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Effect of Hexythiazox 5.45 EC on Developmental Stages of *Oligonychus biharensis* Hirst and *Tetranychus urticae* Koch (Acari: Tetranychidae)

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The effect of the acaricide, hexythiazox 5.45 EC, known for its activity against eggs, and immature stages of spider mites, was evaluated in the laboratory against *Tetranychus urticae* Koch and *Oligonychus biharensis* Hirst, infesting grapes. *O.biharensis* was more susceptible than *T.urticae*. *Hexythiazox 5.45 EC* was highly effective against eggs of *O.biharensis* resulting in zero per cent egg hatch at 10 ppm itself on freshly laid eggs while in *T.urticae* zero per cent egg hatch occurred at 30 ppm only. The per cent egg hatch in untreated control ranged between 80.9 and 88.4 for different age groups of *O.biharensis* and *T.urticae* eggs. Toxicity on eggs depended on the age of eggs, freshly laid eggs being highly susceptible. Similar effects were observed in protonymphs also with more than 80 per cent mortality on the first day of treatment in *O.biharensis* and *T.urticae*, when leaf discs were treated at 30 ppm using spray method.

Key words: Hexythiazox 5.45 EC, ovicidal activity, O.biharensis, T.urticae, life stages

Spider mites are among the major biotic constraints in grapevine cultivation all over the world (Duso *et al.,* 2012). Rather (2008) reported 16 species of phytophagous mites in grape vineyards of Jammu and Kashmir, out of which 11 species belonged to the spider mite family, Tetranychidae. *Oligonychus biharensis* Hirst and *Tetranychus urticae* Koch were the predominant spider mite pests in Coimbatore vineyards. Resistance to pesticides is one of the major limitations, in chemical management of spider mites. Repeated application of the same chemical or chemicals with the same mode of action increases the chances of pest population developing resistance (Ramasubramanian *et al.,* 2004). Another important factor in spider mite population resurgence is lack of toxicity to eggs and many acaricides that are used in mite management, do not have ovidical properties. Hexythiazox 5.45 EC, which belongs to the thiazolidine group is reported to have ovicidal action and highly selective for phytophagous mites (Alzoubi and Cobanoglu, 2010) with growth inhibitory mode of action (Dekeyser, 2005; Nauen and Smagghe, 2006). Hence, its effect on the eggs of *O.biharensis* and *T.urticae* infesting grapevines was studied in the laboratory.

Materials and Methods

O.biharensis were collected from grapevines at Orchard, Tamil Nadu Agricultural University, Coimbatore and reared on grapevine seedlings raised at Insectary, TNAU. *T.urticae* was collected from the laboratory culture at Insectary, TNAU and reared on bhendi. Both the cultures were maintained at 27 ± 5° C and 68 ± 10 % RH.

Five adult females of *O.biharensis* and *T.urticae* were allowed to lay eggs on grapevine and bhendi leaf discs (3.5 cm diameter) kept in plastic containers separately and transferred to fresh leaf discs every 24 hours for 5 days. The number of eggs laid was counted and the leaf discs infested with eggs (freshly laid, 1 day old, 2 days old, 3 days old and 4 days old) were treated with dilutions of hexythiazox 5.45 EC at 50 ppm, 40 ppm, 30 ppm, 20 ppm and 10 ppm by immersion for 5 seconds. Then leaf discs were air dried and kept in plastic cups (4 cm diameter) with minutely perforated lid. The eggs dipped in water served as control. This was replicated four times. Egg hatch was assessed for 5 days and total number of eggs hatched in each age group was enumerated. Per cent egg hatch was then calculated using the number of eggs laid and number of eggs hatched in each age group (Bleicher, 2003).

The method used by Knight *et al*. (1990) for evaluating acaricide bioefficacy against spider mite protonymphs was followed. The test unit consisted of a detached leaf disc of about 3.5 cm diameter (grapevine leaf for *O.biharensis* and bhendi leaf for *T.urticae*) on a filter paper kept over a layer of water-saturated cotton in a plastic container. Twenty numbers of protonymphs, the second active immature stage, were transferred carefully onto the treated leaf surface using a fine camel hair brush and closed with the lid having adequate holes for ventilation. Leaf discs were treated by spraying with the acaricide dilutions using an atomizer and were allowed to dry. The toxicity of hexythiazox 5.45 EC on protonymphs of each species was assessed at 10, 20, 30, 40 and 50 ppm concentrations. Water treated leaf discs

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served as control. The six treatments were replicated four times using 20 protonymphs of each species and number of surviving mites was observed on 1, 3 and 5 days after treatment. Individual mite survival was ascertained by touching each mite with a fine brush. Mites that were unable to move at least a distance equivalent to their body length were considered dead. The data recorded were expressed as per cent mortality.

Results and Discussion

Each *O. biharensis* female laid 2-3 eggs per day and *T. urticae* female laid 4-5 eggs per day. The ovicidal action of hexythiazox 5.45 EC was more pronounced on *O. biharensis* compared to *T. urticae*. The freshly laid *O. biharensis* eggs (0 day old) were highly susceptible and even at its lowest dose of 10 ppm, resulted in 100% mortality of the freshly laid eggs while eggs in control showed 80.9% hatching. As the age of eggs progressed, the hatching per cent increased. At 10 ppm, 35.5% of the 4-days old eggs hatched. At 40 ppm, only 3.0 % of the 4 days old eggs hatched and at 50 ppm, none of the eggs belonging to any of the age group hatched (Table 1).

T. urticae eggs were comparatively more tolerant. There was 14.1% of hatching when freshly laid eggs were treated at 10 ppm which was reduced to 8.4% at 20 ppm and to 0 % at all concentrations above 20 ppm. The per cent egg hatch of 1 day old eggs was 30.03, 15.52, and 2.76 at 10, 20 and 30 ppm respectively and thereafter 0% at 40 and 50 ppm. However, 73.04 % of the 4 days old eggs hatched at 10 ppm. At 40 ppm, only 5.39 and 5.23 % of the eggs hatched in the ages of 3 and 4 days respectively and at 50 ppm none of the eggs at any age, hatched. Similar trend of reduced toxicity, was noticed in *T.urticae* also. The per cent egg hatch in untreated control ranged between 80.9 and 88.4 for different age groups of *O.biharensis* and *T.urticae* eggs (Table -1). This indicated the strong ovicidal action of the acaricide, the toxicity of which depended on the age of the eggs, the freshly laid eggs being the most vulnerable.

The second immature stage of the mite, protonymph was found to be susceptible to at a much lower concentration than the field dose. None of the *O.biharensis* mites survived when treated at 30 ppm and above. At a lower dose of 20 ppm, 20 per cent of the mites could survive one day after treatment while at 10 ppm, 70 and 85.00 per cent mortality were observed, 1 and 5 days after treatment respectively. The mortality at all doses was significantly different from the untreated control which recorded 5.00 – 25.00 per cent mortality only (Table 2). A similar trend was noticed in protonymphs of *T.urticae* but with a little higher survival than those of *O.biharensis*. At 30, 40 and 50 ppm was observed more than 80 per cent mortality even on the 1st day after treatment. At 10 and 20 ppm, 78.75 and 93.75 per cent mortality was observed on 7th day after treatment while in water sprayed control, 13.75 per cent mortality only was observed which was significantly superior to all other treatments. Thus, it is evident that the acaricide, hexythiazox 5.45 EC has an appreciable nymphycidal action also against *O.biharensis* and *T.urticae*.

Table 1. Ovicidal action of hexythiazox 5.45 EC on eggs of *O.biharensis* and *T.urticae*

Concentration	*O.biharensis* - % Egg hatch					*T.urticae* - % Egg hatch				
	0 day	1 day	2 days	3 days	4 days	0 day	1 day	2 days	3 days	4 days
10ppm	0.0 (0.6)$_a$	10.5 (18.9$_b$	21.1 (27.3)$_c$	33.2 (35.2)$_c$	35.5 (36.6)$_c$	14.1 (22.1)$_c$	30.0 (33.2)$_d$	38.0 (38.1)$_d$	47.6 (43.6)$_e$	73.0 (61.6)$_e$
20ppm	0.0 (0.6)$_a$	7.4 (15.8$_b$	13.6 (21.7)$_c$	24.8 (29.9)$_c$	27.4 (31.6)$_c$	8.4 (16.9)$_b$	15.5 (23.2)$_c$	22.8 (28.5)$_c$	29.7 (33.0)$_d$	39.6 (39.0)$_d$
30ppm	0.0 (0.6)$_a$	0.0 (0.6)$_a$	2.8 (9.6)$_b$	10.4 (18.8)$_b$	11.7 (20.0)$_b$	0.0 (0.6)$_a$	2.8 (9.6)$_b$	10.1 (18.5)$_b$	15.5 (23.2)$_c$	20.2 (26.7)$_c$
40ppm	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	3.0 (10.0)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	5.4 (13.4)$_b$	5.2 (13.2)$_b$
50ppm	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$	0.0 (0.6)$_a$
Control	80.9 (65.3)$_b$	86.3 (68.3$_c$	82.6 (65.3)$_d$	86.7 (68.6)$_d$	87.0 (68.9)$_d$	81.6 (66.0)$_d$	84.3 (67.9)$_e$	86.0 (68.0)$_e$	87.2 (69.0)$_f$	88.4 (70.1)$_f$
CD (0.05 %)	1.37	8.33	8.81	10.62	8.69	3.94	5.29	1.55	3.54	5.29
SEd	0.63	3.82	4.04	4.88	3.99	1.81	2.43	0.71	1.62	2.43

Figures in parenthesis are arc sine transformed values.

In a column, means followed by the same alphabet(s) are not significantly different by DMRT (P=0.05).

The result was in accordance with the findings of Hoy and Ouyang (1986) who reported that hexythiazox-treated eggs of the pacific spider mite, *Tetranychus pacificus* Mc Gregor became less susceptible with age. Likewise, Welty *et al.* (1988) found that susceptibility of European red mite, *Panonychus ulmi* (Koch) eggs treated with hexythiazox decreased with age. Similar results were observed by Marris (1988) in *T.urticae*. The reason for this decreased susceptibility with age is unclear. Aveyard *et al.* (1986) suggested that the embryo might become less susceptible or penetration through the chorion might have occurred slowly, thus preventing sufficient active ingredient from reaching the embryo when treated at a later age. The high toxicity of ovicides to the larval stage suggests that the embryo does not increase in tolerance with age and

the reason therefore might be the rate of penetration through chorion.

Most of the immatures which were found dead, were at the next chrysalis stage (deutochrysalis) and never emerged as deutonymphs at 40 and 50 ppm.

Welty *et al*. (1988) also observed that when nymphs of *P.ulmi* were exposed to hexythiazox residues (100 ppm), mites usually died in the nymphochrysalid stage and a few died in the protonymphal or deutonymphal stages.

Table 2. Effect of hexythiazox 5.45 EC on protonymphs of *O. biharensis* and *T. urticae*

Concentration	*O.biharensis* - % mortality			*T.urticae* - % mortality		
	1 DAT	3 DAT	5 DAT	1 DAT	3 DAT	5 DAT
10ppm	70.00 (56.79)$_c$	78.75 (62.55)$_c$	85.00 (67.22)$_b$	56.25 (48.59)$_c$	58.75 (50.04)$_e$	77.50 (61.69)$_c$
20ppm	80.00 (63.44)$_b$	90.00 (71.57)$_b$	95.00 (77.08)$_b$	65.00 (53.73)$_c$	80.00 (63.44)$_d$	86.25 (68.24)$_b$
30ppm	100.00 (89.36)$_a$	100.00 (89.36)$_a$	100.00 (89.36)$_a$	83.75 (66.23)$_b$	92.50 (74.11)$_c$	100.00 (89.36)$_a$
40ppm	100.00 (89.36)$_a$	100.00 (89.36)$_a$	100.00 (89.36)$_a$	91.75 (73.31)$_b$	97.50 (80.90)$_b$	100.00 (89.36)$_a$
50ppm	100.00 (89.36)$_a$	100.00 (89.36)$_a$	100.00 (89.36)$_a$	100.00 (89.36)$_a$	100.00 (89.36)$_a$	100.00 (89.36)$_a$
Control	5.00 (12.92)$_d$	16.50 (23.97)$_d$	21.25 (27.45)$_c$	3.75 (11.17)$_d$	8.75 (17.21)$_f$	11.25 (19.60)$_d$
SEd	0.50	0.44	0.54	4.53	2.83	1.18
CD (0.05 %)	1.05	0.93	1.14	9.52	5.94	2.47

Figures in parenthesis are the arcsine transformed values.
In a column, means followed by the same alphabet(s) are not significantly different by DMRT.
DAT – Days After Treatment.

Hexythiazox 5.45 EC at the field recommended rate of 50 ppm was found to be an efficient acaricide with good ovicidal and nymphicidal action, that can be applied early in the season, when the first symptoms of spider mite occurrence are noticed. The application when coinciding with the early deposition of eggs, would achieve a more effective control and can thus be included in pesticide schedule in viticulture.

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