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Can Phosphine Ever Stand as a Magical Fumigant? Susceptibility of the Red Flour Beetle [*Tribolium castaneum* (Herbst)] to Phosphine

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ABSTRACT

Phosphine is a universal fumigant of store insect pests being used for more than 70 years worldwide. In Sudan it is the most known fumigant that is used for this purpose for the farmers and in commercial stores in particular. The use of this fumigant is generalized in the public sector in replacement of the methyl bromide which will phase out in 2015. An experiment of the susceptibility of the red flour beetle (*Tribolium castaneum* Herbst) for this fumigant was done in the Food Research Center (FRC), Khartoum during 2013. The damaging stages of this insect (larva and adult) were tested. The results reflected that one tablet of Quickphos® (1 gram of phosphine, PH_3) for a period of five days was unable to disinfest the flour from the beetles in two of the four tests done with corrected mortalities (98.98, 100, 100 and 98.98%, respectively). However, this dose succeeded in disinfesting the test flour from larvae and adults (100% corrected mortality) when used for a period of 6 and 7 days and a period of 5 days for the larval stage. Another experiment was conducted using 2 tablets Quickphos® for 5, 4, 3, 2 and 1 days. The obtained results reflected 100% corrected mortality in all tests (1, 2, 3, 4 and 5 days). These results reflect some resistance of *T. castaneum* to this fumigant and may represent an early alert of the injudicious use of this insecticide. Moreover, testing the susceptibility of the other insect pests of store in Sudan is needed considering the strong resistance of some store product insects to phosphine in other countries such as the flat grain beetle [*Cryptolestes ferrugneus* (Stephens)] in Australia. However, these results may infer the dismerits of the reliance on one fumigant and may push forward to test for the introduction of other alternative available fumigants.

Keywords: *Fumigant, phosphine, Quickphos®, Sudan, susceptibility and T. castaneum.*

INTRODUCTION

Fumigant is a chemical which, at required temperature and pressure, can exist in a gaseous state in sufficient concentration that is lethal to a given pest (Monro, 1961). However, another definition is "fumigant is a chemical which, at a particular temperature and pressure, can exist in a gaseous state in sufficient concentration and for sufficient time that is lethal to insects or other animals, weeds or other pests" (Banks, 2012). Fumigants include: methyl bromide, phosphine, cyanogen (Ethane Di Nitrile 'EDN'), carbonyl sulphide, sulphuryl fluoride, hydrogen cyanide (HCN), ethylene bromide, ethylene oxide, ethylene dichloride, carbon disulphide, carbon tetrachloride, chloropicrin, dichlorovos (DDVP, dimethyl dichlorovinyl phosphate), sulphuryl fluoride, acrylonitrile, propylene oxide (Bond, 1984) and ozone (James, 2011). However, the ASEAN members led by Indonesia aggressively pursuing the phase out of methyl bromide as embedded in the Montreal protocol. They mentioned an alternative to it as ECO2phosphine been recognized by the Indonesia agricultural quarantine Agency (AQA) (Tumambing and Dikin, 2013). Lavoisier (1789) recognized phosphine as a combination of phosphorus with hydrogen and described it as "hydruyet of phosphorus, or phosphuret of hydrogen". Phosphine has been in commercial use as a grain fumigant since the mid 1950s. It has developed and retained an important role in the control of storage pests since that time. More recently its use has expanded due to the phase – out of methyl bromide and marketing complications caused by grain protectant chemicals (Annis, 2000).

The dosage is the amount of fumigant applied and is usually expressed as weight of the chemical per volume of space treated. In grain treatments, liquid – type fumigants are often used and the dosage may be expressed as volume of liquid (litres or gallons) to a given volume (amount of grain given as litres or bushels) or sometimes to a given weight (quintals, metric tonnes or tons) (Bond, 1984). Although concentration and exposure time are still the main factors that determine toxicity of this fumigant, the length of the exposure time is of great importance. Phosphine is a slow acting poison that is absorbed slowly by some insects even at high concentrations (Bond, 1967, 1969). However, high concentrations may not increase toxicity; in fact, they may cause insects to go into a protective narcosis. It is reported that the LD_{50}, $LD_{99.9}$ and the discriminating dosage for Tribolium castaneum (Herbst) are 8.4, 11.7, and 12 for 5 hours, respectively, whereas the corresponding figures for 20 hr exposure are 0.009, 0.028 and 0.04 mg/ L, respectively (Bond, 1984). Discriminating dose assays determined eight out of nine T. castaneum populations, and all five populations of R. dominica, contained phosphine – resistant individuals, and highest resistance frequencies were 94 and 98%, respectively. However, the export standards often have a zero tolerance standards for insects (Driscoll and Srzednicki, 2012). Based on United States Department of Agriculture estimates, Pennsylvania farmers may lose as much as 10 percent of the grain crop from the time of harvest until the grain is fed or processed. This percentage roughly equates to losses of $31.4 million annually to insects infesting stored grains. Most of the loss from insect damage and/ or contamination could be avoided if farmers would practice several relatively easy management techniques Calvin et al. (1989). In Africa the postharvest grain losses may exceeds 30% whereas in Sudan it was estimated to reach up to 25% (Kabbashi, 2006).

Study Objectives

(1) To check the susceptibility of T. castaneum to phosphine gas.
(2) Stimulate the awareness about insecticide resistance.
(3) Alerts to use alternative fumigants with the commonly used ones alternately to delay the resistance build up.
(4) Push into enriching the baseline data of store product insects (SPI) to phosphine.
(5) Enrich the knowledge about insecticide resistance to the extensionists, stakeholders and beneficiaries as well.

Expected Outcome

(1) Better judicious use of dose and dosage of the test gas.
(2) Updating the knowledge about any resistance of SPIs to phosphine.
(3) Economic use of phosphine.

MATERIALS AND METHODS

Materials

Quickphos® tablets were brought from the CTC Company, Khartoum; vials with punctured neck (tiny holes with a pin); wheat bread flour; electronic sensitive balance; magnifying lens; binuclear microscope; fine camel hair brush; 0.32 inch aperture fine mesh (Tyler Standard Screen Scale®); plastic basins; plastic funnel; spatulas; stainless steel spoons, big glass and plastic bottles for insect rearing; larvae and beetles of *T. castaneum*; a hygrometer; a body of a deep freezer (116 (length) X 58 (width) X 80 (height) Cm^3); cello tape; small melamine plate for the tablets etc….. were all used in the experiment.

Methods

A weight of 10 grams of wheat bread flour was put in a plastic vial with punctured neck where an addition of 10 beetles or worms was done for the experiments of these two stages. A tablet (1 gram PH_3) or two tablets (2 grams PH_3) of Quickphos® were put in a melamine dish in deep freezer box (116 (length) X 58 (width) X 80 (height) $Cm^3 = 0.54\ Cm^3$), which was already loaded with the treatment vials and sealed tightly with cello tape. The test then left for the specified time (1, 2, 3, 4, 5, 6 or 7 days). The experiment was begun from the longest time and then descending to the shortest. Each treatment was replicated four times for verification. Corresponding control were also set for each treatment. The beetles or worms were transferred to the vials from the mesh using a stainless spoon and a camel hair fine brush through a plastic funnel. The Readings were taken after the planned time through sieving and counting of the live and dead insects using naked eyes, magnifying lens and a binuclear. The readings were corrected using Abbott's formula (Tatterfield and Morris, 1924). The average temperature and relative humidity of the laboratory (Food Research Center, Shambat, Khartoum North) were 30 ± 1ºC and 13 ± 5%, respectively.

RESULTS AND DISCUSSION

Two groups of experiments were done using one and two Quickphos® tablets. The results of these groups of experiments are summarized in Tables 1 and 2, respectively. However, all the dose/ time combinations used disinfest the test flour from all the test insects (beetles and larvae) except in two tests (one tablet and 5 days) the corrected mortality was 98.98 for these two tests (Tables 1 & 2). However, the dosage used in these tests were very high *i.e.* 1 g/ 0.54 Cm^3. However, the AlP formulations come in a number of different forms. They evolve phosphine slowly, over a day or more, by reaction with ambient moisture, leaving a grey powdery residue, typically containing 3 – 4% of unreacted AlP. Formulations include pellets releasing 0.2g PH_3, tablets releasing 1 g PH_3 and sachets releasing 11 g PH_3 (Banks, 2012). However, a lot of work on resistance of *T. castaneum* to phosphine was done internationally, e.g. Opit. (2012) reported: Discriminating dose assays determined eight out of the nine *T. castaneum* populations, and all five populations of *R. dominica*, contained phosphine-resistant individuals, and highest resistance frequencies were 94 and 98%, respectively. Dose – response bioassays and logit analyses determined that LC_{99} values were approximately 3 ppm for susceptible and 377 ppm for resistant *T. castaneum*, and approximately 2 ppm for susceptible and 3,430 ppm for resistant *R. dominica*. The most resistant *T. castaneum* population was 119 – fold more resistant than the susceptible strain and the most resistant *R. dominica* population was over 1,500 – fold more resistant. Results suggest a substantial increase in phosphine resistance in these major stored – wheat pests in the past 21 yr, and these levels of resistance to phosphine approach those reported for other stored – grain pest species in other countries. However, Shazali and Reichmuth (1999) reported, there have been no studies on the efficiency of phosphine on eggs of *S. cerealella.* The study was designed to assess and compare the toxicity of phosphine to the eggs of *Sitotroga cerealella* (OLIV.) compared to *Ephestia cautella* (Walker). Three phosphine concentrations were used (01 mg/ L, 0.5 mg/ L and 1.0 mg/ L). They found that the increase of exposure period was more effective than the increase in the gas concentration to achieve complete mortality. Moreover, the following combinations are the minimum required to achieve a very high level of kill in almost all stored product beetles (excluding *Trogoderma* spp.), psocids and moths, excluding known

Table 1. Results of one Quickphos® tablet against *T. castaneum* (an average of 4 tests)

Test No./ Time	Larvae								Adults							
	Test Mort. (%)				Corrected Mort. (%)				Test Mort. (%)				Corrected Mort. (%)			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
1 (1 day)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2 (2days)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3 (3 days)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4 (4 days)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5 (5 days)	100	100	100	100	100	100	100	100	99	100	100	99	98.98	100	100	98.98
6 (6 days)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
7 (7 days)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

N.D. = Not Done *i.e.* the 5 days time was not satisfactory to disinfest the test flour from test beetles, therefore was no need to test less time.

Table 2. Results of two Quickphos® tablets against *T. castaneum* (an average of 4 tests)

Test No./ Time	Larvae									Adults								
	Test Mort. (%)				Corrected Mort. (%)					Test Mort. (%)				Corrected Mort. (%)				
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	overall	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	Overall
1 (1 day)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2 (2days)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
3 (3 days)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4 (4 days)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
5 (5 days)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
6 (6 days)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
7 (7 days)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = Not Done *i.e.* the 5 days time was satisfactory to disinfest the test flour from test beetles, therefore no need to test for more time.

Table 3. Recommended phosphine dosage guide for ECO_2fume®

Minimum Duration	Rate of ECO_2FUME®/1,000 cu.ft	PH_3 Concentration Maintained	Temperature
Do not fumigate	Do not fumigate	Do not fumigate	Below 32 ^{0}F (0 ^{0}C)
6 days	0.88 – 4.41 lbs	200-1,000 ppm	32-39 ^{0}F (0-4 ^{0}C)
4 days	0.88 – 4.41 lbs	200-1,000 ppm	40-53 ^{0}F (5-12 ^{0}C)
3 days	0.88 – 4.41 lbs	200-1,000 ppm	54-59 ^{0}F (12-15 ^{0}C)
2 days	0.88 – 4.41 lbs	200-1,000 ppm	60-79 ^{0}F (16-25 ^{0}C)
36 hours	0.88 – 4.41 lbs	200-1,000 ppm	80 ^{0}F & Above (=26 ^{0}C)
24 hours	2.20 – 4.41 lbs	500-1,000 ppm	80 ^{0}F & Above (=26 ^{0}C)

Resistant strains: 10,000 ppm for 1.5 days, 1,200 ppm for 2 days, 1,000 ppm for 8 days, 200 ppm for 10 days, 35 ppm for 20 days, 10 ppm for 30 days (Annis, 2000).

The results of this study raise the vigilance and alert for phosphine resistance in this pest and the other pests in general. However, from a survey in Sudan we reported a lot of mistakes in the storage system that may accelerate the resistance in these pests such as the uncontrolled tightness, temperature, humidity and the dosage used. However, the application of phosphine is usually accompanied by a carelessness in handling the left powder of this insecticide as well (Mahmoud, personal communication). This work must be followed by a thorough research for this pest and the other pests in silos and granaries, hopefully. Table 3 shows the recommended doses of phosphine (Gurusinghe, 2008). However, this work is part of research and training components of a work plan on return (WPR) a post course component of a residential training supported by the AAA (Australian Awards in Africa) program.

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