Mortality time of immature stages of susceptible and resistant strains of *Sitophilus oryzae* (L.) exposed to different phosphine concentrations

Wang, D-X.*#, Ma, X-H., Bian, K.
School of Food Science and Technology, Engineering Research Center of Grain Storage and Security of Ministry of Education, Henan University of Technology, Zhengzhou, 450052, Henan P. R. China.
Email: wangdx@haut.edu.cn

* Corresponding author
\# Presenting author

DOI: 10.5073/jka.2010.425.170

Abstract

The mortality time on egg, larvae and pupae of four strains with resistance factor 1, 69, 160 and 295 to phosphine of *Sitophilus oryzae* (L.), which were expressed in R_1, R_{69}, R_{160} and R_{295} in this report, respectively, were investigated with stable concentrations of 100, 300, 500, 700 and 900 mL m^{-3} of phosphine in a well sealed fumigation chamber. The mortality time on all immature stages was about 10 d for strain R_1, more than 15 d for all resistance strains exposed to 100 mL m^{-3} of phosphine. Mortality time on egg and larvae of R_1 was 9 and 6 d at 300 and 700 mL m^{-3}, respectively. But it was only 4 d and 2 d for pupae of R_1 at 700 and 900 mL m^{-3}, respectively. The mortality time on immature stages of R_{69} was 12 and 5 d with the 300 and 700 mL m^{-3}, respectively. And that on immature stages of strain R_{160} and R_{295} was 15 and 10 d with phosphine of 300-700 mL m^{-3}, respectively. With the fumigant of 900 mL m^{-3}, the full death time were 5 d for larval of all strains, 5d for pupae and egg of R_1 and more that 8 or 9 d for pupae and egg of three resistance strains. The egg and pupae of *S. oryzae* were the most tolerant stages to phosphine both for susceptible and resistance strains.

Keywords: *Sitophilius oryzae*, Immature stage, Phosphine, Mortality time

1. Introduction

The importance of phosphine usage to control stored-grain insect pests has increased due to international phasing out of the fumigant methyl bromide and the difficult to develop new fumigants in recent years and in the future. Phosphine has several advantages that have made it attractive for use in the grain industry. It is relatively easy to apply (compared with other fumigants), versatile and inexpensive, with international acceptance as a near residue-free treatment (Emery et al. 2003) or readily available without restrictions. High-level resistance to phosphine reported in Bangladesh (Tyler et al., 1983) and later in India and other countries (Zettler, 1993; Rajendran and Narasimhan, 1994; Chaudhry et al., 1997; Daglish and Bengston, 1998; Zeng, 1999; Collins et al., 2002; Wang et al., 2004; Pimentel, 2009), threatens the useful life of this fumigant and causes control failures in many species and cases. To protect the long-term use of phosphine in the grain storage industry and continue to market low residue product in the world grain trade it is important to manage development of phosphine resistance in stored-grain insects (Newman, 1998). Knowing the mortality time or full death time on insect population during fumigation is a key for the resistance management. The exposure time to the fumigant is more important than its dosage in many cases (Annis, 1993). The concentration and exposure time product are usually different owing to variable concentration, insect species, strains for same species or population (Price, 1985) and different stages in a species (Hole et al., 1976). Exposure time needed to control insects using phosphine is becoming longer due to resistance. For instance it was 7 d (Taylor and Harris, 1994; Bengston et al., 1997; Rajendran et al., 2001; Rajendran and Muralidharan, 2001), later 8 d (Rajendran and Gunasekaran, 2002; Collins et al., 2005), 6-9 d (Price and Mills, 1988; Liang, et al., 1999; Collins et al., 2002) and more than 7 d (Sayaboc, et al., 1998) for resistance *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) under the concentration of 0.2, 0.3, 0.5 and 0.7 g m^{-3}. There are several reports about phosphine resistance on other insect species (Daglish et al., 2002; Wang et al., 2006). It was recommended that exposure time should be more than two, three and four weeks relatively with 100 to 350 mL m^{-3} of phosphine concentration according to the China national recommended standard on phosphine fumigation (Wang et al., 2002). But, in some cases, when even applying these recommended standards, insect survival was still encountered. Furthermore, the mortality time in insects is useful reference to successful fumigation while maintaining phosphine at effective level. Although *Sitophilus* *oryzae* (L.) (Coleoptera: Curculionidae) is one of the world's most serious pests in stored grain, there are a few data on the practical significance of phosphine resistance on this species. An Australian susceptible strain, a homozygous resistant strain exhibiting a level of resistance common in Australia and an unselected field strain from China with a much stronger resistance were investigated (Daglish et al., 2002). The objective of the present work was to study the mortality time on egg, larvae and pupae of a susceptible strain and three different levels of resistant strains of *S. oryzae* to phosphine, from three provinces of China.

2. Materials and methods

2.1. Insects

Four strains of *S. oryzae* with different levels of resistance to phosphine were received from Department of Employment, Economic Development and Innovation, Queenland, Australia (QDEEDI), and three grain depots in China. All strains were maintained without further exposure to phosphine in the Stored Product Insect Research Laboratory, Henan University of Technology, Henan, China. Resistance factor was examined followed the standard FAO test method to phosphine (Anonymous, 1975). Resistance factor of Strain LS_2 from (QDEEDI) was ×1 (as reference to susceptible strain) and marked with R_1 here, collected in 1965 from Brisbane, south-east Queensland (Daglish et al., 2002). That of Chinese strain SCXD from Xindu Grain Depot in Sichuan Province, was ×69 and marked with R_{69};. strain CQTL from Tongliang Grain Depot in Chongqing City was ×160 and marked R_{160}. ; of strain HBSY from Shiyan Grain Depot in Hubei Province was ×295 and marked R_{295}. These populations were reared on wheat (13% m.c.) in glass jars under controlled conditions (28±1ºC, 70 ± 5 % r.h.).

2.2. Fumigation chamber

The fumigation was carried out in a rectangle chamber (dimensions of 60×35×40 cm) that was made of armor plate except for transparent top side which was made of plexiglass. There was one operating opening on one vertical side that could be sealed with rubber glove. A sampling cylinder was inserted on another vertical side that can be sealed by two screwed caps which were 80 mm in diameter and 200 mm in length. The insect cages can be taken out through this cylinder during fumigation that avoids the fumigant leaking. The size of insect cage was 10 mm in diameter and 70 mm in length. The airtightness of the chamber was maintained by an airproof mat bolted between rectangle bin and transparent top. The fumigant in the chamber could be re-circulated and monitored by an electronic phosphine monitor with a pump and two rubber pipes controlled by valves. The phosphine monitor could detect phosphine concentration in a range of 0-1000 mL m^{-3} and in precision of 0.01 mL m^{-3} (model HL-210, Xinjialiang Co., Beijing, P.R. China). A supersaturated solution of sodium chloride in a Petri dish placed on the bottom in the chamber was used for maintaining 70% r. h. The pressure decay time at 500 Pa was more than two min for the chamber.

2.3. Phosphine, monitoring and concentration control

The phosphine source was generated from zinc phosphide in acidified water based on FAO method (Anonymous, 1975). The fumigant was injected using a gastight syringe through the recirculation rubber pipes. The phosphine concentration was determined by the monitor after the insect cages and chamber were ready for the test. There were six fumigation chambers maintained at constant concentrations of 0, 100, 300, 500, 700 and 900 mL m^{-3} of phosphine. Phosphine supplementation was necessary if there was a decay in the concentration after a daily check.

2.4. Fumigation of eggs

Five thousand two-week-old adults were delivered into three kg of wheat (14% m.c.). Eggs of the same age were selected from infested kernels. Fifty kernels with egg plugs dyed red with acidic fuchsine solution were put into the cages for exposure to the fumigant. Three replication of egg cages were taken out at 3 d intervals during 12 d fumigation. The fumigated and control wheat contained eggs were dissected with penknife after each checking time. The rate of hatch was counted through dissection.

2.5. Fumigation of larvae

Infected wheat seeds with dyed egg plugs were reared until insects reached the larval stage. Fifty kernels with larvae were placed into the cages for each different fumigation regime. Three cages that served each for a replicate were taken out at 5 d intervals during 12 d fumigation. The fumigated larvae in the kernels were incubated under controlled conditions until the day there was no new adult emergence. The rate of pupation was counted after kernels dissection.

2.6. Fumigation of pupae

Insects inside the infested wheat seeds, identified by the dyed egg plugs were reared to pupa stage. Fifty pupa kernels were placed into the cages for exposure to each different fumigation regimes. Three cages that served each for a replicate were taken out in 2 d interval during 8 d fumigation. The fumigated pupae in the seed were incubated under controlled conditions until the day there was no new adult emergence. The mortality of pupae was determined by dissecting the infested kernels.

2.7. Statistical methods

The statistical analysis was performed using DPS 3.11 software and Microsoft Excel 2003.

3. Results

3.1. Mortality time on eggs

The mortality time on eggs of different strain of *S. oryzae* was expressed by the eclosotion rate of adult through egg reared after a series of fumigations (Fig. 1).

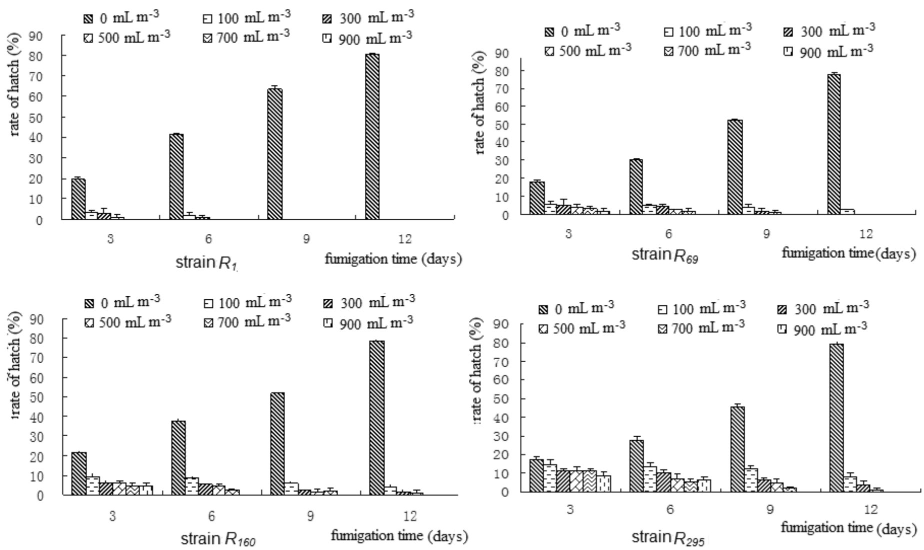

Figure 1 Rate of egg hatch of *Sitophilus oryzae* strains exposed to different phosphine concentrations.

There was more than 80% eclosion rate of adult from kernel infected by eggs for all strains in control. Eclosotion rate of control for all strains increased with time. There were no obvious differences in of egg hatch rates among the four strains at each similar time. There was a sharp decreasing in adult eclosion rate for all strain at any fumigated concentration; there was no adult emergence for any longer times or higher concentrations. It indicates that phosphine can kill hidden eggs by penetrating kernels and/or the egg plug.

The least mortality time for strain R_1 were 3 d at 700 mL m^{-3} of fumigant, 6 d at 500 mL m^{-3}, 9 d at 100 mL m^{-3} and more. The least mortality time for strain R_{69} were 6 d at 900 mL m^{-3}, 9 d at 700 mL m^{-3}, 12 d at 300 mL m^{-3} and more. The mortality time for strain R_{160} was 6 d at 900 mL m^{-3} and 12 d at 700 mL m^{-3} and more. The mortality time for strain R_{295} was 9 d at 900 mL m^{-3} and 12 d at 700 mL m^{-3} and more. The resistance factor was larger and mortality time longer at similar concentrations for different strains. The mortality time was shortened with increased phosphine concentration for the same strain of the insect.

3.2. Mortality time on larvae

The mortality time on larvae of different strains of *S. oryzae* was obtained according to the full mortality of tested insects in wheat seed. The mortality (Fig. 2) was checked by seed dissection after rearing and complete eclosion of adults. Figure 2 indicates that there was less than 5% mortality of larvae for unfumigated kernels infested by eggs of all strains. There was an increase in larva death rate for fumigated kernels of all strains at any tested phosphine concentration and exposure time. Phosphine can kill the larvae hidden in the seed through the penetrating kernel and/or egg plug in a short time. With 100 mL m^{-3} phosphine, the mortality time was 10 d for R_1 and 15 d for the three resistance strains. There seems to be no difference in mortality times among of resistance strains. With 300 mL m^{-3} phosphine, mortality time was 5 d for strain R_1, 10 d for R_{69} and 15 d for R_{160} and R_{295}. at least five days was required to cause mortality at concentrations above 300 mL m^{-3} for R_1, above 700 mL m^{-3} for strain R_{69}, 900 mL m^{-3} for R_{160} and R_{295}. Ten days was required for above 300 mL m^{-3} for strain R_{69}, and above 500 mL m^{-3} for R_{160} and R_{295} The effect of resistance on extending mortality time was clearly demonstrated to control larvae (Fig. 2).

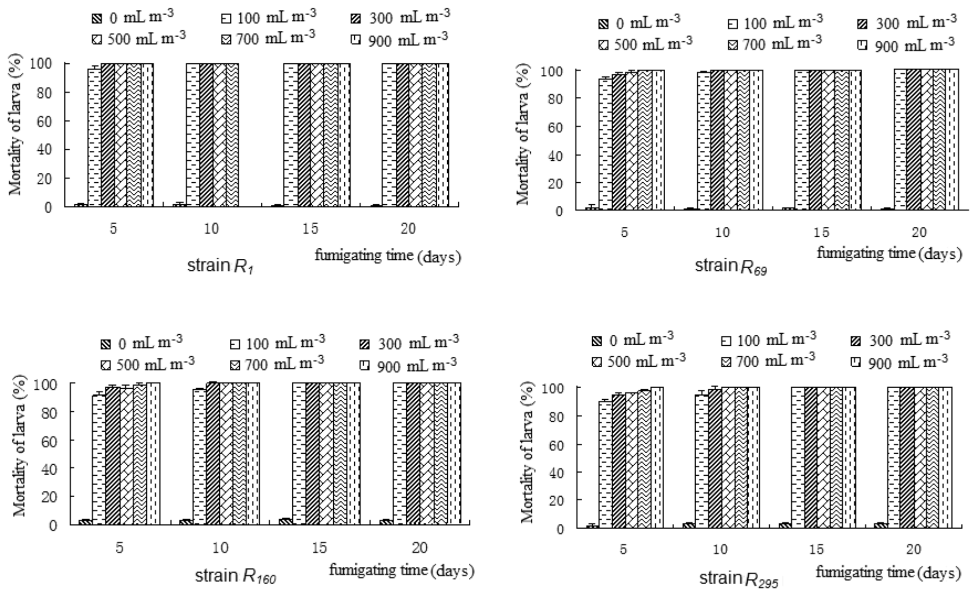

Figure 2 Mortality of larva of four *Sitophilus oryzae* strains exposed to different phosphine concentrations.

3.3. Mortality time on pupae

The mortality time on pupae of different strains of *S. oryzae* was detected according to the mortality of insects in wheat seed. The death numbers (Fig. 3) were checked by dissecting each kernel after incubation and adult emergence was completed. For R_1 the mortality times were 2 d in 900 mL m^{-3} of phosphine, 4 d in 700 mL m^{-3}, 6 d in 500 mL m^{-3}, 8 d in 300 mL m^{-3} and 10 d in 100 mL m^{-3}. The exposure time became shorter with the increase in concentration. The mortality time for pupae of resistance strains was longer than that of strain $R_{1,}$ obviously. The time was postponed with the resistance level in the same concentration. The higher concentration made the mortality time shorter in the tested range of phosphine.

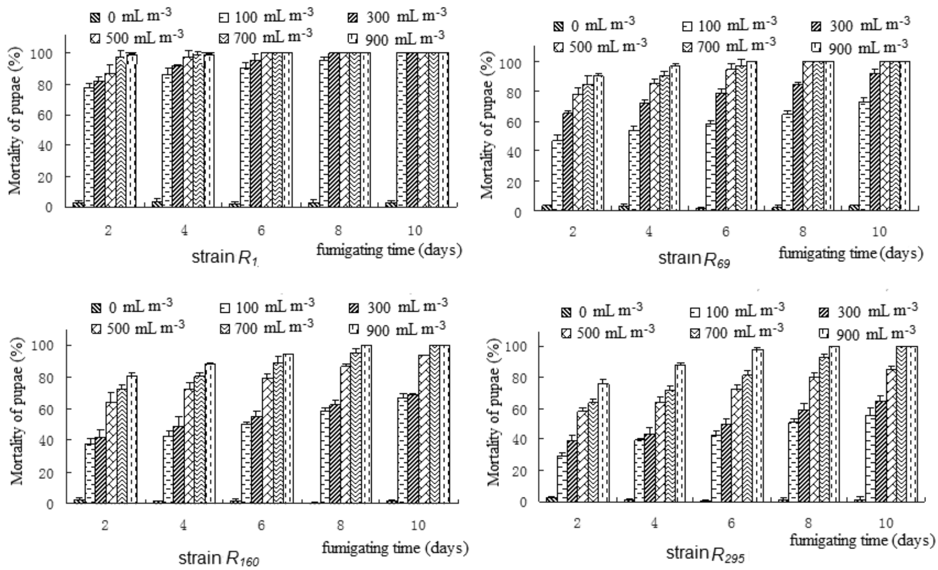

Figure 3 Mortality of pupa of four *Sitophilus oryzae* strains exposed to different phosphine concentrations.

3.4. The effect of developmental stages on the mortality time

For the comparison on different developmental stages of *S. oryzae*, the mortality time was shown in Table1. Table 1 indicates that the order of tolerance to phosphine was from egg to pupae for all strains.

Table1 The mortality time (days) for different strains and life stages exposed to five phosphine concentrations.

strain	stage	Mortaly time (d)				
		100 (mL m^{-3})	300 (mL m^{-3})	500 (mL m^{-3})	700 (mL m^{-3})	900 (mL m^{-3})
R_1	Egg	9	9	6	6	6
	larvae	10	5	5	5	5
	pupae	10	8	6	6	6
R_{69}	Egg	—	12	12	9	6
	larvae	15	10	10	5	5
	pupae	—	—	8	8	6
R_{160}	Egg	—	—	—	12	6
	larvae	15	15	10	10	5
	pupae	—		10	10	6
R_{295}	Egg	—	—	—	12	9
	larvae	15	15	10	10	5
	pupae	—	—	10	10	8

"—":There still were some survivals at the tested concentration

4. Discussion

Sitophilus oryzae is a major pest of stored grain, but little is known about mortality time at specific concentration of phosphine against this species, particularly in regard to immature stages and phosphine-resistant strains. Daglish et al. (2002) investigated the effects of exposure period and phosphine concentration on mortality of a strain with a resistance factor of ×77, collected from Santai County from Sichuan Province, China in 1998. We examined the mortality time of susceptible and resistance strains at different elevated of phosphine efficacy against immature stages hidden with in wheat kernels. Mortality time could be shortened by increasing in phosphine concentration. The impact of resistance on insect killing was nearly relative to the concentration levels. Population mortality could be achieved with lower concentrations combining with longer exposure times. Time was more important than concentration, especially in fumigation practice where the dosage of fumigant or cost could be reduced. That validated to equations of the form $C^n t=k$ time again. In all cases n<1, indicating that time was a more important variable than concentration (Daglish et al., 2002), is verified again. The egg and pupae of *S. oryzae* were very tolerant of phosphine both for susceptible and resistance strains. Therefore, fumigation must be aimed at tolerant stages in order to control all stages of the population. Although there is nothing inherently different between constant and changing concentration on adults of a phosphine-resistant strain of *S. oryzae* (Daglish et al., 2004), the basic phosphine concentration is necessary for a successful fumigation. The findings of this study will be useful in modifying fumigation recommendations. The fumigating concentration should be higher than existing data in the range of 100 to 350 mL m^{-3} of phosphine for quick killing, especially for management and control high level of the resistance in insect pests control.

Acknowledgments

We thank P. J. Collins and G. J. Daglish from Department of Employment, Economic Development and Innovation, Queensland, Australia, for providing the susceptible strain of *S. oryzae* during the project (PHT98-137) cooperation of Integrating effective phosphine fumigation practices into grain storage systems in China, Vietnam and Australia, Mr. Liu Guoshi from Henan University of Technology for providing the writing support for this paper.

References

Annis, P.C., Banks, H.J., 1993. A predictive model for phosphine concentration in grain storage structures. In: Navarro, S., Donahaye, E.J. (Eds), Proceedings of International Conference on Controlled Atmosphere and Fumigation in Grain Storages, June 1992, Winnipeg, Canada. Caspit Press, Jerusalem, Israel, pp. 299-312.

Anonymous, 1975. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major pest species of stored cereals methyl bromide and phosphine. FAO method No.16 FAO Plant Protection. Bullatin 23, 12-25.

Bengston, M., Sidik, M., Halid, H., Alip, E., 1997. Efficacy of phosphine fumigations on bagged milled rice under polyethylene sheeting in Indonesia. In: Donahaye, E.J., Navarro, S., Varnarva, A. (Eds), Proceedings of the International Conference on Controlled Atmospheres and Fumigation in Stored Products, 21-26 April 1996, Nicosia, Cyprus, Printco Ltd., Nicosia, Cyprus, pp. 225-233.

Chaudhry, M.Q., Macnicholl, A.D., Mills, K.A., Price, N.R., 1997. The potential ofmethyl phosphine as a fumigant for the control of phosphine-resistant strains of four species of stored-product insects. In: Donahaye, E.J., Navarro, S., Varnarva, A. (Eds), Proceedings of International Conference on Controlled Atmospheres and Fumigation in Stored Products, , 21-26 April 1996, Nicosia, CyprusPrintco Ltd., Nicosia, Cyprus, pp.45-58.

Collins, P.J., Daglish, G.J., Bengston, M., Lambkin, T.M., Pavic, H., 2002. Genetics of resistance to phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Journal of Economic Entomology 95, 862–869.

Collins, P.J., Daglish, G.J., Pavic, H., Kopittke, R.A., 2005. Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. Journal of Stored Products Research 41, 373-385.

Daglish G.J., Bengston, M, 1998. Phosphine resistance in Asia.Stored Grain in Australia In: Banks, H.J., Wright, E.J., Damcevski, K.A. (Eds), Proceedings of the Australian Postharvest Technical Conference.26-29 May 1998, Canberra, ACT, Australia. pp.63-65.

Daglish, G.J., Collins, P.J., Pavic, H., Kopittke, R.A., 2002. Effects of time and concentration on the mortality of phosphine-resistant *Sitophilus oryzae* (L.) fumigated with phosphine. Pest Management Science 58, 1015-1021.

Daglish, G.J., Kopittke, R.A., Cameron, M.C., Hervoika, P., 2004. Predicting mortality of phosphine-resistant adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in relation to changing phosphine concentration. Pest Management Science 60, 655-659.

Emery, R. N., Collins, P. J., Wallbank, B. E., 2003. Monitoring and managing phosphine resistance in Australia. In: Wright, E.J., Webb, M.C., Highley, E. (Eds), Canberra, Proceedings of the Australian Postharvest Technical Conference. Australia.pp.142-151

Hole, B. D.,Bel, C.H.,Mills, K.A., Goodship, G., 1976. The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. Journal of Stored Products Research 12, 235-244.

Liang, Y., Yan, X., Qin, Z., Wu, X., 1999. An alternative to the FAO method for testing phosphine resistance of higher level resistance insects. In: Jin, Z., Liang, Q., Liang, Y., Tan, X., Guan, L. (Eds), Stored Product Protection. Proceedings of the Seventh International Working Conference on Stored-Product Protection, 14-19 October 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, People's Republic of China, pp. 607-611.

Newman, C. R., 1998. A model for improved fumigant use on farms in Australia. Stored Grain in Australia In: Banks, H.J., Wright, E.J., Damcevski, K.A. (Eds), Proceedings of the Australian Postharvest Technical Conference.26-29 May 1998, Canberra, ACT, Australia. pp.150-153.

Pimentel, M.A,G., Faroni, L.R.D.A., Guedes, R.N.C., Sousa, A.H.,Totola, M.R., 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Products Research 45, 71-74.

Price N R.,1985. The mode of action of fumigants. Journal of Stored Products Research. 21, 157-164

Price, L.A., Mills, K.A., 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 Products Research 24, 51-59.

Rajendran, S., Narasimhan, K.S., 1994. The current status of phosphine fumigations in India. In: Highley E., Wright, E.J., Banks, H.J., Champ, B.R. (Eds), Stored Products Protection. Proceedings of the Sixth International Working Conference on Stored-product Protection, 17-23 April 1994, Canberra, Australia, CAB International, Wallingford, UK, pp. 148–152.

Rajendran, S. 2001. Insect resistance to phosphine -- challenges and strategies. International Pest Control.43, 118-123.

Rajendran, S., Muralidharan, N., 2001. Performance of phosphine in fumigation of bagged paddy rice in indoor and outdoor stores. Journal of Stored Products Research 37, 351-358.

Rajendran, S., Gunasekaran, N., 2002.The response of phosphine resistant lesser grain borer *Rhyzopertha dominica* and rice weevil *Sitophilus oryzae* in mixed-age cultures to varying concentrations of phosphine. Pest Management Science 58, 277-281.

Sayaboc, P.D., Gibe, A.J.G., Caliboso, F.M., 1998. Resistance of *Rhizopertha dominica* (Coleoptera: Bostrychidae) to phosphine in the Philippines. Philippines Entomology 12, 91–95.

Tyler, P.S., Taylor, R.W.D., Rees, D.P., 1983. Insect resistance to phosphine fumigation in food warehouses in Bangladesh. International Pest Control 25, 10-13, 21.

Taylor, R.W.D., Harris, A.H., 1994. The fumigation of bag-stacks with phosphine under gas-proof sheets using techniques to avoid the development of insect resistance. In: Highley E., Wright, E.J., Banks, H.J., Champ, B.R. (Eds), Stored Products Protection. Proceedings of the Sixth International Working Conference on Stored-product Protection, 17-23 April 1994, Canberra, Australia, CAB International, Wallingford, UK pp. 210-213.

Wang, D.,Collins, P. J.,Gao, X., 2006. Optimising indoor phosphine fumigation of paddy rice bag-stacks under sheeting for control of resistant insects. Journal of Stored Products Research 42, 207-217

Wang, D., Song, W., Qin, Z., Bai, X., Cao, Y., Xu, Y., Wang, P., Deng, H., 2002. LS/T1201-2002 Fumigation Regulation of Phosphine Recirculation (in Chinese), The Standard of State Administration of Grain, Standard Publishing House of China, Beijing, P.R. China.

Wang, D., Yuan, K., Wu, Z., Wang, H., Dong, W., Gao, X., 2004. Relative tolerance to phosphine of *Cryptolestes ferrugineus* compared with several other species of stored product insects. Journal of the Zheng zhou Institute of Technology (in Chinese) 25, 4-8.

Zeng, L., 1999. Development and countermeasures of phosphine resistance in stored grain insects in Guangdong of China. In: Jin, Z., Liang, Q., Liang, Y., Tan, X., Guan, L. (Eds), Stored Product Protection. Proceedings of the Seventh International Working Conference on Stored-Product Protection, 14-19 October 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, P.R. China, pp. 642-647.

Zettler, J.L., 1993. Phosphine resistance in stored-product insects. Navarro, S., Donahaye, J. (Eds) Proceedings of the International Conference on Controlled Atmospheres and Fumigation in Grain Storages. Caspit Press Ltd, Jerusalem, Israel pp. 449-460.