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EFFICACY OF PHOSPHINE AND NEEM OIL AS FUMIGANT AGAINST PULSE BEETLE

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ABSTRACT

A laboratory experiment was carried out to understand the toxicity of phosphine and neem oil on adults of pulse beetles *Callosobruchus maculatus* and *C. analis*. Phosphine gas at 0.0125 mg/L dosage was relatively more toxic to *C. maculatus* (80% mortality) than *C. analis* (56.67%). Among neem oil and solvents combinations, neem oil in ethanol at 135 mg a.i/L dosage, resulted in 83.33% and 73.33% mortality in *C. maculatus* and *C. analis*, respectively. Probit analysis revealed neem oil in ethanol (151.24 a.i mg/l) and water (85.04 mg a.i/l) had low LC_{50} values with *C. maculatus*, while *C. analis* was observed with high LC_{50} (176.59 mg a.i /l and 102 mg a.i /l) with neem oil in ethanol and water, respectively. Thus an organic fumigant neem oil at high dosage led to more or equal adult pulse beetle mortality compared to inorganic phosphine at low dosage.

Keywords: *Callosobruchus maculatus, C. analis*, adults, fumigant, LC_{50}, phosphine, neem oil, ethanol, water, bioassay

The pulses in both field and storage suffer due to damage by the pulse beetles *Callosobruchus* spp. (Bruchidae: Coleoptera). Losses caused by these have been estimated to be about 30-40% in storage (Mahendran and Mohan, 2002). Phosphine is the primary fumigant used in grains and other stored commodities against insect pests (Daglish, 2004; Collins et al., 2005). Development of resistance to phosphine in storage beetle pests *Tribolium castaneum, Rhizopertha dominica* and *Trogoderma granarium* is known (Bell and Wilson, 1995), and hence, substitute fumigant is required. Neem (*Azadiracta indica*) seeds are a rich storehouse of 100 tetranotriterpinoids and diverse non isoprenoids (Devakumar and Sukhdev, 1993). Neem based products have different mode of action and are medium to broad spectrum insecticides. The present study is an attempt to evaluate the neem oil as a fumigant against the adults of *C. maculatus* and *C. analis*, as an alternative to phosphine.

MATERIALS AND METHODS

Experiments were conducted with three replications using adults from the same generation and cohort of *C. maculatus* and *C. analis* picked-up from the cultures. These were maintained at 30±2°C and 70±5% R.H on green gram (brought from local market) seeds at the Division of Entomology laboratory, ICAR–Indian Agricultural Research Institute, New Delhi. The apparatus for generation of phosphine gas consisted of a 5l beaker, a gas collecting tube (cylinder), an inverted funnel, 5% H_2SO_4 (sulphuric acid) solution, aluminium phosphide tablets and muslin cloth (FAO, 1975) following standard method. One day after setup, PH_3 gas was collected at top end of collecting tube, using a Hamilton applicator for exposing it to insects, with bubbles and blockages in syringes removed by injecting air into acetone. Appropriate dose volumes of phosphine (PH_3) gas was withdrawn from collecting tube of this gas generation apparatus for each dose and injected using Hamilton applicator into the suitable desiccator through septum, with the time of application recorded in each. Dose volumes of phosphine source were calculated using the equation (AISRF, 2012) as follows: $d_1(\mu L) = [x_1\ (mg/l) \times v_1\ (l)] / x_2\ (mg/l) \times 1000 \times 1000$, where $d_1(\mu l)$ = volume of phosphine gas to inject, x_1 (mg/l) = desired concentration in desiccator, v_1 (l) = volume of desiccator, and x_2 (mg/l) = concentration of PH_3 source (measured or 1200 mg/l, if GC not available).

Initially for knowing the discriminating dose of phosphine, the lower 0.03 mg/l and higher 0.25 mg/l were taken, as recommended by FAO for resistance monitoring in *T. castaneum, R. dominica* and *T. granarium* (FAO, 1975). Ten adults each of *C. maculatus* and *C. analis* of same age and generation were taken in culture tube having their mouths tied with muslin cloth. These were placed in the desiccator and closed with lid having septum. PH_3 gas bioassay was carried out at six dosages i.e. 1 µl (0.0005 mg/l), 5 µl

(0.0025 mg/l), 10 µl (0.005 mg/l), 15 µl (0.0075 mg/l), 20 µl (0.01 mg/l) and 25 (0.0125 mg/l) µl. Control was without PH_3 in the desiccator. PH_3 gas concentration was assumed to 1200 mg/l (AISRF, 2012). Fumigation with PH_3 gas was done at different dosages for 24 hr, after which lids of desiccators were removed, culture tubes were taken out and desiccators ventilated. A small quantity of culture medium (green gram seed) was added to each culture tube before the tubes were transferred to maintain at 25°C and 55% RH, for 7 days to assess adult mortality.

Five doses (135 mg a.i/L, 120 mg a.i/L, 105 mg a.i/L, 90 mg a.i/L and 75 mg a.i/L) of neem oil 0.03% (MultiNeem, Multiplex Co.) were prepared with two solvents *viz.,* water and ethanol along with one control (either only ethanol or water). Adults of *C. maculatus* and *C. analis* of same age and generation were released into 250ml capacity volumetric fumigation flask (VFF). For each dose three VFF were taken and in each, 10 adults from same generation were released. Later 1 x 1 cm sized filter paper was tied with thread at the center to hang inside VFF. By placing 10 µl quantity on filter paper using micropipette each dosage was imposed. For each VFF, two such filter papers were hung inside, which finally delivered a quantity of 20 µl per 250 ml volume of VFF. After 24 hr exposure, adults were removed to a culture tube with green gram seeds, to maintain at 25°C and 55% RH, with mortality observed after 7 days after exposure.

Data obtained was analyzed using one-way ANOVA in completely randomized design (Gomez and Gomez, 1983). Mortality and survival data were subjected to arcsine transformation, and to compare means Duncan Multiple Range Test (DMRT) was used in MSTAT software. Results were also interpreted to the resistance/ susceptibility classification for PH_3 gas proposed by AISRF (2012).

RESULTS AND DISCUSSION

PH_3

To find discriminating dose, range finding test was conducted initially by testing four-day-old *C. maculatus* and *C. analis* against PH_3 gas at lower (0.03 mg/l) and higher (0.25 mg/l) doses. This showed 100% mortality in both the doses, and interpretation reveal that the two species fall under susceptible category. Therefore, still lower doses of 0.003mg/l (low) and 0.01 mg/l (high) were tried and 40 and 60% mortality, respectively of *C. maculatus* observed; in *C. analis* adults it was 20 and 60%, respectively. Based on these results, 0.003 and 0.01 mg/l doses were considered for further toxicity studies.

Toxicity of six doses revealed that highest (80%) mortality in *C. maculatus* was observed at 0.0125 mg/l, which significantly differed from other doses; it was followed by 63.33% mortality at 0.01 mg/l, and there was no significant variations in mortality with other doses, with the least value being with 0.0005 mg/l. With regard to *C. analis* maximum mortality was at 0.0125 mg/l (56.67%), which significantly differed from other doses; the least mortality (16.67%) was observed at 0.0005 mg/l (Table 1).

Neem oil

Bioassay as fumigant did not reveal any overlap of toxicity between the neem oil and solvents (ethanol and water) combinations. The evaluated doses of neem oil in water viz., 135 mg a.i/l, 120 mg a.i/l and 105 mg a.i/l revealed 36.67, 26.67 and 23.33% mortality in *C. maculatus*, which are on par with each other. However, in *C. analis* 23.33, 16.67 and 10.00% mortality was observed at these doses. Maximum mortality of 83.33 and 73.33% was achieved with neem oil and ethanol combination at 135 mg a.i/l in *C. maculatus* and *C. analis*, respectively (Table 1).

The log dose-probit assay obtained from the above data for the neem oil along with solvent combinations gave LC_{50} and LC_{90} values. These revealed that with *C. analis*, it was maximum (LC_{50} of 176.59 mg a.i /l) over *C. maculatus* (151.24 a.i mg/l) in neem oil and water combination. Similarly, with ethanol combination, with *C. analis* it was maximum LC_{50} of 102 mg a.i/l compared to *C. maculatus* (85.04 mg a.i/L). Neem oil in ethanol resulted in low LC_{50} values in both the species compared to that with water. Perhaps, ethanol as organic polar solvent dissolved the neem oil appropriately, provided good fumigant action. And *C. analis* resisted the high concentration of neem oil compared to *C. maculatus*. For neem oil and water combination, a dosage of 249.48 mg a.i/l was enough to attain LC_{90} value in *C. maculatus*, but a higher dose of 293.12 mg a.i/l is required to achieve the same in *C. analis*. An elevated dosage 205.43 mg a.i/l is required for *C. analis* to reach LC_{90} value in neem oil and ethanol combination.

To conclude, phosphine gas at very low dose (0.0125 mg/l) itself led to 80% and 56.67% mortality in *C. maculatus* and *C. analis*, respectively (Table 2). Sohail et al. (2002) reported 100% adult morality of *C. maculatus* at 200 mg/l with PH_3. Neem oil in ethanol gave

Table 1. Toxicity of phosphine and neem oil against *Callosobruchus* spp. (adults)

	Phosphine					Neem oil (% mortality)			
	C. maculatus		*C. analis*			*C. maculatus*		*C. analis*	
Dose (mg/l)	% Mortality	% Survival	% Mortality	% Survival	Dose (mg a.i/l)	Neem oil (water)	Neem oil (ethanol)	Neem oil (water)	Neem oil (ethanol)
0.0005	26.67^{d}±2.08 (89.26)	73.33^{d}±2.08 (155.97)	16.67^{e}±0.58 (73.3)	83.33^{f}±0.58 (167.66)	135.0	36.67^{a}±5.77 (12.4)	83.33^{a}±5.77 (66.12)	23.33^{a}±5.77 (9.59)	73.33^{a}±5.77 (58.98)
0.0025	53.33^{c}±2.52 (131.03)	46.67^{c}±2.52 (121.26)	26.67^{d}±0.58 (93.56)	73.33^{e}±0.58 (156.98)	120.0	26.67^{a}±11.55 (10.26)	66.67^{b}±5.77 (54.76)	16.67^{a}±11.55 (7.95)	63.33^{b}±5.77 (52.75)
0.005	53.33^{c}±0.58 (133.35)	46.67^{c}±0.58 (124.55)	36.67^{c}±0.58 (110.12)	63.33^{d}±0.58 (145.61)	105.0	23.33^{a}±5.77 (9.59)	63.33^{b}±5.77 (52.75)	10.00^{a}±5.77 (5)	43.33^{c}±5.77 (41.14)
0.0075	53.33^{c}±0.58 (133.35)	46.67^{c}±0.58 (124.55)	33.33^{c}±1.53 (103.38)	66.67^{c}±1.53 (148.92)	90.0	6.67^{b}±5.77 (4.1)	53.33^{c}±5.77 (46.9)	6.67^{a}±5.77 (4.1)	36.67^{d}±5.77 (37.21)
0.01	63.33^{b}±0.58 (145.61)	36.67^{b}±0.58 (110.12)	43.33^{b}±1.15 (119.34)	56.67^{b}±1.15 (137.22)	75.0	3.33^{cb}±5.77 (2.05)	43.33^{c}±5.77 (41.14)	0.00^{b}±5.77 (0)	33.33^{d}±5.77 (35.2)
0.0125	80.00^{a}±1.00 (164.03)	20.00^{a}±1.00 (79.45)	56.67^{a}±0.58 (137.54)	43.33^{a}±0.58 (119.93)	0.0	0.00^{e}±5.77 (0)	0.00^{d}±5.77 (0)	0.00^{b}±5.77 (0)	0.00^{e}±5.77 (0)
0.00	0^{e}±0.00 (0)	100^{e}±0.00 (184.28)	0^{f}±0.00 (0)	100^{g}±0.00 (184.28)					
C.D (p=0.01)	4.84	4.77	3.41	2.49	C.D (p=0.01)	21.45	10.12	22.42	9.64
S.Em±	1.21	1.10	0.79	0.57	S.Em±	4.52	2.13	4.72	2.03

Means followed by same letters do not differ significantly (p=0.01) DMRT; Values in parentheses arcsine transformed

Table 2. Toxicity of neem oil 0.03% as fumigant against *Callosobruchus* spp.

	LC_{90} (mg a.i/L)	LC_{50} (mg a.i/L)	95% confidence limits		Regression equation (Y = a + bX)	Fit of probit line		df	n
			Lower bound	Lower bound		Heterogenity factor ($\chi 2$)	Slope (±S.E)		
Water as solvent									
C. maculatus	249.48	151.24	132.38	219.78	Y=12.85+5.89X	1.002	5.89±1.59	3	30
C. analis	293.12	176.59	145.24	421.76	Y=13.08+5.82X	0.872	5.82±1.96	3	30
Ethanol as solvent									
C. maculatus	179.99	85.04	61.921	96.41	Y=7.59+3.93X	0.92	3.93±1.19	3	30
C. analis	205.43	102.80	90.02	117.39	Y=8.576+4.26X	1.687	4.26±1.18	3	30

n- number of larvae; df- degree of freedom; $\chi 2$- insignificant for all the assays

maximum mortality of *C. maculatus* and *C. analis* (83.33 and 73.33%, respectively) at 135 mg a.i/l. In contrast, neem oil in water gave a maximum of 36.67 and 23.33% mortality in *C. maculatus* and *C. analis*, respectively at 135 mg a.i/l. These results reveal that neem oil in ethanol is having considerable fumigant action, rather than with water as solvent. There are some advantages of neem oil as fumigant rather than phosphine, as it is ecofriendly, non-toxic, and less polluting.

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