coleoptera: Laemophloeidae): its characterisation, a rapid assay for diagnosis and its distribution in Australia. Pest Management Science **69:** 48-53.
- OPIT G.P., PHILLIPS T.W., AIKINS M.J. AND M. M. HASAN, 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. Journal of Economic Entomology 105: 1107–1114.
- PRICE L. A. AND K. A. MILLS, 1988: The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. Journal of Stored Product Research 24: 51-59.
- RAHIM M. AND Z. SULAIMAN, 1999: Survey of resistance of stored grain pests to phosphine in Malaysia. Proceeding of International Conference on Plant Protection in the Tropics **5:** 226–229.
- RAHIM M., FARIDAH, M.E. AND M. RASALI, 2004. Current status of phosphine resistance in stored grain insects in Malaysia. Journal of Tropical Agriculture and Food Science 32(1): 101–107
- REICHMUTH CH. 1986. A quick test to determine phosphine resistance in toredproducts

research. GASGA Newsletter 15: 14-15.

SAS. 2002. User's Guide, v. 8. SAS Institute, Cary, NC.

- SCHLIPALIUS D.I., VALMAS N., TUCK A.G., JAGADEESAN R., MA L. AND R. KAUR, 2012: A core metabolic enzyme mediates resistance to phosphine gas. Science 338: 807–810.
- TAYLOR R.W.D. 1986: Response of field strains of some insect pests of stored products. (In) Proceedings GASGA Seminar on Fumigation Technology in Developing Countries, Tropical Development Research Institute, London pp 132–140.
- TYLER P.S., TAYLOR R.W.D. AND D.P. FLEES, 1983: Insect resistance to phosphine fumigation in food warehouses in Bangladesh. International Pest Control 25: 10–13.

Phosphine resistance in Saw-toothed Grain Beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) in the United States

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