0611

Response of *Trogoderma granarium* (Everts) to Different Combinations of Phosphine and *Acorus calamus* Oil

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Abstract: The response of *Trogoderma granarium* (Everts) to different combinations of phosphine concentrations (100ppm, 200ppm and 300ppm) and *Acorus calamus* oil doses (30, 50 and 70 μL) was studied. Percent mortality was observed for different exposure periods of 3, 5 and 7 days. Population build – up was observed after 30 and 60 days. Percent mortality increased with the increase in phosphine concentration and exposure time. In case of *Acorus calamus* oil treatments, exposure period appeared to be the most important factor affecting the percent mortality. Population build – up was reduced both with increase in dose of *Acorus calamus* oil and exposure period.

Key words: *Trogoderma granarium*, phosphine, fumigation, *Acorus calamus*, essential oil

Introduction

"Khapra" beetle, *Trogoderma granarium*, is the world's worst pest of the stored grains. (Christensen and Kaufmann, 1969). Losses caused by khapra beetle have been reported to range from 0.2% to 2.9% over a period of 1 to 10.5 months (Irshad *et. al.*, 1988). Although a native of India, this pest has gone abroad and has been reported from England, Germany, Israel, and the USA. In the Indo-Pak subcontinent, it is a very destructive pest of wheat and other stored grains, particularly in the northwestern dry regions of Pakistan and Indian states of Rajasthan, Haryana and the Punjab. Apart from wheat, the insect has also been recorded on sorghum, rice, barley, gram, maize, poppy, pulses, pistachio, walnut and other dried fruits (Azeem *et al.*, 1976; Ramzan and Chahal, 1989; Hamed *et al.*, 1989; Atwal, 1994; Khattak *et al.* 1995; Ram and Singh, 1996). Losses caused by khapra beetle have been reported to range from 0.2% to 2.9% over a period of 1 to 10.5 months (Irshad *et al.*, 1988).

Phsophine is most frequently used to protect stored agricultural products. Various stored grain insect pests including *Trogoderma granarium* (Everts.) have become tolerant to this fumigant. The substandard techniques of phosphine fumigation have led to the development of phosphine resistance in major insect pests of stored grains (Taylor, 1989; Mills *et al.*, 1990). Borah and Chahal (1979) reported that phosphine failed to control khapra beetle, *Trogoderma granarium* in warehouses in India. Tyler *et al.* (1983) documented the development of resistance in stored grain insects pests against useful insecticide, phosphine, in warehouses in Bangladesh. Appreciably high resistance was recorded in *Trogoderma granarium* strains collected from Punjab and Sindh (Alam *et al.*, 1999).

Rhizomes of sweet flag, *Acorus calamus* L. (Aracae), possesses insecticidal properties against a wide variety of insect pests. The powder and extracted oil of rhizomes act as stomach or contact poison, anti-feedant and repellent. The toxic and sterilizing effects of vapors of rhizome oil against certain insect pests have also been observed. (Sexena and Mathur, 1976; Schmidt *et al.*, 1991). Its most effective component is β – asaron (Schmidt, 1986).

In the present study response of *Trogoderma granarium* (Everts) to different combinations of phosphine and *Acorus calamus* oil has been evaluated.

Materials and Methods

T. granarium collected from various godowns of Punjab Food Department located in Faisalabad district was reared in one litre capacity glass jars containing wheat. These glass jars were placed in an incubator maintained at 30 ± 2℃ and 60% ± 5 % relative humidity.

The rhizomes of sweet flag, *Acorus calamus*, were collected from northern hilly areas of Pakistan where it grows naturally. The rhizomes were cleaned, dried and ground to a fine powder (30 mesh) and then extracted with n-hexane in the soxhlet extraction apparatus. Extracts were

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concentrated in a rotary evaporator and finally made solvent free in vacuum desiccator to obtain pure oil. The oil was stored in a refrigerator at 4℃.

Acetone was used as a solvent for *Acorus calamus* oil. Different dilutioins were prepared as under:

999 ml of acetone + 1 mL of oil = 1 liter of solution.

1 mL of solution = 1μL of oil

Phosphine gas was generated by FAO's method (Anonymous 1975). A funnel tied with thread was hanged over a cylinder with 5% H_2SO_4 solution. A pellet of aluminum phosphide wrapped in muslin cloth was dropped in solution under the funnel. Open – end burette was taken in the solution on the funnel as solution rises into the burette. As the burette filled with generated gas the level of solution go down. 5 mL gas was sucked out of it and injected into the glass jar of known volume. Again 100 mL of gas taken out from the glass jar was injected into phosphine meter to get the concentration.

Glass jars of 1 150mL capacity were used as exposure chamber. 25 grams of wheat grains were taken for each treatment. 30 grubs of *Trogoderma granarium* were placed in each glass jar. These grubs were exposed to different combinations of phosphine concentrations and *Acorus calamus* oil. Phosphine concentrations were 100, 200 and 300 ppm. The doses of *Acorus calamus* oil were 30, 50 and 70 L.

All treatments were replicated three times. There was one untreated control for each treatment.

Dosing of *Acorus calamus* oil was carried out by releasing the required volume of appropriate oil solution from an automatic pipette to a disk of 4cm diameter filter paper attached to lower surface of the lid of the glass jar.

The amount of phosphine gas required was calculated using formula as under.

Concentration × Volume of glass jar ×836. 81

Exposure periods for each treatment were 3, 5 and 7 days.

At the end of exposure period, jars were opened. The grubs of *Trogoderma granarium* were separated from the grains and mortality was assessed.

The survivors were transferred to glass jars containing untreated grains. The jars were kept at 32 ±2℃ and 65% ±5 % relative humidity. Data were collected for insect population build-up after 30 and 60 days. At the end, data were analyzed statistically by the analysis of variance, CRD test and Duncan's Multiple Range Test (Steel and Torrie, 1980).

Results and Discussion

Results of the present studies are given in table-1. Results revealed that significant difference existed between interaction of phosphine and exposure time. Percent mortality of *T. granarium* was increased with the increase in concentration of phosphine and exposure period. Maximum mortality (87. 78%) was observed at 300 ppm concentration with exposure period of seven days and minimum mortality (23. 34 %) was observed at 100ppm concentration with exposure period of three days.

According to Kashi (1982) the level of mortality increased with the increase in concentration and also by extending the exposure period. This is in accordance with our results. Present findings are also partially at par with those of Banks and Cavanaugh (1985), Winks (1986), Rajendran (1994), Proctor (1994), Irshad and Iqbal (1994) and Ahmad (1999). Our results are, however, contrary to those of Price and Mills (1988).

Results also revealed that significant difference existed between interaction of doses of *Acorus calamus* oil and exposure time. In case of *Acorus calamus* oil, mortality increased with the increase in exposure period. This indicates that the duration of exposure is more important than the dose applied.

Our findings are in accordance with the findings of Chandel *et al.* (2001); Kumari *et al.* (1999); Risha (1993) and Rasool *et al.* (2002) who observed that exposure period was much more important than dose at the levels tested.

The results of present study differ from the findings of Schmidt and Risha (1990); Risha *et al.* (1990); Schmidt *et al.* (1991) and Pierce and Schmidt (1993).

Results of present studies also revealed that when phosphine and *Acorus calamus* oil were used in combination, mortality was increased both with the increase in phosphine concentration and *Acorus calamus* oil dose as well as with the increase in exposure period.

Population build-up studies revealed that population build was reduced with the increase in dose of *Acorus calamus* oil and exposure period.

There was no effect of phosphine concentration on the population build up of the test insect.

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Table 1. Response of *Trogoderma granarium* (Everts) to different combinations of phosphine and *Acorus calamus* oil at various exposure times.

1(a): Percent Mortality of *Trogoderma granarium* (Everts)

		Phosphine concentrations						*Acorus calamus oil*					
		Phosphine used alone			Phosphine used in combination with Acorus calamus oil			Acorus calamus oil used alone			Acorus calamus oil used in combination with phosphine		
		100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	30μL	50μL	70μL	30μL	50μL	70μL
Exposure	3 Days	23.34	41.11	50.00	33.33	55.19	62.59	10.00	11.11	12.28	12.2	12.42	14.00
	5 Days	34.45	45.56	62.22	42.21	60.00	67.04	17.78	18.89	31.11	18.00	18.59	24.00
	7 Days	40.00	68.79	87.78	51.19	72.59	92.59	38.77	43.33	40.00	39.00	44.32	41.2

1(b): Population build up of *Trogoderma granarium* (Everts)

		Phosphine concentrations								*Acorus calamus oil*							
		Phosphine used alone				Phosphine used in combination with Acorus calamus oil				Acorus calamus oil used alone				Acorus calamus oil used in combination with phosphine			
		0 ppm	100 ppm	200 ppm	300 ppm	0ppm	100 ppm	200 ppm	300 ppm	0μL	30μL	50μL	70μL	0μL	30μL	50μL	70μL
Exposure	3 Days	12.99	12.36	12.37	12.21	12.92	7.85	7.39	7.90	12.61	8.72	7.39	5.44	12.69	7.52	5.81	4.77
	5 Days	12.93	12.41	12.52	12.20	12.91	7.22	6.41	7.08	12.69	6.97	5.47	4.70	12.64	5.92	4.84	3.97
	7 Days	12.94	11.51	12.89	11.31	12.93	6.36	6.04	6.82	12.56	5.45	4.16	3.31	12.55	4.91	4.31	3.69