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Research Note

Ovicidal action of different fungal pathogens against two spotted spider mite, *Tetranychus urticae* (Koch) under laboratory conditions

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ABSTRACT: Bioassay studies were performed with ten different fungal isolates of six species against two spotted spider mite, *Tetranychus urticae*. Among different formulations, *Hirsutella thompsonii* caused significantly higher mortalities of 61.1, 35.8 and 22.1 per cent at conidial concentrations of 971, 196 and 36 conidia mm^{-2}, respectively. At a conidial concentration of 1457 conidia mm^{-2}, *Beauveria bassiana* isolate Bb101 caused significantly higher mortality rate of 46.9 per cent followed by *B. bassiana* isolate B2 (36.1%) and *Cladosporium cladosporioides* isolate Cc101 (32.1%). Based on the probit estimates, *H. thompsonii* was found to exert high ovicidal activity with the lowest LC_{50} of 674 conidia mm^2 against *T. urticae* eggs.

KEY WORDS: Entomopathogenic fungi, ovicidal effect, *Tetranychus urticae*

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The Two Spotted Spider Mite (TSSM), *Tetranychus urticae* Koch, is a ubiquitous agricultural pest with a global distribution. It is a major concern in vegetables causing heavy damage leading to 7 to 48 per cent yield loss (Srinivasa and Sugeetha, 1999). Its phytophagous nature, high reproductive potential, extremely short life cycle and arrhenotokous mating system facilitate rapid resistance development to many acaricides often after a few applications. Therefore, screening of suitable biocontrol agents is a step in developing new or improving existing environment friendly strategies offering an alternative to conventional pest control. Entomopathogenic fungi may play a major role in the natural regulation of spider mite populations and could be used in biological control programme, either as a replacement for synthetic acaricides or as a component of integrated mite management.

Several studies have been conducted for identifying the ovicidal action of entomopathogenic fungi against mites. Hanchinal and Manjunatha (2000) reported the pathogenicity of *Metarhizium anisopliae* on eggs of *T. neocaledonicus*. The per cent mortalities of eggs were 21.37, 38.50 and 87.82, at one, two and three days, respectively. Irigaray *et al*. (2003) reported that a commercial *Beauveria bassiana* formulation, Naturalis, had high ovicidal action against eggs of *T. urticae* within 1-3 days of its application containing 1,400-22,800 viable conidia ml^{-1}.

Acevedo *et al*. (2003) reported that the exudates produced by the *Hirsutella thompsonii* inhibited egg production by female spider mites. Topical application of the metabolite resulted in 100 per cent reduction in fecundity in *T. urticae* over a 6 day post treatment period. Kumar and Singh (2007) found that *T. urticae* eggs treated with the exudate from sporulating culture of *H. thompsonii* showed very significant (64.6%) reduction in hatching. In the fecundity test, an adult female feeding on leaves treated with exudates laid only 30.7 eggs compared to untreated leaf (51eggs/female) laid over a period of 7 days.

In the present study, ten different fungal isolates were evaluated for their ovicidal action against TSSM, *Tetranychus urticae.*

Preparation of mite eggs

Around 20 adult females of *T. urticae* were arbitrarily taken from the plants and transferred onto a detached okra leaf disc (50 mm) placed on 1.5 per cent agar in a Petri dish and allowed to lay eggs for 24 h. Subsequently, the females were removed, leaving 30 eggs per leaf disc, for bioassays.

The detached leaf disc on agar remained turgid for over seven days so as to allow normal hatching of the mite eggs.

Source of the fungal isolates

Pure cultures of the entomopathogenic fungi viz. *Paecilomyces fumosoroseus* (Thom.) and *Hirsutella thompsonii* (Fisher) were obtained from the National Bureau of Agricultural Insect Resources (NBAIR), Bangalore and *Beauveria bassiana* (Balsamo) Vuillemin (B2 strain) from Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore. Local isolates *viz., Cladosporium cladosporioides* (Fresenius) de Vries*, Fusarium pallidoroseum* Cooke (Sacc)*, B. bassiana* and *Metarhizium anisopliae* (Metchinkoff) Sorokin were obtained during surveys.

Bioassays on eggs of *Tetranychus urticae*

Spore suspensions of six different fungal pathogens and ten isolate (*Beauveria bassiana* - Bb101, Bb102, Bb103, B2, *Metarhizium anisopliae* - Ma101, *Fusarium pallidoroseum* - Fp101, *Cladosporium cladosporioides* - Cc101, Cc102, *Paecilomyces fumosoroseus*- Pfr and *Hirsutella thomsoni* Ht) were prepared separately and standardized at the concentration of $1x10^8$, 10^7 and 10^6 spores ml^{-1}. Bioassay was done with the slight modifications as per the procedure of Shi and Feng (2004a). For each fungal isolate, three detached leaves with eggs in uncovered Petri dishes were placed onto the bottom of a large bucket (48 cm diameter and 60 cm height). Three glass cover slips (20 x 20 mm) were triangularly placed on the bucket bottom (besides each of the Petri dishes) to collect the sprayed conidia while the eggs were exposed. Then, counts of conidia on each cover slip were made from five randomly sampled view fields under a compound microscope at 400 x magnification and averaged as the number of conidia per mm^2. Thus, the three different concentrations of each fungal isolate resulted in low, intermediate and high concentrations of conidia attached to the eggs and leaves. A spray of 0.05 per cent Tween 80® served as blank control. All the treatments were replicated three times.

After spray, all the treated Petri dishes were maintained in an incubator at 25°C and examined daily for three days. Later, the eggs were individually examined under a stereo zoom binocular microscope (Carl zeiss Stemi 2000) at 40x magnification for verification of fungal infection. Finally, all unhatched eggs were transferred to moist chambers for two or three days to observe fungal outgrowth if any, as an evidence of egg mortality due to fungal infection.

Ovicidal activity of ten fungal isolates studied against *T. urticae* under laboratory conditions revealed that *H. thompsonii* was more effective against *T. urticae* eggs with pronounced ovicidal activity. A significant reduction (61.1 %) in hatchability was observed at the higher conidial concentration of 971 conidia mm^{-2}. It was concurrent with the results of Kumar and Singh (2007), which treated the *T. urticae* eggs with the *H. thompsonii* exudates and noticed a significant reduction (64.6%) in hatching.

The isolate Bb101 caused significantly higher egg mortality rate of 46.9 per cent followed by B2 (36.1%) and Cc101 (32.1%) at higher conidial concentration of 1457, 1231 and 856 conidia mm^{-2}, respectively. In other isolates, mortality per cent ranged from 29.3 to 14.7 and was found to be low, which may be due to less sporulation of the isolates. Perhaps the duration of the egg stage (1-2 days) was too short for the fungal mycelium to cause infection of less sporulated cultures (Mazet and Vey, 1995). Based on the LC_{50} estimates determined by the concentration mortality relationships for all fungal pathogens, *H. thompsonii* was more lethal to the eggs of *T. urticae* with lower LC_{50} (674 conidia mm^{-2}). The two isolates Ht and Bb 101 were highly infective to spider mite eggs. The Ht isolate had a LC_{50} apparently lesser than 1718 conidia mm^{-2} of the Bb 101 isolate, indicating its stronger ovicidal action and Bb 101 was moderately infective to eggs. The high ovicidal activity of *H. thompsonii* isolate may be due to its insecticidal and acaricidal metabolite Hirsutellin A, a toxic compound secreted in the broth culture (Liu *et al.,* 1995).

The results obtained in the present study also corroborates with the findings of Shi and Feng (2004 a) who reported that application of *B. bassiana* conidia to *T. cinnabarinus* eggs at the concentrations of 58, 298 and 1306 conidia mm^{-2} resulted in corrected egg mortalities of 20.4±4.2 per cent, 36.0±7.6 per cent and 64.6±12.5 per cent, respectively. Shi and Feng (2004 b) found that *B. bassiana* Bb2860 was highly infectious to *T. cinnabarinus* eggs with the lowest LC_{50} of 548 conidia mm^{-2}, followed by *P. fumosoroseus* Pfr116 (848 conidia mm^{-2}) and Pfr153 (913 conidia mm^{-2}).

Unlike active immature and adult stages, the eggs are immobile, making it difficult to determine their mortality status. Slight changes in egg morphology such as shape and colour were not sufficient to judge the mortality. Thus, the unhatched eggs observed on a given day after spray could not be classified as dead or alive until fungal outgrowths were visible. Fortunately, counts of the hatched eggs could be easily attained, generating an overview to the effect of different fungal sprays on their hatch rates over time which can be used to assess the egg mortalities at different conidial concentrations.

Entomopathogenic fungi have evolved with the insects,

a hundred million years ago and are still evolving**.** They are part of our natural environment and have interacted with pest management as long as their existence. In the present study, utilization of native strains with the increased virulence and pathogenicity provides specific action on host and adaptation to the environment. Successful use of entomopathogenic fungi as microbial control agents of mites depends on the virulent strains, formulation, and compatibility with other control agents, better delivery system and timing of applications. Hence, the use of fungal entomopathogens serves as an alternative to acaricides and offers a great scope in bio intensive pest management with safety to environment as an added advantage over chemicals.

Table 1. Ovicidal action of different fungal isolates against *Tetranychus urticae*

Fungal isolate#	No. of Conidia mm^{-2}	Egg Mortality (%)	Fungal isolate	No. of Conidia mm^{-2}	Egg Mortality (%)
B. bassiana Bb101	1457	46.9^{a}	*F. pallidoroseum* Fp 101	984	29.3^{a}
	539	30.2^{b}		239	17.4^{b}
	87	8.8^{c}		65	6.05^{c}
	Control	3.0^{d}		Control	3.5^{c}
Bb102	1384	14.7^{a}	*C.cladosporioides* Cc101	791	16.9^{a}
	593	7.9^{b}		282	6.4^{b}
	75	2.9^{c}		84	6.2^{b}
	Control	3.0^{c}		Control	3.6^{b}
Bb103	1144	19.4^{a}	Cc102	856	32.1^{a}
	509	12.0^{b}		248	24.3^{b}
	68	3.4^{c}		79	19.9^{b}
	Control	3.1^{c}		Control	3.6^{c}
B2	1231	36.1	*P. fumosoroseus* Pfr	1168	15.0^{a}
	474	26.5		476	12.3^{a}
	64	13.6		64	6.8^{b}
	Control	6.6		Control	4.3^{b}
M. anisopliae Ma 101	1147	25.8^{a}	*H. thompsonii* Ht	971	61.1^{a}
	294	17.6^{b}		196	35.8^{b}
	57	10.6^{c}		36	22.1^{c}
	Control	2.9^{d}		Control	4.9^{d}

Number of eggs used per treatment - 30
Means followed by a common letter (s) are not significantly different at P = 0.05 by LSD

Table 2. Concentration mortality response of *Tetranychus urticae* eggs to different fungal isolates

Fungal Isolates	No. of eggs used	2*	Regression equation	Lc_{50} (No. of conidia mm^{-2})	95 % Fiducial Limits (No. of conidia mm^{-2})
B. bassiana Bb101	30	0.034	Y=1.042X - 3.37	1718	1126 - 3471
Bb102	30	0.264	Y=0.67X - 3.20	5755	8259 - 22589
Bb103	30	1.303	Y=0.001X - 1.72	2169	1617 - 4071
B2	30	0.037	Y=0.576X-2.147	5380	1907-12167
M. anisopliae Ma 101	30	0.608	Y=0.001X - 1.19	2229	1488 - 66665
F. pallidoroseum Fp 101	30	0.466	Y=0.818X - 2.69	4196	1726 -38952
C. cladosporioides Cc101	30	1.518	Y=0.024X - 1.547	1930	1317 - 5008
Cc102	30	0.134	Y=0.12X - 0.849	1851	1097 - 205020
P. fumosoroseus	30	0.601	Y=0.11X - 1.43	3908	2345 - 6321
H. thompsonii	30	1.673	Y=0.12X - 0.688	674	524.3 - 896.6

*All lines are significantly good fit at P ≤ 0.05

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