J. Biol. Control, 21(Special Issue): 197-202, 2007

Acarotoxicity of *Hirsutella thompsonii* Fisher exudate with reference to the two-spotted spider mite, *Tetranychus urticae* Koch

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ABSTRACT: The exudate from sporulating culture of the mite-specific fungal parasite *Hirsutella thompsonii* Fisher was tested for potential acarotoxicity through its effect against different stages of *Tetranychus urticae* Koch, the two-spotted spider mite. *T. urticae* eggs treated with the exudate showed very significant reduction (64.6%) in hatching. The exudate exhibited ovicidal effect through desiccation in up to 28 per cent of the eggs. A significant number (38 %) of treated eggs remained intact and unhatched even after 4 days of incubation, compared with control (4%). The exudate showed toxicity to both nymphs and adults of *T. urticae* that were fed separately on treated cowpea leaves. A maximum of 33.3 per cent mortality was obtained in nymphs before they turned into adults. Adult mortality increased from 55.6 to 77.8 per cent between the fifth and seventh days of treatment. In the fecundity test, an adult female feeding on exudate-treated leaf could lay only 30.7 eggs compared with 51 eggs laid by the mite feeding on untreated leaf over a period of 7 days. Hatching of those eggs was also lower (18.5%) in comparison with control (38.3%).

KEYWORDS: Acarotoxicity, exudate, *Hirsutella thompsonii*, *Tetranychus urticae*

INTRODUCTION

Hirsutella thompsonii Fisher (Mitosporic fungi: Hyphomycetes) is known to infect a wide range of eriophyid and tetranychid mites both in nature and when artificially inoculated (Chandler *et al.*, 2000; Kumar and Singh, 2000; van der Geest *et al.*, 2000). The fungus is known to produce metabolites toxic to several mite and insect species (Vey *et al.*, 1993; Krasnoff and Gupta, 1994; Mazet and Vey, 1995; Omoto and McCoy, 1998). Certain isolates are known to exude droplets (Cabrera and Lopez, 1977; Samson *et al.*, 1980), which have recently been demonstrated to inhibit oviposition by the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (Rosas-Acevedo *et al.*, 2003). Our work with several isolates of *H. thompsonii* also indicated the production of exudate in cultures on a variety of media.

T. urticae is a major pest of many horticultural crops including vegetables and ornamentals. Since biological control is preferred to chemical control in agricultural and horticultural ecosystems wherever feasible, the utility of fungal pathogens such as *H. thompsonii* assumes more importance. The objective of the present study was to find out if the exudate secreted by sporulating cultures of an isolate of *H. thompsonii* derived from the coconut eriophyid mite (*Aceria guerreronis* Keifer) showed acarotoxicity to various stages in the lifecycle of *T. urticae*.

MATERIALS AND METHODS

Mite culture

Healthy stock cultures of the two-spotted spider mite, *T. urticae* were multiplied and maintained on pot-grown cowpea plants [*Vigna unguiculata* (L.) Walp.] and on detached cowpea leaves in Petri-dishes lined with moist cotton or sponge in the laboratory. The initial culture originated from mites collected from Bangalore in

2003. Different stages of the mite were obtained from cultures kept separately. To obtain fixed-age adults for the bioassays, quiescent deutonymphs were separately collected and put on leaf discs. For getting eggs of same age, adult females were placed on detached cowpea leaf cultures in Petri-dishes for 24 or 48 h to deposit eggs.

Fungus culture

H. thompsonii [isolate MF(Ag)5], originally isolated from the coconut mite, was used in the present studies. The test fungus was propagated on freshly prepared potato dextrose agar (PDA) so as to get a constant supply of the same for the experiments.

Exudate production and collection

Sporulating mycelial discs (6-mm diam.) were cut from 1-month-old PDA cultures of *H. thompsonii* and were added to 500-ml Erlenmeyer flasks containing 200 ml of Sabouraud dextrose broth at the rate of five discs per flask. The flasks were run on an orbital shaker [Orbitek-L, Scigenics Biotech (Pvt.) Ltd., Chennai] at 150 rpm for seven days. At the end of the run, 1-ml aliquots of the biomass were transferred to PDA in Petri-dishes and incubated upside down in an incubator set at 25 °C and 12-h photoperiod. Sporulating colonies after 20-30 days incubation were used for collecting the exudate. The biomass was produced in several batches and plated for obtaining the exudate as and when required. The exudate in 1-5 ml quantities was collected on different occasions from 86-115 colonies measuring 10-13-mm in diameter (average 11.6 mm) with the help of a sterile, disposable 1-ml syringe (Fig. 1). In all the experiments, the spore-free exudate was used immediately after extraction.

Experimental methods

All the experiments were performed during 2005 under laboratory conditions (28 ± 2 °C daytime temperature) following the leaf disc culture technique. Moist chambers were created with clean, pre-sterilized, glass Petri-dishes (90 x 15 mm) holding a sponge disc (90-mm diam.) and a layer of country filter paper disc of the same size over that. Sterile deionized water was used to moisten the sponge. Cowpea leaf discs (30-mm diam.) placed on the damp filter paper with the under surface facing up. Moisture was increased as and when required during the experiment period. Fresh and healthy cowpea leaves grown separately under mite-free conditions were

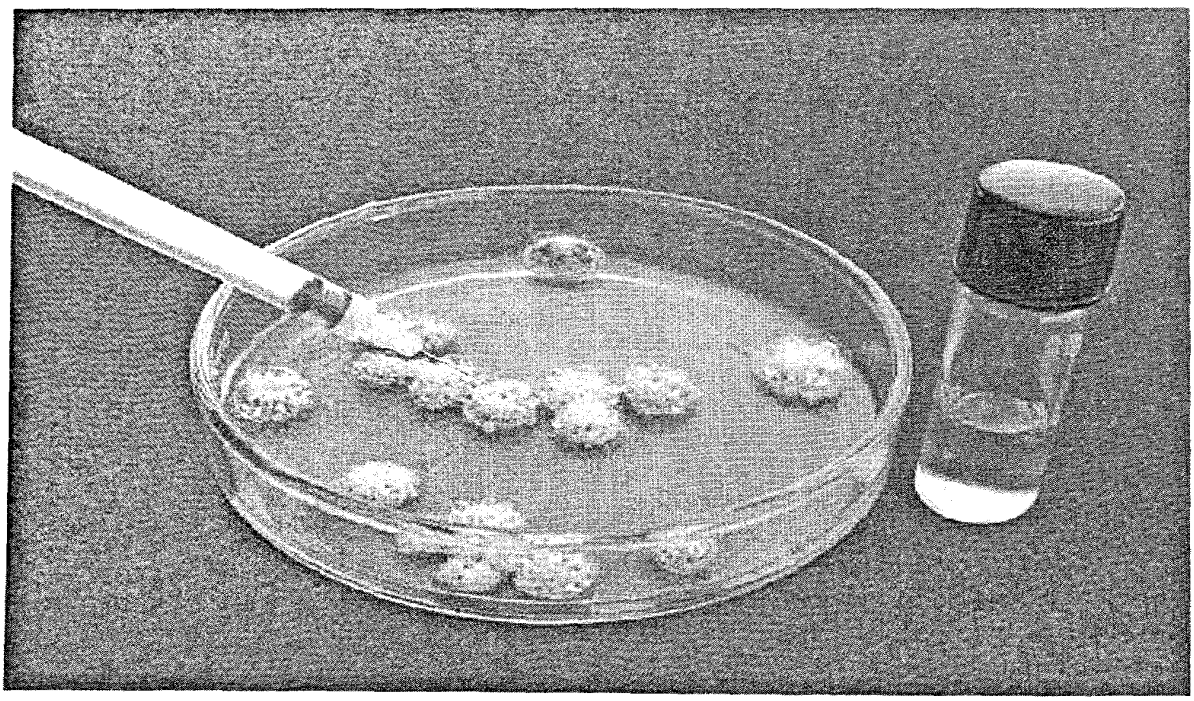

Fig. 1. Collection of exudate from *H. thompsonii* colonies

used throughout the present study. Eggs are usually laid near the veins of leaves and so the discs were cut out with the midrib passing from the centre.

The effect of the exudate on eggs was studied first. Freshly laid *T. urticae* eggs were picked from the laboratory-reared culture and immersed in the exudate for 30 minutes. Ten eggs were then carefully removed with the help of a fine brush and put onto a 30-mm circle of a cowpea leaf. Another set of eggs was similarly treated with sterile deionized water. Both sets consisted of five replicate discs. The eggs during the incubation period of four days were classified as hatched, intact unhatched and desiccated on each day. For studying the effect on both the nymphal and adult stages, the mites were fed on host leaf circles applied with the exudate. The exudate was applied on the disc with a fine brush and allowed to air-dry for 20-30 minutes before releasing the mites. Healthy nymphs (a mixture of proto- and deutonymphs) were released at the rate of 30 individuals per disc. Three leaves per treatment were maintained. In control, leaf discs were smeared with sterile deionized water. The mortality percentage was calculated every 24 h by recording the number of live and dead mites for four days. Healthy *T. urticae* adults (female majority) of approximately the same age were also tested in a similar manner as for nymphs. Mortality assessment was done on each day for seven days. A quiescent female deutonymph closest to emergence along with a male clinging on to it was picked up and transferred to exudate-treated disc at the rate of one mating pair per disc. Fifty such pairs were set up initially and only 30 pairs that survived on the second day after treatment were retained as individual replicates for the exudate treatment. A similar number of replicates were maintained for the control. The number of eggs laid by the female per day was recorded for a week.

RESULTS AND DISCUSSION

The exudate from sporulating culture of *H. thompsonii* was found to have acarotoxicity through experiments done on its effect against different stages of *T. urticae*, which is one of the potential polyhouse- or field-level targets in future. The exudate (*ca.* pH 9) was odourless, but had a pale greenish grey colouration, which was not very evident all the time. However, Rosas-Acevedo *et al.* (2003) described the exudate secreted by strain HtM 1201 as 'rose-coloured'. *T. urticae* eggs treated with the exudate showed very significant reduction (64.6%) in hatching (Table 1), which commenced only after the end of 24 h of incubation. The exudate exhibited ovicidal effect through desiccation in up to 28 per cent of the eggs. Desiccation commenced from within a day of treatment and increased on successive days of observation, with a drastic 8-fold increase by end of the second day (Table 2). In contrast, there was no desiccation in control eggs. A significant number (38 %) of treated eggs remained intact and unhatched (Table 3) even after 4 days of incubation, compared with control (4%).

The exudate showed toxicity to both nymphs and adults of *T. urticae* that were fed separately on treated cowpea leaves. A maximum of 33.3 per cent mortality was obtained in nymphs before they turned into adults (Table 4). Adult mortality was observed between the second and third days after treatment, and a drastic increase from 55.6 to 77.8 per cent happened between the fifth and seventh days (Table 5). In the fecundity test, an adult female feeding on exudate-treated leaf could lay only 30.7 eggs compared with 51 eggs laid by the mite feeding on untreated leaf over a period of 7 days (Table 6).

Prior to the present study on the deleterious effect of *H. thompsonii* exudate on the two-spotted spider mite,

Table 1. Effect of *H. thompsonii* exudate on *T. urticae* eggs: hatched

Treatment	Hatched eggs (% ± SEM) after (days)		
	2	3	4
Exudate	4 ± 2.45 (7.4)	24 ± 10.30 (25.8)	34 ± 13.64 (31.8)
Control	36 ± 9.27 (36.0)	74 ± 7.48 (60.3)	96 ± 2.45 (82.6)
't' value	3.83*	3.53*	6.49**

Figures in parentheses are arcsine-transformed values; *Highly significant ($P = 0.01$); **Very highly significant ($P = 0.001$); ^{NS}Not significant

Table 2. Effect of *H. thompsonii* exudate on *T. urticae* eggs: intact unhatched

Treatment	Intact unhatched eggs (% ± SEM) after (days)			
	1	2	3	4
Exudate	98 ± 2.00 (86.3)	80 ± 6.32 (64.5)	56 ± 8.12 (48.6)	38 ± 7.35 (37.6)
Control	100 ± 0.00 (90.0)	64 ± 9.27 (54.0)	26 ± 7.48 (29.7)	4 ± 2.45 (7.4)
't' value	1.00^{NS}	1.41^{NS}	2.70*	4.74**

Figures in parentheses are arcsine - transformed values; *Significant ($P = 0.05$); ** Highly significant ($P = 0.01$); ^{NS} Not significant

Table 3. Effect of *H. thompsonii* exudate on *T. urticae* eggs: desiccated

Treatment	Desiccated eggs (% ± SEM) after (days)			
	1	2	3	4
Exudate	2 ± 2.0 (3.69)	16 ± 8.12 (18.18)	20 ± 8.94 (23.02)	28 ± 7.35 (31.16)
Control	0 ± 0.0 (0.00)	0 ± 0.0 (0.00)	0 ± 0.0 (0.00)	0 ± 0.0 (0.00)
't' value	1.00^{NS}	2.23^{NS}	3.02*	6.49**

Figures in parentheses are arcsine-transformed values; *Significant ($P = 0.05$); ** Very highly significant ($P = 0.001$); ^{NS} Not significant

Table 4. Effect of *H. thompsonii* exudate on *T. urticae* nymphs

Treatment	Mortality (% ± SEM) after (days)			
	1	2	3	4
Exudate	5.6 ± 1.11 (13.5)	11.1 ± 2.94 (19.2)	17.8 ± 1.11 (24.9)	33.3 ± 1.93 (35.3)
Control	1.1 ± 1.11 (3.5)	7.8 ± 1.11 (16.1)	10.0 ± 1.92 (18.3)	11.1 ± 1.11 (19.4)
't' value	2.62^{NS}	1.05^{NS}	3.26*	10.30**

Figures in parentheses are arcsine-transformed values; *Significant ($P = 0.05$); ** Very highly significant ($P = 0.001$); ^{NS} Not significant

Table 5. Effect of *H. thompsonii* exudate on *T. urticae* adults

Treatment	Mortality (% ± SEM) after (days)				
	3	4	5	6	7
Exudate	13.3 ± 3.33 (21.1)	40.0 ± 10.18 (38.9)	55.6 ± 5.89 (48.3)	76.7 ± 3.84 (61.3)	77.8 ± 4.01 (62.0)
Control	2.2 ± 2.22 (5.0)	16.7 ± 3.85 (23.8)	18.9 ± 4.84 (25.4)	35.6 ± 2.22 (36.6)	36.7 ± 3.33 (37.2)
't' value	2.75^{NS}	2.17^{NS}	4.53*	8.38**	7.39**

Figures in parentheses are arcsine-transformed values; *Significant ($P = 0.05$); ** Highly significant ($P = 0.01$); ^{NS} Not significant

Table 6. Effect of *H. thompsonii* exudate on the fecundity of *T. urticae*

Treatment	Fecundity (no.± SEM /female) after (days)						
	1	2	3	4	5	6	
Exudate	0.57 ± 0.26	3.83 ± 0.59	8.43 ± 1.13	17.77 ± 1.79	23.37 ± 2.53	27.43 ± 2.70	30.67 ± 2.84
Control	0.53 ± 0.30	6.03 ± 0.50	10.97 ± 0.86	19.00 ± 1.41	24.93 ± 1.43	39.73 ± 1.38	50.97 ± 1.83
't' value	0.08^{NS}	2.86*	1.78^{NS}	0.54^{NS}	0.54^{NS}	4.06**	6.01**

*Highly significant ($P = 0.01$); **Very highly significant ($P=0.001$); ^{NS} Not significant

the only information available was on the oviposition suppression property (Rosas-Acevedo *et al.*, 2003). *H. thompsonii* has been reported to produce insecticidal and acaricidal metabolites such as hirsutellin A, hirsutellin B, and (+) phomalactone, 6- (1-propenyl)-5, 6-dihydro-5-hydroxypyran-2-one in spent liquid media (Vey *et al.*, 1993; Krasnoff and Gupta, 1994; Mazet and Vey, 1995; Omoto and McCoy, 1998).

The mode of action of the exudate is still not clear although its alkaline nature might have an impact on the mite metabolism. It has to be seen if the action of the exudates on the mite is dose-dependent. It is also presumed that topical application of the exudate will also have a significant impact on the different life stages of the mite. In the present study, *T. urticae* was taken up only as a model target, although several other tetranychid, eriophyid or related species of mites could be more susceptible to the exudate. Eriophyid mites could show better susceptibility because the fungus used in the study originated from an eriophyid and also because *H. thompsonii* is more pathogenic to them than to tetranychids in nature. It has been discovered that the exudate produced by *H. thompsonii* during and/or after sporulation possesses acarotoxic properties vis-à-vis its effect on the eggs, nymphs, adults and fecundity of *T. urticae*. The only report available prior to this research suggested oviposition inhibition property of the exudate in the same target mite (Rosas-Acevedo *et al.*, 2003). In the next stage of research, the effect of topical application of the exudate on the test mite/s could be investigated. Although hirsutellin A has been extracted and purified from the culture filtrate (Liu *et al.*, 1995) the same has not been achieved for the exudates. Parallel studies should, therefore, be directed at characterizing the toxic compounds secreted by the fungus in the form of the exudate, and their acute and chronic effects on mites.

ACKNOWLEDGEMENTS

The authors are grateful to the Project Director, Project Directorate of Biological Control, Bangalore, for the facilities provided during the study.

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