INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–2–276–279
http://www.fspublishers.org

Toxicity of Culture Filtrate of *Hirsutella thompsonii* Fisher Against Citrus Rust Mite, *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae) and Two Spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidea)

S. AGHAJANZADEH[1], B. MALLIK AND S.C. CHANDRASHEKAR†
Department of Entomology and † Plant Pathology, University of Agricultural Sciences, GkVK, Bangalore -560065, India
Present address: Iran Citrus Research Institute, P.O.Box 46915-335, Motahari St. Ramsar, Mazandaran, Iran
[1]Corresponding author's e-mail: sirous_in@yahoo.com, Tell: +981925229081, Fax: +981925223282

ABSTRACT

The efficacy of HtCRMB isolate of *Hirsutella thompsonii* Fisher a fungal Pathogen of eriophyid mites was evaluated in tests with its culture filtrate against citrus rust mite, *Phyllocoptruta oleivora* Ashmead and two spotted spider mite, *Tetranychus urticae* koch. The results revealed that the crude filtrate obtained from 14 days old broth culture of HtCRMB isolate was toxic to citrus rust mite. Mite mortality inceased from 41.99 to 55.90%, 1, 3 and 9 days after treatment, but mortality decreased with serial dilutions of the crude filtrate. Similarly, culture filtrate of HtCRMB isolate was found to be toxic to the two spotted spider mite, maximum mortality, 27.97%, was observed eight days after treatment. The mycelia suspension induced highest mortality (57.70%) compared to undiluted filtrate (27.97%). The filtrate of culture, which was 20 days old and had high mycelia production, (0.721 g dry wt/50 mL) induced higher mortality (44.68%). Hence there is a positive correlation between age of the culture, mycelia production and toxicity.

Key Words: *Hirsutella thompsonii;* Mites; Crude filtrate; Toxicity

INTRODUCTION

Hirsutella thompsonii Fisher is a fungal pathogen of eriophid and tetranychid mites and is considered an important biological control agent of the citrus rust mite, *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae) (McCoy, 1981 & 1996; McCoy & Couch, 1982). Vey *et al*. (1993) showed that crude filtrates of shake cultures of *H. thompsonii* var. *thompsonii* contain toxic metabolites, which were toxic to *Galleria melonella* (L.) larvae and *Drosophila melanogaster* Migen adults. Broth cultures of *H. thompsonii* have been demonstrated to contain insecticidal activity (Mazet, 1992) and detected two protein toxins, hirsutellin A (HtA) and B in the filtrates of *H. thompsonii* strain HtF- 87. Liu *et al.* (1995) analyzed the *in vitro* production of HtA by *H. thompsonii* strain JAB- 04 under submerged fermentation conditions and demonstrated that HtA was the major exocellular metabolite, and it was produced throughout the active growth phase. The HtA Preparations from both *H. thompsonii* strains were highly toxic to *G. melonella* larvae (Mazet, 1992; Liu *et al*. 1995) and to mosquito larvae, aphids and mites (McCoy *et al*., 1992). In addition to having insecticidal activity, HtA was reported to be cytotoxic to a cell line of *Bombyx mori* (Vey *et al*., 1993). Krasnoff and Gupta (1994) demonstrated toxicity of HtA to apple maggot flies, *Rhagoletis pomonella* (Wash) and Mediteranian fruit fly, *Ceratitis capitata* (Widemann). HtA is the first mycotoxin of an invertebrate mycopathogen determined to possess ribosomal inhibiting activity and appears to possess some specificity to invertebrate cells (Liu *et al*., 1996). Omoto and McCoy (1998) isolated the toxic protein, hirsutellin A, from filtrate during liquid fermentation of *H. thompsonii* and tested against citrus rust mite. Mite mortality increased with increase in hirsutelin A concentration. They also suggested that fecundity was affected prior to the death of the host. In the present study, we discuss the effect of crude filtrate of HtCRMB isolate of *H. thompsonii* collected from India against citrus rust mite and two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae).

MATERIALS AND METHODS

Mite Colonization

Citrus rust mite. The original citrus rust mite population was collected from a citrus grove in Bangalore and reared on mosambi seedlings, *Citrus reticulata* Blandco var. *sinensis*, in the green-house for nine months prior to the experimentation according to the procedures described by Omoto *et al*. (1994). Mosambi fruits were used for bioassay and to infest them, heavily mite infested leaves were detached from each seedling cut into small pieces, and placed on fruits by the method described by Read *et al*. (1964). As the pieces of leaves dried, mites crawled on to the fruit surfaces.

Two spotted spider mite. The original two spotted spider mite population was collected from bean farm in Bangalor

and reared on bean potted plants in the green-house. Mulberry leaves were used for test and to infest them, two spotted spider mites transferred on leaves in Petri plates containing moist cotton at bottom. Single layer of newspaper placed over the cotton was used for maintaining the mulberry leaves fresh and suitable for development of the mites.

Mite bioassays. HtCRMB isolate of *H. thompsonii* was collected from citrus rust mite, *P. oleivora* on citrus fruits in Bangalore, India. The fungus was isolated from the mite by removing dead mites exhibiting mycelial strands attached to their bodies from the fruit and leaf samples. Pure cultures of the fungus were obtained after 2 - 3 transfers and 72 h between each transfer on potato dextrose agar (PDA). In order to identify the *H. thompsonii* isolates, their virulence was confirmed through pathogenicity test and their growth patterns, colony characters and microscopic observations were recorded on PDA medium. HtCRMB isolate of *H. thompsonii* tested in the laboratory against citrus rust mite and two spotted spider mite, *T. urticae* for pathogenicity as per Villalon and Dean (1974). Fungal colonies about two weeks old culture on the potato dextrose agar (PDA) medium, containing mycelia mat and conidia were ground in a homogenizer and diluted to 20 mL with sterilized water. HtCRMB isolate of pathogen was cultured on a liquid medium (potato dextrose broth). Fifty milliliters of medium was transferred to a 250 mL Erlenmeyer flask and incubated at 28°C on a shaker at 160 rpm for two days after inculcation with 5 mm dia. mycelial mat, and then the flasks were transferred to an incubator maintaining 28°C. After 14 days of incubation, the mycelia were harvested by filtration using Watman No. 1 filter paper disks. The mycelia retained by the filter paper were dried for 24 h at 60°C and weighed. About 2.0 mL of the crude filtrate and fungal suspension was sprayed on fruits and leaves infested with healthy mites using an atomizer and Potter Precision Spray Tower. The samples sprayed with distilled water served as control. These treated samples were held at 22 ± 2°C temperature and a photoperiod of 13:11 (L.D) conditions.

The experimental design for all bioassays was completely randomized design with six treatments and three replications. The data were analyzed statistically for comparing treatments following the analysis of variance (ANOVA) technique and the results are interpreted at 5% level of significance. Mortality was calculated as T - C × 100/100 - C (T-mortality in treatment; C-mortality in control) (Abbott, 1925).

Two fruits were used for each treatment. The number of citrus rust mites on one cm^2 of the two points of each fruit were counted one day before (Table I) and deed ones 1, 3, 6 and 9 days after spraying, under a stereo binocular microscope.

Twenty adult two spotted spider mites were transferred on mulberry leaf for each treatment. The number of dead mites was counted 1, 3 and 8 days after spraying.

In a similar experiment the crude filtrate of HtCRMB isolate of *H. thompsonii* obtained from 11, 14 and 20 days old culture was tested against two spotted spider mite with six replications. The numbers of deed mites were counted 2, 3 and 4 days after treatment.

RESULTS AND DISCUSSION

Morphological characters of HtCRMB isolate of *H. thompsonii.* HtCRMB isolate of *H. thompsonii* was found to be effective against citrus rust mite. The morphological characters of this isolate are as follows:

The fourteen day old colonies on PDA characteristically greyish white in colour with fluffy mycelial growth protuberant and greyish brown substratum. Aerial mycelia white and powdery. Colony growth slow, attaining a diameter of 1.6 - 2 cm. Vegetative mycelium smooth walled, hyaline, hyphae 3 - 4 μm wide. Conidiogenous cells polyphialidic, arising singly from aerial hyphae and hyaline. Basal part of the phialide 9.5 - 11.5 x 3.5 - 4 μm, ellipsoid or flask shaped, the neck 2.5 - 4 μm long. Phialides proliferating giving rise to two to three necks. Conidia usually produced singly or in groups of two or three, hyaline, globose, verrucose, 4 - 4.5 μm in diameter. These characters agree with the descriptions by Samson *et al*. (1980) of *H. thompsonii* Fisher. In addition, the general morphological characters of the fungus agreed with descriptions of Fisher (1950).

Toxicity of culture filtrate of *H. thompsonii* to citrus rust mite. Crude filtrate obtained from 14 days old broth culture with 0.313 g dry wt./50 mL mycelial production of HtCRMB isolate of *H. thompsonii* were found to be toxic to citrus rust mites (Table II). Pure crude filtrate of fungus caused maximum mortality in the mite, increasing from 41.99% to 55.09% 1, 3, 6 and 9 days, after treatment. The differences in % mortality of citrus rust mite among the different concentrations of the filtrate were statistically significant. Mite mortality decreased with serial dilution of crude filtrate of the fungus culture (Table II). Almost less mortality was produced when the culture filtrate was diluted ten times with distilled water.

Omoto and McCoy (1998) isolated a toxic protein, hirsutellin A, from filtrate during liquid fermentation of *H. thompsonii* and tested against citrus rust mites at concentration of 0, 10, 32, 56 and 100 g/mL. Mite mortality increased with an increase in hirsutellin A concentration. The present results conform with that of Omoto and McCoy (1998) and indicate the possibility of increasing the mortality by either growing the fungus for a longer time or obtaining a further concentrated extract of the culture filtrate, by improving the culturing process.

Toxicity of culture filtrate of *H. thompsonii* to the two spotted spider mite. The significant difference observed in adults of two spotted spider mites mortality when exposed to different concentrations of crude filtrate of 14 days old broth culture with 0.313 g dry wt./50 mL of mycelial production and mycelial suspension of HtCRMB isolate of *H. thompsonii* (Table III). Mortality percent three days after

Table I. Mean number of citrus rust mites in one square centimeter area of the mosambi fruit

Treatment	Replication		
	I	II	III
1ml F (100% filtrate)	20	16	14
1ml F + 1ml DW (50% filtrate)	17	13	28
1ml F + 5ml DW(20% filtrate)	20	22	11
1ml F + 10ml DW(10% filtrate)	12	14	16
Control	13	19	22

Figures in the table are averages of four points counting on two fruits
F = Culture filtrate; DW = Distilled water

Table II. Mortality percentage of citrus rust mite treated with filtrate of 14 days culture of HtCRMB isolate of *H.thompsonii*

Concentration	1 DAS	3 DAS	6 DAS	9 DAS
1 ml F (100% filtrate)	46.73^{a}	41.99^{a}	53.66^{a}	55.09^{a}
1 ml F + 1 ml DW (50% filtrate)	14.23^{b}	8.18^{b}	24.22^{b}	17.5^{ab}
1ml F + 5 ml DW (20% filtrate)	0.0^{b}	0.0^{b}	1.41^{b}	14.85^{b}
1 ml F + 10 ml DW (10% filtrate)	0.0^{b}	1.40^{b}	3.37^{b}	5.96^{b}

F = Culture filtrate; DW = Distilled water; DAS: Days after spraying
Means with the same superscript within each column are not significantly different at the P = 0.05 according to Duncan's Multiple Range Test.

Table III. Mortality percentage in adults of *Tetranychus urticae* treated with filtrate of 14 days culture of HtCRMB isolate of *H. thompsonii*

Treatment	1 DAS	3 DAS	8 DAS
HtCRMB	13.68^{NS}	26.14^{a}	57.07^{a}
1ml F (100% filtrate)	0.0^{NS}	15.70^{ab}	27.97^{b}
1ml F + 1ml DW (50% filtrate)	1.67^{NS}	1.75^{b}	1.75^{c}
1ml F + 5ml DW (20% filtrate)	0.0^{NS}	0.0^{b}	1.75^{c}
1ml F + 10ml DW (10% filtrate)	1.75^{NS}	0.0^{b}	0.0^{c}

F = Culture filtrate; DW = Distilled water; DAS: Days after spraying; NS = Non-significant.
Means with the same superscript within each column are not significantly different at the P = 0.05 according to Duncan's Multiple Range Test.

Table IV. Mycelial production and mortality percentage of spider mites treated with crude filtrate taken from broth culture of different age

Culture broth age	Mycelial yield Mean (dry wt./50 ml)	Mean mortality	Mortality recorded after treatment (days)
11 days	0.273g	15.18%	4
14 days	0.313g	15.70%	3
20 days	0.721g	44.68%	2

treatment was in the range of 0.0 to 26.14. The mycelial suspension caused highest mortality (26.14%) followed by 1 mL undiluted filtrate (15.70%). Mortality ranged from 0.0 to 57.07% eight days after treatment during this observation. The mycelial suspension also induced highest mortality (57.07%), followed by 1 mL undiluted filtrate (27.97%), 50% filtrate (1.75%), 20% filtrate (1.75%) and 10% filtrate (0.00).

The results obtained from broth cultures of different age and mycelial production of HtCRMB isolate indicate that mortality was highest (44.68% in two days) in spider mites treated with the filtrate of culture, which was 20 days old and had 0.721 g dry wt./50 mL, followed by 15.70%, in three days by the 14 days old culture filtrate with 0.313 g dry w./50 mL and 15.18% in four days by eleven days old culture filtrate, with 0.273 g dry wt./50 mL (Table IV). These results supported the views of Vey *et al*. (1993) that culture filtrate of *H. thompsonii* obtained from Czapek-Dox + 1% yeast extract, Czopak-Dox + peptone and soil fungus medium (SFM) media is toxic to *G. melonella* and produced 98.5, 14.3 and 9.5% mortality, respectively.

The culture filtrate of HtCRMB isolate to the two spotted spider mite adult was not as toxic as the mycelial suspension of the same fungus. Even eight days after treatment the extent of mortality was fifty % of what was observed in mycelial suspension treated mites, and as the filtrate was diluted with water the toxicity reduced. In general, the results revealed in this study were similar to previous bioassays reports. Aghajanzadeh (2003) reported that suspension spray of HtCRMB isolate of *H. thompsonii* on two spotted spider mite caused 70.85% mortality eight days after treatment. Gerson *et al*. (1979) observed that adult carmine spider mites, *T. cinnabarinus* (Boisduval), are susceptible to *H. thompsonii* and mite mortality reached 84% five days after treatment. Rosas-Acevedo *et al*. (1995) found that *H. thompsonii* strain HtMOR can cause 95% mortality of *T. urticae*.

The age of the broth culture was also observed to influence the effectiveness. This could be related to the growth of the fungus. Vey *et al*. (1993) observed that the mortality of *G. melonella* peaked with eight day old culture filtrate but 15 day old culture filtrate showed reduction in mortality. In our studies, the filtrate of culture which was 20 days old and had high mycelial production, (0.721 g dry wt/50 mL) induced higher mortality (44.68%) and the filtrate from broth culture, which was just eleven days old, producing 0.273 g mycelia, induced relatively less mortality(15.18%) even four days after treatment. Hence there is a positive correlation between age of the culture, mycelial production and toxicity. However, since any fungal growth on the culture broth will reach a peak and drop when the nutrients are exhausted, the optimum time for extracting the filtrate need to be studied, which may vary with isolates and culturing conditions.

Acknowledgments. The authors acknowledge the financial support and Ph.D. scholarship by Agricultural Research, Education and Extension Organization, Ministry of Jihad-e-Agriculture, Islamic republic of Iran (to SA) and financial support from Indian Council for Agricultural Research, Government of India (to BM) through NATP.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*., 18: 265–7

Aghajanzadeh, S., 2003. Characterization and mass culturing of some isolates of *Hirsutella thompsonii* (Deuteromycete) and their bioefficacy against citrus rust mite, *Phyllocoptruta oleivora* Ashmead

(Acari:Eriophyidae). *Ph.D. Thesis*, University Agriculture Science, Bangalore. P: 176

Fisher, F.E., 1950. Two new species of *Hirsutella* Patouillard. *Mycologia*, 42: 290–7

Gerson, U., R. Kenneth and T.I. Muttath, 1979. *Hirsutella thompsonii* a fungal pathogen of mites. II. Host-pathogen interaction. *Annu. Appl. Biol*., 91: 29–40

Krasnoff, S.B. and S. Gupta, 1994. Identification of the antibiotic phomalactone from the entomopathogenic fungus, *Hirsutella thompsonii* var. *synnematosa*. *J. Chem. Ecol*., 20: 293–302

Liu, J.C., D.G. Boucias, J.C. Pendland, W.Z. Liu and Marunlak, 1996. The mode of action of hirsutellin A on eukaryotic cells. *J. Invertebr. Pathol*., 67: 224–8

Liu, W., D.G. Boucias and C.W. McCoy, 1995. Extraction and characterization of the insecticidal toxin, hirustellin A produced by *Hirsutella thompsonii* var. *thompsonii* during submerged fermentation conditions. *Exp. Mycol*. 19: 254–62

Mazet, L., 1992. Researches sure les *Hirsutellines*, Toxins Protetques Produttes per *Hirsutella thompsonii* Fisher, Champignon Pathgence d' Acareins Phytophages. *Doctoral Dissertation*, *University Mon Pellier*, France

McCoy, C.W. and T.L. Couch, 1982. Microbial control of the citrus rust mite with the mycoacaricide, Mycar. *Fla. Entomol*., 65: 117–26

McCoy, C.W., 1981. Fungi pest control by *Hirsutella thompsonii*. *In*: Burges, H.D. (ed.), *Microbial control of insects, mites and plant diseases*. Pp: 499–512. Academic Press, London, UK

McCoy, C.W., 1996. Pathogens of eriophyoid mites. *In*: Lindquist, E.E., M.W. Sabelis and J. Bruin (eds.), *Eriophyoid mites, their biology, natural enemies and control*. Pp: 481–90. Elsevier Sciences Publication, Amsterdam. The Netherlands

McCoy, C.W., C. Omoto, I. Mazet and A. Vey, 1992. *Biological activity of crude filtrates and hirsutellin A to mosquitoes and phytophagous arthropods*. Abstract 120 XXV Annual meeting Society Invertebrate Pathology, Heidelberg, Germany

Omoto, C. and C.W. McCoy, 1998. Toxicity of purified fungal toxin hirsutellin A to the citrus rust mite, *Phyllocoptruta oleivora* (Ash.). *J. Invert. Pathol*., 72: 319–22

Omoto, C., T.J. Dennehy, C.W. McCoy, S.E. Crane and J.W. Long, 1994. Detection and characterization of the interpopulation variation of citrus rust mite (Acari: Eriophyidae) resistance to dicofol in Florida citrus. *J. Econ. Entomol*., 87: 566–72

Reed, D.K., A.K. Burditt and C.R. Crittenden, 1964. Laboratory methods for rearing rust mites, (*Phyllocopruta oleivora* (Ashmead) and *Aculus pelekassi* Keifer on citrus. *J. Econ. Ent*., 57: 130–3

Rosas-Acevedo, J.L., R.R. Alatorre, K.L. Sampedro and J. Valdez-Carrasco, 1995. Sporulation of the entomogenous fungi *Hirsutella thompsonii* and *H. nodulosa* in mixed culture. *Revista-Latinuamericana de-microbiologica*. 37: 59–64

Vey, A., J.M. Quiot, I. Mazet and C.W. McCoy, 1993. Toxicity and pathology of crude broth filtrate produced by *Hirsutella thompsonii* var. *thompsonii* in shake culture. *J. Invertebr. Pathol*., 61: 131–7

Villalon, B. and H.A. Dean, 1974. *Hirsutella thompsonii*, a fungal parasite of the citrus rust mite, *Phyllocoptruta oleivora* in the Rio Grande Valley of Texas. *Entomophaga*, 19: 431–6

(Received 20 November 2005; Accepted 18 February 2006)