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

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Abstract: For studying the bioefficacy of six isolates of *Hirsutella thompsonii* Fisher a fungal pathogen of eriophyid mites collected from different agro-climatic regions of India, fungal suspension was sprayed on fruits and leaves infested with healthy citrus rust mite, *Phyllocoptruta oleivora* Ashmead and two spotted spider mite, *Tetranychus urticae* Koch. The results revealed that all isolates of *H. thompsonii* were effective against citrus rust mite. HtEMC isolate of *H. thompsonii* brought about maximum mortality of 48.05% ten days after treatment followed by HtCRMB (46.87%), HtCRMK (44.27%), HtCRMC (42.09%), HtPMG (39.82%) and HtPDBC (31.22%), however, this difference was not significant statistically. These isolates were also found to be effective against adult two spotted spider mite. HtEMC isolate of *H. thompsonii* brought about maximum mortality of 83.33% after eight days of treatment, followed by HtCRMB (70.85), HtCRMK (39.48), HtPMG (33.05), HtCRMC (31.86) and HtPDBC (19.64). The HtCRMB isolate of *H. thompsonii* was found to be effective against nymphal stage of the two spotted spider mite with 88.85% mortality six days after treatment. These results indicate that *H. thompsonii* isolates exhibit differences in their virulences, suggesting the requirement for screening the *H. thompsonii* isolates for their effectiveness against mite species before recommending as biological control agent.

Key words: *Hirsutella thompsonii*, *Phyllocoptruta oleivora*, *Tetranychus urticae*, mites, citrus

INTRODUCTION

Hirsutella thompsonii Fisher was first reported by Spears and Yothers (1924) in association with the citrus rust mite, *Phyllocoptruta oleivora Ashmead* (Acari: Eriophyidae). Fisher (1950a) described the fungus associated with the citrus rust mite as *H. thompsonii*. Fisher and Griffiths (1950) and Fisher (1950b) reported that on occasions this fungus is capable of almost completely eliminating rust mites from a grove. *H. thompsonii* caused a high mortality in wild population of citrus rust mite and seems to be a key factor in natural control of this pest (Fisher *et al*., 1949; Fisher, 1950b, Muma, 1955). Although citrus rust mite seems to be the chief host of *H. thompsonii* this fungus also attacks other mites. It caused relatively high mortality of the eriophyid, *Aceria vaccine,* infesting blueberry. Abundance of the blueberry bud mite in North Carolina was inversely correlated with seasonal deaths caused by *H. thompsonii* (Baker and Neunzig, 1968).

Selhime and McCoy (1971) reported that spray formulation of the fungus *H. thompsonii* applied to citrus groves controlled the rust mite upto twelve weeks as against six weeks control obtained by chlorobenzilate. The mycelia of *H. thompsonii* were applied as a foliar spray to orange trees infested with high populations of the citrus rust mite. The mean number of mites per leaf decreased and the rate of mite infection increased one week after the treatment. Mite populations remained at low levels for 10 to 14 weeks (McCoy *et al*., 1971). A 0.5 mL fungal suspension of *H. thompsonii* was sprayed on leaves and fruit containing healthy mites using a DeVilbiss atomizer. Evidence indicated 40% of the mites were infected with *H. thompsonii* on the sixth day (Villalon and Dean, 1974). Laboratory studies by Van Brussel (1975) showed considerable decline in rust

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mite population within five days by a single application of *H. thompsonii* suspension (mycelium and conidia) 0.5-1 g L^{-1} and remained suppressed for tree weeks. In China, laboratory produced mycelium was applied at dosage of 0.5 to 1.0 g L^{-1} to citrus trees (Yen, 1974). One application caused 90% death of citrus rust mite in three days.

Gerson *et al.* (1979) reported that *H. thompsonii* grown on potato-dextrose agar (PDA) or on sterile wheat bran, was highly pathogenic to the carmine spider mite, *T. cinnabarinus*. The fungus killed most mites quickly usually by the second day, at 25, 27 and 30°C. McCoy and Couch (1982) in their studies on microbial control of the citrus rust mite with the mycoacaricide MYCAR as WP or dust found it to be effective in stimulating premature fungal epizootics in the citrus rust mite populations in Valencia orange groves located in central and South Florida in 1979-80.

Winkelhoff and McCoy (1984) found that *H. thompsonii* var. *synnematosa* from Ivory coast (Htle) was virulent causing 32.5% infection to adult citrus rust mite when sprayed on citrus foliage. Results of field trails with commercial preparation of *H. thompsonii* applied at two to four pounds formulated product per acre have been effective when weather conditions were optimal for establishment of the growing fungal propagules in citrus fruit and foliage. There was no difference in the percent fruit injury at the end of the season between fungal and standard chemical applications (McCoy, 1996). In the present study, we discuss the differences in virulence of *H. thompsonii* isolates of eriophyid mites affecting citrus and palms, collected from different agro-climatic zones of India against citrus rust mite and two spotted spider mite.

MATERIALS AND METHODS

Citrus rust mite colonization: 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 greenhouse 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 Reed *et al.* (1964). As the pieces of leaves dried, mites crawled on to the fruit surfaces.

Two spotted spider mite colonization: The original two spotted spider mite population was collected from bean farm in Bangalor and reared on bean potted plants in the greenhouse. 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 news paper placed over the cotton was used for maintaining the mulberry leaves fresh and suitable for development of the mites.

Mite bioassays: Six isolates of *H. thompsonii* (Table 1) were tested, in the laboratory, against the citrus rust mite, *Phyllocoptruta oleivora* Ashmead (Eriophyidae) and the two spotted spider mite, *Tetranychus urticae* Koch (Tetranychidae) for pathogenicity as per Villalon and Dean (1974). Fungal colonies about two weeks old cultured on the Potato Dextrose Agar (PDA) medium, containing mycelial mat and conidia were ground in a homogenizer and diluted to 20 mL with sterilized water. About 2.0 mL of the 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 incubated separately within air filled polythene bags for creating high humidity and 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. The mortality rate was assessed 2, 4, 6, 8 and 10 days after treatment and microscopic examinations were carried out for the presence of *H. thompsonii* in dead mites. Mortality was calculated as T-C × 100/ 100-C (T-mortality in treatment; C-mortality in control) (Abbott, 1925).

Table 1: Name and origin of the isolates of *H. thompsonii*

Name	Origin of the isolates
HtCRMB	*P. oleivora* on citrus fruits in Bangalore, Karnataka
HtPMG	*A. guerreronis* on palmyra nuts in Govindapuram, Tamil Nadu.
HtCRMC	*P. oleivora* on citrus fruit in Chettahalli, Karnataka
HtCRMK	*P. oleivora* on citrus leaf in Kolkata, West Bengal
HtEMC	Obtained from the Microbial Technology Institute, Chandigarh, Panjab
HtPDBC	*A. guerreronis* on coconut, sprayed with Mycohit (product by Project Directorate Biological Control (PDBC)) in Bangalore, Karnataka

Table 2: Mean number of citrus rust mites in one square centimeter area of the mosambi fruit

	Replication		
Treatment	I	II	III
HtCRMB	28	65	41
HtEMC	24	20	12
HtPMG	43	17	18
HtPDBC	31	30	19
HtCRMK	22	19	38
HtCRMC	28	69	42
Control	25	58	43

Figures in the table are averages of four points counting on two fruits

Table 3: Mortality percentage of citrus rust mite 10 days after treatment of different isolates of *H. thompsonii*

Isolate	Mortality percentage*
HtEMC	48.05^{NS}
HtCRMB	46.87^{NS}
HtCRMK	44.29^{NS}
HtCRMC	42.09^{NS}
HtPMG	39.82^{NS}
HtPDBC	31.27^{NS}
SE±	±5.44

*Mean percentage mortality based on three replications of mite population per cm^2 of mosambi fruit ±SE. NS = Non significant at the p = 0.05 according to Duncan's multiple range test

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 2) and deed ones ten 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 were counted 2, 4 and 8 days after spraying.

In a similar experiment the HtCRMB isolate of *H. thompsonii* was tested against nymphal stages of the two spotted spider mite with six replications. The number of deed mites were counted 2, 4 and 6 days after treatment.

RESULTS AND DISCUSSION

Effect of *H. thompsonii* isolates on citrus rust mite: The results revealed that all *H. thompsonii* isolates cause mortality in the citrus rust mite (Table 3). HtEMC isolate showed maximum mortality of 48.05% followed by HtCRMB (46.87%), HtCRMK (44.29%), HtCRMC (42.09%) and HtPMG (39.82%) isolates and the least being HtPDBC (31.27%) on treated citrus rust mites. The differences in the pathogenicity among different isolates of *H. thompsonii* against citrus rust mite were not significant, statistically.

HtCRMB, HtCRMC and HtCRMK strains of *H. thompsonii* isolated from citrus rust mite and induced more than 42% mortality. These findings concur with those of Villalon and Dean (1974) who reported that the fungal colonies of about 2.5 cm diameter containing mycelial mat and conidia on PDA diluted with 25 mL distilled water, 40% of the mites were infected with *H. thompsonii* on the sixth day after treatment. However, HtPMG and HtPDBC strains of *H. thompsonii* isolated from coconut mite, *Aceria guerreronis* Keifer and induced less than 40% mortality, this could be due to the differences in host species of mite and host plant.

Table 4: Mortality percentage of adults *T. urticae* 2, 4 and 8 days after treatment with different isolates of *H. thompsonii*

Isolate	2 DAS	4 DAS	8 DAS
HtEMC	44.68^{a}	62.92^{a}	83.33^{a}
HtCRMB	43.76^{a}	57.06^{ab}	70.85^{ab}
HtPMG	20.35^{ab}	26.67^{bc}	33.05^{bc}
HtCRMK	17.66^{ab}	16.72^{c}	39.48^{bc}
HtCRMC	3.33^{b}	3.33^{c}	31.86^{bc}
HtPDBC	1.67^{b}	6.67^{c}	19.64^{c}
SE±	10.65	10.71	13.01

DAS : Days after spraying

Means followed by the same letters within columns are not significantly different according to Duncan's multiple range test at 0.05 probability level

In general, the results revealed for the *H. thompsonii* isolates in this study were similar to that reported by McCoy *et al.* (1971) and Van Brussel (1975) for considerable decline in citrus rust mite population by single application of *H. thompsonii* suspension. In China, laboratory produced mycelia was applied at a dosage of 0.5 to 1.0 g L^{-1} to citrus trees, this caused 90% deaths of citrus rust mite in three days (Yen, 1974). McCoy (1978) sprayed *H. thompsonii* at three concentrations (2.5, 5 and 10 g/500 mL water) and reported that at the highest rate, the mean number of citrus rust mites after four weeks (9.6/leaf) was significantly lower than in control (26.3/leaf). Later McCoy and Couch (1982) reported that the fungus had established on treated fruit and foliage of Valencia orange groves in Florida and was providing excellent crop protection. Kumar *et al.* (2001) reported that 17.19% of coconut mite infested with *H. thompsonii* in Coimbatore district.

Previous bioassays using different isolates and concentrations of *H. thompsonii* and observations of this study demonstrated that various strains of this pathogen could be effective against citrus rust mite at different level of mortality (McCoy *et al.*, 1984; Winkelhoff and McCoy, 1984; Chen *et al.*, 1978; McCoy, 1996).

Effect of *H. thompsonii* isolates on two spotted spider mite: Six isolates of *H. thompsonii* were found to be effective against adult two spotted spider mite. There was significant difference in extent of mortality of the spider mite treated with these isolates (Table 4). HtEMC isolate of *H. thompsonii* brought about maximum mortality of 44.68, 62.97 and 83.33% after 2, 4 and 8 days of treatment,

respectively. This was followed by HtCRMB isolate which caused 43.76, 57.06 and 70.85% mortality, respectively. Lowest mortality was brought about by HtPDBC isolate 1.67, 6.67 and 19.64%) respectively. The other three isolates (HtPMG, HtCRMK and HtPDBC) caused intermediate levels of mortality. This shows that the different isolates of *H. thompsonii* can exhibit difference in virulence and hence while selecting the *H. thompsonii* for biological control there is need to screen the isolates.

Mortality increased with time, maximum mortality was caused by HtEMC isolate four (62.92%) and eight (83.33%) days after treatments. Similarly HtCRMB caused 57.06 and 70.85% mortality. However the differences between the strains were not significant, statistically. HtCRMC and HtPDBC caused the least mortality on spider mite, 31.86 and 19.64%, respectively, eight days after treatment. Since the isolate HtCRMB was found to be effective against the adult stage, it was tested against the nymphal stage, the mortality of nymphs of the spider mites was 53.72% two days after treatment and it increased to 81.65 and 88.85% four and six days after treatment. This isolate brought about 88.85% mortality six days after inoculation, which was slightly higher than the mortality rate observed on adults. Thus the nymphal stages appear to be susceptible for infection than the adult stages. These results supported the views of Gerson *et al*. (1979) that both adults and nymphs of carmine spider mite, *T. cinnabarinus* (Boisduval), are susceptible to *H. thompsonii*. Adult mite mortality reached 84% five days after treatment and nymph mortality was almost the same as in adults.

In laboratory infectivity studies on *T. cinnabarinus, Eutetranychus orientalis* (Klein) and *T. turkestani* (Ugarou and Nikolshi) were found to be susceptible to *H. thompsonii* (McCoy, 1978, 1981). Rosas-Acevedo *et al*. (1995) found that *H. thompsonii* strain HtMOR at the lowest concentration can cause 95% mortality of *T. urticae*. At the same time, the strain with lower virulence were HtM2, HtC59 and HtC77. Chernin *et al*. (1997) found that 80% and 35% mortality was caused by isolates 414 (originating from Zimbabwe) and 255 (originating from New Guinea) of *H. thompsonii,* three days after spraying on *T. cinnabarinus,* respectively. As pointed out above, susceptibility was detected among the spider mite species to different *H. thompsonii* isolates. However, results of this study indicate that *H. thompsonii* isolates exhibit differences in their virulences, suggesting the requirement for screening the *H. thompsonii* isolates for their effectiveness against mite species before recommending as biological control agent.

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