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Toxicological studies of phosphine against the lesser grain borer, *Rhyzopertha dominica* (Fabr.) (Coleoptera Bostrychidae)

INTRODUCTION

Phosphine (PH_3) is currently used as a fumigant in the control of insect pests in stored agricultural products. It is a respiratory poison which causes a reduction in oxygen uptake and induces narcosis (Nakakita et al., 1974). An influence on the mitochondrial oxidation has been experimentally proved both in the grain weevil (Chefurka et al., 1976) and in susceptible and resistant strains of the lesser grain borer (Price, 1980b and 1981). Both the enzymatic activity of the cytochrome c and its physical properties can be affected by phosphine (Kashi and Chefurka, 1976; Nakakita, 1976; Price, 1980a). Published data on the toxicity of phosphine to *R. dominica* are still scarce in relation to concentration and exposure period, Most investigators reported only the toxicity of the fumigant for a fixed period of exposure, mostly at a single temperature. Such data are not enough for commercial fumigation which needs varying periods of exposure at varying concentrations. Outstanding in these efforts are FAO-Publication, 1975; Hole et al., 1976; Bell et al., 1977).

Thus, it seems to be essential to explore thoroughly the toxicity of fumigants to insects at conditions simulating practical situations, as much as possible, in the laboratory. In the present work a study on the toxicity of phosphine to the adults of *R. dominica* is carried out over the practical ranges of concentrations, periods of exposure and temperatures. It is actually an examination of the validity of the concentration - time product relationship under varying conditions.

MATERIALS AND METHODS

The original culture used in the present study was started with adult beetles obtained from a laboratory stock culture maintened for several generations at

the Insect Research Section, Ministry of Agriculture, Cairo, Egypt. Rearing was carried out in a constant room temperature maintened at 30 ±1°C and 65±5%R.H. The food used to rearing the insect was a mixture of wheat grain and whole meal wheat in a ratio of 4:1.

Phosphine was obtained by hydrolysis of the aluminium phosphide in Phostoxin pellets(R). These pellets contained 56% aluminium phosphide and each yielded approx. 0,2 g of phosphine. Gas evolved was collected by displacement of an aqueous solution containing approx. 5% (v/v) sulphuric acid. The collection tube was fitted with a rubber septum through which phosphine could be withdrawn with a gas-tight syringe. For determining the concentration of phosphine a GLC (PYE 104) with a flame ionization detector was used. The chromatographic conditions were: Column temp. 80°C, detector temp. 125°C, gas flow rate; nitrogen carrier gas, 30 cm^3; hydrogen 30 cm^3 and air flow rate through the detector was optimized for maximum sensitivity to phosphorus compounds. The retention time was 55 sec.

The experiments were designed to determine the values of LC 50 and 95 and Lc.t.50 and 95 for 15-days old adults by the usual probit method of analysis. The periods of exposure selected were 24, 48, 72 and 96 hours and the tempertures were 10, 20 and 30°C. The c.t-product was calculated by multiplying the period of exposure by the average concentration of the fumigant throughout the period of exposure as monitored by GLC. The average of the initial and final concentrations was considered as the actual concentration to which the insects were exposed.

The investigation on response of *R. dominica* to phosphine spanned a range of concentrations from appox. 2 μg/l to 4 mg/l. This was achieved by using a 5-Litre capacity glass container, a gas-tight rubber under the lid and a septum through which phosphine could be injected. To preparing the insects for fumigation, batches of adults were counted (100-200 adults each) in cuttings of glass tubes, each 5 cm long and 3.5 cm diameter, covered on both ends with fine muslin and secured firmly with a string.

Preparation of insects for fumigation, fumigation procedure, pre- and postfumigations of insects and estimation of mortality were conducted as described by Osman (1980). Results on mortality upon dosage for different exposure periods at different temperatures, were determined by probit analysis (Finney, 1970). Some insects appeared to be dead but moved soon when disturbed or activated by exposing to the light heat of an electric lamp. Such insects hold their legs and antennae close to their bodies and appeared smaller than usual size. Least significant difference (*L.S.D.*-Test) procedure (Snedecor and Cochran, 1971) and *t*-test were used to compare the significance of differences due to the different factors, considered in this study.

RESULT AND DISCUSSION

Before starting the main tests, some preliminary tests were carried out by counting mortalities 3, 7 and 14 days after fumigation (fig. 1). The main conclusions of these tests were:

a) The analysis of variance: the results obtained indicated that the 14th day counts were not significantly different from those of 7th day counts, whereas those on 7th day varied significantly from the 3rd day counts. The 14th day

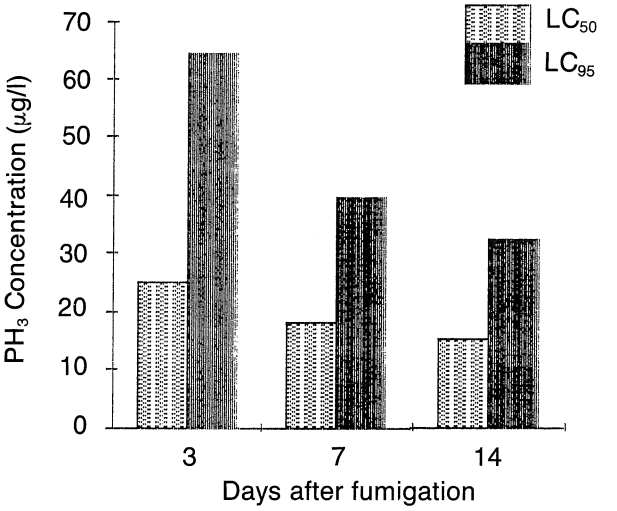

Fig. 1 - The end-point mortality obtained for *Rhyzopertha dominica* adults due to estimating mortality at different intervals after fumigation with phosphine for 4 and 12 hours period of exposure at 30°C.

counts appeared to be feasible holding period for final mortality counts, in which the mortalities of adults of *R. dominica* in the present study were assessed in the main fumigation tests.

b) This delayed effect of phosphine is more pronounced with low concentrations as well as long exposure periods (72 and 96 hours).

The fumigation tests with phosphine against adults of *Rhyzopertha dominica* over a wide range of concentrations and periods of exposure at different temperatures revealed that the c.t. product required for fixed mortality levels, represented here by 50 and 95%, did not remain fairly constant by varying the components of the c.t. product, i.e. concentration and time of exposure.

1 - *Relationship between concentration, exposure time and mortality*

The data in Tables 1 and 2 contain the regression equations of phosphine for the adults of *R. dominica* a t different temperatures and different exposure periods which show that the LC50 and LC95 values decrease as the period of exposure increases from 4 to 96 hours. The rate of decrease in the concentration of the fumigant required to cause a certain level of mortality, i.e. 50% and 95%, being more rapid between 4 to 48 h than between 48 to 96 hours of exposure. The analysis of variance in Table 3 shows that the averages of LC50 and LC95 at each period of exposure calculated across the three tested temperatures decreased significantly as the period of exposure was increased from 24 to 72 hours but the decrease was not significant between 24 and 48 hours and between 72 and 96 hours.

A comparison on the basis of the c.t. products (data in Tables 2 and 3) shows that the Lc.t. 50 and L.c.t. 95 values are not constant, which were found to increase as the period of exposure was increased. The rate of decrease in the concentration of phosphine, responsible for a fixed level of mortality, is not always proportional to an increase in the period of exposure in achieving a certain c.t. product. This is, in fact, an indication of the interaction between concentration and time of exposure.

A general indication of these results is that the concentration-time rule breaks down under the conditions of the present tests, apparently due to the interaction between concentration and period of exposure and their effects on the test organism at different temperatures.

Results confirming these findings were found by Estes 1965; Hole et al., 1976 and Muthu et al., 1975, as well. In the laboratory, Bell and Glanville (1973) studied the effect of concentration and exposure period on the efficiency of methyl bromide and phosphine on *Ephestia elutella* (Huebner) as an insect test and found that the c.t. product required for 50% mortality was the same within a certain range of concentration. Outside this range the Lc.t. value increased showing a decline in the efficiency of the fumigants. Winks (1982) found that both dosage and concentration influenced the speed of action of phosphine on adults of *Tribolium castaneum* (Herb.). The median response time is inversely correlated with dosage but directly with concentration. Thus, the speed of action of phosphine over the dosage range producing up to 100% mortality increased with increasing period of exposure for a given concentration.

2 - *Effect of temperature on the efficiency of phosphine*

The situation is further complicated by interaction of the temperature which found to act with each of the period of exposure in double interactions or with

both of them acting together in triple interactions. The data in Tables 1, 2 and 4 show that phosphine became less toxic to the adults of *R. dominica* as the temperature decreased from 30 to 10°C. To achive 50% mortality at 10°C, a concentration of 23.96 μg/l was necessary which was 3.8 times higher than that at 30°C. The rate of increase in concentration with decreasing the temperature differed between the two steps of reduction in temperature tested, i.e. 30 - 20 and 20 - 10°C.

By comparing the values of Lc.t. 50 and Lc.t. 95 for each temperature at different periods of exposure (data in Table 4), the interaction between temperature and the period of exposure could be detected. The changes in Lc.t. values as well as the concentration and the period of exposure at each temperature indicated different trends at both 50 and 95 levels.

These differences within the tested temperatures are, in fact, an indication of triple interaction between temperature, fumigant concentration and period of exposure, adding more complications to the c.t.-product principle. Osman (1980), in his studies on the effects of methyl bromide on the different stages of *R. dominica* concluded that the c.t. factor is not always constant at different

Table 1 - Regression equation of phosphine for the adult of Rhyzopertha dominica *(Fabr.) fumigated at different temperatures and different exposure periods (mortality assessed 14 days after fumigation).*

Tested Temp. °C	Exposure period (hrs)	Regression equations Y = a + bx where Y = probit unit transformation x = log of conc.	Injected conc. (μg/l)	*t* - Test of regression		
				d.f.	value of «*t*»	probability P<0.05* P<0.01**
10	24	Y = 7.15x—5.79	30.0-60.0	3	4.18	*
	48	Y = 6.32x—4.03	25.0-55.0	4	12.15	**
	72	Y = 4.84x—1.07	10.0-30.0	5	8.03	**
	96	Y = 5.06x—1.22	12.0-30.0	4	12.80	**
20	24	Y = 3.87x + 0.50	5.0-25.0	4	5.03	**
	48	Y = 3.05x + 2.19	3.5-16.0	5	5.80	**
	72	Y = 2.63x + 2.92	3.0-16.0	5	5.83	**
	96	Y = 2.73x + 3.06	2.5-12.0	5	9.60	**
30	4	Y = 4.29x—2.06	20.0-60.0	4	9.13	**
	12	Y = 5.11x—1.24	8.3-26.5	5	4.26	*
	24	Y = 3.84x + 1.31	4.5-16.5	5	4.50	*
	48	Y = 3.37x + 2.22	3.0-14.0	5	7.34	**
	72	Y = 3.58x + 2.48	2.6-12.1	5	7.78	**
	96	Y = 3.51x + 2.73	2.4- 9.6	5	8.4	**

Table 2 - Lethal concentration of phosphine (LC values for 50 and 95% mortality) and lethal c.t.-product (Lc.t.50 and Lc.t.95 values) of each period of exposure for 15-days old adults of Rhyzopertha dominica *(Fabr.) fumigated at different temperatures (mortality assessed 14 days after fumigation).*

Temperature °C	Exposure period (hrs)	Lethal concentration		c.t. - product	
		LC 50 µg/l	LC 95 µg/l	Lc.t. 50 µg h/l	Lc.t. 95 µg h/l
10	24	32.00	52.00	768.00	1248.00
	48	28.90	46.50	1387.20	2232.00
	72	17.94	39.13	1291.68	2817.36
	96	17.00	35.50	1632.00	3408.00
20	24	14.20	36.00	340.80	864.80
	48	8.37	28.85	401.76	1384.80
	72	6.17	25.93	444.24	1866.96
	96	5.00	20.40	480.00	1958.40
30	4	42.0	105.00	168.00	420.00
	12	17.00	35.20	204.00	422.40
	24	9.10	25.00	218.40	600.00
	48	6.69	20.49	321.12	983.52
	72	5.07	14.52	365.04	1045.44
	96	4.42	12.97	424.32	1245.12

Table 3 - Time of exposure and mortality of the adults of Rhyzopertha dominica *(Fabr.) fumigated with phosphine at different temperatures (averages of the 3 tested temperatures).*

Period of exposure (hrs)	Aver. LC50 µg/l	Aver. LC95 µg/l	Aver. Lc.t.50 µg h/l	Aver. Lc.t.95 µg h/l
24	18.43	37.67	442.40	904.00
48	14.65	41.95	703.65	1533.44
72	9.73	26.53	700.32	1909.92
96	8.81	22.96	845.44	2203.84
f - Test	*	**	n.s.	*
L.S.D. - Test				
at 5%	7.44	3.91	—	678.65
at 1%	11.20	5.90	—	1024.80

periods of exposure varying from 8 to 48 hours, Methyl bromide becomes less effective as the temperature decreases.

Thus, it appears that the relationship between concentration, time of ex-

Table 4 - The effect of temperature on the efficiency of fumigation tests with phosphine (averages of the 4 tested periods of exposure).

Temperature °C	Aver. LC50 µg/l	Aver. LC95 µg/l	Aver. Lc.t.50 µg h/l	Aver. Lc.t.95 µg h/l
10	23.96	43.23	1269.72	2426.34
20	8.44	27.80	416.70	1028.94
30	6.32	18.25	332.22	968.52
f - Test	**	**	**	**
L.S.D. - Test				
at 5%	3.20	6.08	392.15	678.65
at 1%	4.80	9.18	592.23	1024.80

posure and mortality is more complex than assumed. The factors involved include the insect species, the developmental stages, the temperature, the levels of concentrations and periods of exposure. The c.t. principle is further complicated by the enormous variation in the tolerance of insects as development proceeds some stages being tolerant for short periods and therefore, it should be carefully used as a guide for practical fumigation work according to the very specific conditions of each fumigation.

SUMMARY

The toxicity of phosphine (hydrogen phosphide, PH_3) has been determined on the adults of *R. dominica* (Fabr.) at different temperatures (10, 20 and 30°C) over a wide range of concentrations at different periods of exposure (4-96 hours). The efficiency of phosphine against the adult beetles was less toxic as the temperature was decreased from 30 to 10°C. The rate of decrease in concentration with an increase in exposure period to achieve a fixed level of mortality (50% and 95%), was much more rapid between 4 to 48 h than between 48 to 96 h exposures. The rate of increase in both Lc.t. 50 and 95 values (Lethal concentration-time product) with increasing the period of exposure varied at the tested temperature, especially at shorter periods of exposure. This indicates that the relationship between the c.t.-product and mortality varied with these factors acting either single or in double and triple interactions.

RIASSUNTO

Attività tossica della fosfina su adulti di Rhyzopertha dominica *(Fabr.) (Coleoptera Bostrychidae).*

È stata valutata la tossicità della fosfina (PH_3) su adulti di *R. dominica* a differenti

temperature (10, 20 e 30°C), a concentrazioni variabili e tempi di esposizione compresi tra 4 e 96 ore. L'efficacia del gas diminuisce quando la temperatura decresce da 30 a 10°C. Il rapporto tra la riduzione della concentrazione e l'aumento del tempo di esposizione, per raggiungere un prefissato livello di mortalità (50% e 95%) ha un valore piú elevato tra 4 e 48 ore di trattamento che tra 48 e 96 ore. La L.c.t. 50 e 95 (rapporto tra concentrazione letale e tempo di trattamento) varia, alle temperature saggiate, in relazione al protrarsi del trattamento stesso, ma specialmente quando il periodo di esposizione alla fosfina è breve. I dati ottenuti evidenziano nel complesso che la concentrazione del gas, la durata del trattamento e le temperature esercitano un ben distinguibile influsso sulla mortalità dell'insetto con reciproche interrelazioni.

ZUSAMMENFASSUNG

Toxische Wirkung von Phosphin auf Rhyzopertha dominica *(Fabr.) (Coleoptera Bostrychidae).*

Die toxische Wirkung von Phosphin auf Adulte *R. dominica* wurde bei verschiedenen Temperaturen (10, 20 und 30°C) und während unterschiedlicher Begasungszeiten (4, 12, 24, 48, 72, und 96 Stunden) bestimmt. Die Zucht des Insekts erfolgte bei 30 ± 1°C und 70 ± 5% rel. Luftfeuchte. Während der Begasungszeit wurde die aktuelle Konzentration von Phosphin mit Hilfe eines Gas-Chromatographen (GLC; PYE 104) verfolgt. Nach der Begasung bei den jeweiligen Temperaturen und nach den Behandlungszeiten wurden die Tiere für 14 Tage auf einem Gemisch von Weizenkörnern und -vollmehl (80%:20%) gehalten. Es zeigte sich, daß die Toxizität von Phosphin bei niedriger Temperatur (10°C) stark reduziert war und die LC 50 und LC 95 bei Begasungszeiten zwischen 4 - 48 h schneller abnahmen als bei Begasungszeiten zwischen 48 und 96 h. Das errechnete Produkt letale Konzentration x Begasungszeit (Lc.t.) ist nicht konstant; es steigt mit zunehmender Besagungszeit bei allen Untersuchungstemperaturen an. Die Ergebnisse weisen darauf hin, daß die Konzentration des Gases, Begasungszeit und Temperatur auf die Beziehung von c.t.-Produkt und Mortalitätsrate unterschiedlich Einfluß nehmen.

Key words: Fumigation tests, Phosphine, *Rhyzopertha dominica.*

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