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Susceptibility of two-spotted red spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) to entomofungal pathogens

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ABSTRACT: **Studies were conducted on the susceptibility of red spider mite, *Tetranychus urticae* (Koch) against different fungal pathogens belonging to the group Deuteromycetes like *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Verticillium lecanii* and *Hirsutella thompsonii*. In bioassay studies *B. bassiana*, isolated from naturally infected population of *T. urticae* was found to be more virulent against *T. urticae* adult. The lowest LC_{50} ($3.61x10^6$ conidia /ml) and LT_{50} value (85.4 hours) were recorded for *B. bassiana*, followed by *V. lecanii* (6.30 conidia/ml and 106.3hrs), *H. thompsonii* (19.91conidia/ml and 124.3hrs) and *P. fumosoroseus* (29.92 conidia/ml and 119.5 hrs). In glasshouse studies on French beans, spore suspension of *V. lecanii* (@ $1x10^{12}$/ ha) was found to be more effective in reducing mite population up to 87. 44% after 10 days of spraying followed by *B. bassiana* (85.47%), *P. fumosoroseus* (84.22%) *and H. thompsonii* (80.47%). In contrast, highest population reduction of 87.50% was recorded in dicofol. *V. lecanii* proved to be an effective fungal pathogen against RSM in reducing population under glasshouse condition probably because of their rapid colonizing capacity in soft bodied micro-arthropods inhabiting humid microclimate. Effective commercial formulation of *V. lecanii* could be a suitable fungal pathogen for the management of red spider mites in different field crops.**

KEY WORDS: *Beauveria bassiana*, bioassay, *Hirsutella thompsonii*, *Paecilomyces fumosoroseus*, *Tetranychus urticae*, *Verticillium lecanii*

INTRODUCTION

The red spider mite (RSM), *Tetranychus urticae* (Koch) (Acari: Tetranychidae) has become a major pest of different horticultural crops grown both under polyhouse and open field conditions, as well as plantation crops. The increased abundance of red spider mite is thought to have resulted from the adverse effects of insecticides on their natural enemies. In modern agriculture, management of red spider mites by chemical insecticides is becoming ineffective and costly due to the development of resistance to most of the insecticides in a short time. Hence there is a need to develop an effective alternative for sustainable control of RSM (Evans, 1992). Of late, the role played by parasitic fungi in mite populations has received world wide attention. In theory, Acari make good hosts for fungal pathogens because they are generally soft bodied and many inhabit environments with humid micro-climates which favour infection and disease transmission (Hajek and St. Leger, 1994). In India, there are very few reports about the natural incidences of fungal pathogens against tetranychid mites (Ramaseshiah, 1971).

Efficacy of Entomophthorales has already been established against tetranychids but the systematic information on the efficacy of Deuteromycetes fungal pathogens against *T. urticae* is very scanty (Chandler *et al*, 2000). The present paper discusses the susceptibility of *T. urticae* against different deuteromycetes, viz.,

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Beauveria bassiana (Bals.) Vuillemin, *Verticillium lecanii* (Zimn) Viegas, *Paecilomyces fumosoroseus* (Wize) Brown & Smith and *Hirsutella thompsonii* Fisher under both laboratory and glasshouse conditions.

MATERIALS AND METHODS

Mite culture

The identified adult mites were collected from Acarology division of University of Agricultural Sciences, GKVK, Bangalore and reared on mulberry leaves under laboratory conditions. Detached leaf rearing technique (Krishnamoorthy, 1989) was followed to maintain the two-spotted spider mite culture under laboratory conditions. The mulberry leaves were placed dorsally on wet cotton wads in Petri dishes to avoid quick drying of the leaves. The older leaves were replaced regularly by new ones at an interval of 3-4 days.

Fungus culture

Test fungal pathogens *V. lecanii* and *P. fumosoroseus* were isolated from naturally infected aphids and whiteflies from surrounding farmer's field of BCRL, Bangalore respectively and *B. bassiana* was isolated from naturally infected RSM (Shivaprakash *et al.*, 2004) collected from GKVK campus, Bangalore. Similarly, *H. thompsonii* was isolated from a product (Mycohit), developed by Project Directorate of Biological Control, Bangalore against coconut mites. The pathogens were passaged through RSM under laboratory conditions for 2-3 times and were made as a pure culture from dead cadavers before they were used for experimental purpose. Fungal cultures were maintained on Sabouraud Dextrose Agar plus 2 per cent yeast extract (SDAY) at 25± 1° C. Fungal conidia were scraped from the surface of 14-20 days old cultures and suspended in Tween 80 (0.01%) by vortexing for 2 minutes. Fungal suspension was filtered through double layered muslin cloth to remove clumps and hyphal fragments. Spore concentration was estimated by using a haemocytometer and adjusted as needed by using additional Tween 80 (0.01%). *T. urticae* adults were treated only with the suspending medium (0.01% Tween 80) in each assay as control.

Laboratory bioassay

Bioassays were conducted using newly eclosed *T. urticae* adults reared in the laboratory on French bean leaves. The host plant used in this bioassay was french beans (*Phaseolus vulgaris* L.). Four to eight leaf stage plants were first sprayed to runoff with 10 ml of different treatment suspensions with a hand operated atomizer. Control plants was sprayed with 0.01per cent Tween 80 spray fluid. Randomly selected leaves were removed from control and treated plants after the foliage of the sprayed plants was allowed to air dry. Leaf discs of 4 cm diameter were cut from these excised leaves and placed on a moist polyethylene sponge disc in covered Petri dishes to prevent mites from crawling off the disc and to maintain high humidity levels. Ten discs were used per treatment, for a total of 100 mites per treatment. The Petri dishes were placed in transparent plastic box (34 x 22 x 12 cm) closed with lids and placed in a BOD incubator at 26±1° C and >90 per cent relative humidity.

Mites dying within 24h of transfer were removed and were not included in statistical analysis. Leaf discs were checked daily for cumulative mortality due to the test fungus and assessed 7 days after treatment. The mycosis was confirmed by microscopic examination of cadavers. The experiment was repeated twice using identical methods. Mite mortality results for corresponding dosage were similar in both experiments hence data were pooled before statistical analysis. Cumulative per cent mortalities after 7 days were collected and all data were subjected to probit analysis to know the susceptibility of *T. urticae* against different fungal pathogens.

Glasshouse trial

A glasshouse trial was conducted during May–June, 2005 at Bio-Control Research Laboratories, Bangalore at 28-32°C temperature and 45-80% relative humidity. The experiment was conducted on French bean, designed with 6 treatments and 4 replications were maintained for each treatment with a control. The French beans were grown in earthen pots @ 3 plants/ pot. Spore suspension of test fungal pathogens was prepared from two weeks old Petri plates' culture in 0.01per cent Tween 80 emulsion. The application rate of fungal pathogens was $1x10^{12}$/ ha and spraying was done only one time in the evening hours with a hand sprayer. Controls were treated with 0.01 per cent Tween 80 solution only. Observations were made randomly by picking up 3 leaves from bottom, middle and top of each treatment. Mite populations were counted per cm^2 in the middle portion of leaf under stereoscopic binocular microscope and the average of three leaf counts was taken as number of mites/cm^2 leaf area. Observations were recorded on 5, 7 and 10 days after spray and the data were analyzed

statistically.

RESULTS AND DISCUSSION

The results of these studies indicate that the susceptibility of *T. urticae* against entomofungal pathogens varies from fungus to fungus and virulence of the fungal pathogen changes with their host interaction.

Lab efficacy

The results of bioassays indicate the differences in the susceptibility of adult *T. urticae* against different entomofungal pathogens. The lowest median lethal concentration (LC_{50}) was recorded for *B. bassiana* (3.61×10^{6} conidia/ml) against *T. urticae* adult which increased 1.8 times for *V. lecanii* (6.5×10^{6} conidia/ml), 5.51 times for *H. thompsonii* (19.9×10^{6} conidia/ml) and 8.28 times for *P. fumosoroseus* (29.9×10^{6} conidia/ml) (Table 1).

Median lethal time (LT_{50}) of different fungal pathogens was 85.4 h, 106.3 h, 119.5 h and 124.3 h for *B. bassiana, V. lecanii, H. thompsonii and P. fumosoroseus* respectively (Table 2). *B. bassiana* was found to be more virulent against *T. urticae* probably because this pathogen was isolated from the body of naturally infected *T. urticae*.

Glasshouse efficacy

The variation in the pre-treatment population on *T. urticae* in different treatments was statistically non-significant, indicating that the population was uniformly distributed before the different treatments were imposed. The mean population of *T. urticae* 24 hours before imposing the treatments was from 8.85 to 9.30 mites/ cm^2 leaf area. The average population of *T. urticae* was recorded after 5, 7 and 10 days of spray (Table 3). All the treatments were significantly superior in controlling *T. urticae* after 5^{th}, 7^{th} and 10^{th} day of spray. Highest population reduction over control in ten days after spray was recorded in dicofol (87.50%) followed by *V. lecanii* (87.44%), *B. bassiana* (85.47%), *P. fumosoroseus* (84.22%) and *H. thompsonii* (80.47%), respectively. In control, a continuous population build up was recorded which increased from 9.01 to 15.21mites / cm^2 leaf area.

Verticillium lecanii was found to be better in bringing down *T. urticae* population under glasshouse condition though *B. bassiana* had shown higher

Table 1. Dose-mortality response of *T. urticae* adults against different fungal pathogens

Fungal pathogens	Regression equation Y= a + bx	LC_{50} (conidia/ml) (x 10^{6})	Fiducial limit x10^{8}- x10^{5} conidia / ml)	χ^2(df)
Beauveria bassiana	Y = - 1.731+ 0.73x	3.61	0.13-8.99	3.81(26)
Verticillium lecanii	Y = - 3.35 + 0.57 x	6.50	2.29-15.10	1.43 (26)
Hirsutella thompsonii	Y = - 2.08 + 0.33x	19.91	1.43-93.19	2.79 (26)
Paecilomyces fumosoroseus	Y = - 4.93 + 0.76x	29.92	15.79-56.80	1.68 (26)

a- Three assays for each pathogen, 25 adults per replicate; 6 replicates per dose, 4 dosages per assay; b- Analysis done on Log_{10} spore per ml

Table 2. Time-mortality response of *T. urticae* adults against different fungal pathogens

Fungal pathogens	Regression equation (Y = a + bx)	LT_{50} (h)	Fiducial limit (h) at 95%CI	χ^2
Beauveria bassiana	Y = 2.48 + 4.50x	85.4	74.4 - 96.9	9.27
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Table 3. Efficacy of different entomofungal pathogens against *T. urticae* on French bean under glasshouse conditions

Treatments	Dosage (conidia / ha)	*T. urticae* population (Mites /cm^2)				Population reduction
		Pre-treatment	Post -treatment			
			5DAS	7DAS	10DAS	
B. bassiana	1x 10^{12}	9.3 (3.31)	4.81 (2.30)^d	3.71(2.05)^c	2.21(1.65)^b	85.47%
H. thompsonii	1x10^{12}	8.91(3.32)	4.37 (2.20)^c	3.92 (2.10)^d	2.97 (1.86)^d	80.47%
P. fumosoroseus	1x10^{12}	8.88 (3.18)	4.19 (2.16)^b	3.95 (2.12)^c	2.40 (1.71)^c	84.22%
V. lecanii	1x10^{12}	8.90 (3.19)	4.18(2.16)^b	3.36 (1.96)^b	1.91(1.55)^a	87.44%
Dicofol	0.25% Conc.	8.95 (3.25)	3.94 (2.10)^a	2.12 (1.61)^a	1.90 (1.54)^a	87.50%
Control	Water spray	9.01 (3.27)	11.43 (3.45)^e	13.14 (3.69)^f	15.21 (3.95)^e	-
F-Test		NS	*	*	*	
SEM ±			0.016	0.018	0.064	
CD (P=0.05)			0.048	0.052	0.026	

NS - Non-significant; Figures in parenthes are (" x+ 0.5) transformed values; * Means within separate treatment followed by the same letter are not significantly different at 5% level by DMRT

virulence in laboratory studies. Selection of effective bioagent should be based on the glasshouse performance instead of relying on only laboratory results (Rangeshwaran and Prasad, 2000). The better efficacy of *V. lecanii* in glass house conditions is probably because of higher colonizing capacity of *V. lecanii* in small bodied insects like mites due to high hydrophilic properties which result in strong adhesion to the insect cuticle. Similarly, Xian *et al.* (2001) reported that a wettable powder formulation of *V. lecanii* with 0.05 per cent Tween 80 could bring down *T.urticae* population by 70-80% after 14 days of spraying on green house vegetable crops. Studies on effective commercial formulation of *V. lecanii* with additional adjuvants, which stimulate its growth and sporulation, could be a further approach for the non-chemical pest management of red spider mites in different crop systems.

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