

Pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin isolate MZ674187 against two spotted spider mite, *Tetranychus urticae* Koch

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ABSTRACT

The two spotted spider mite, *Tetranychus urticae* Koch is a polyphagous pest causing severe yield loss to an extent of 50 per cent in vegetables. The management of two spotted spider mite mostly rely on acaricides. The continuous use of acaricides resulted in rapid development of resistance in mites. In this study, an investigation was made to assess the pathogenicity of the *Beauveria bassiana* isolate (MZ674187) against two spotted spider mites. The pathogenicity of the isolate was assessed by treating the two spotted spider mite with different conidial load of *B. bassiana* MZ674187 ranging from 1×10^3 to 1×10^9 conidia/ml in laboratory conditions. Mortality of the mites were observed at 24 hours interval and the results indicate that the mite mortality increased when the conidia concentration was increased. The mortality of the mites was in the range of 14.4 per cent to 82.22 percent at the conidial load of 1×10^3 conidia/ml and 1×10^9 conidia/ml respectively at 9th day after inoculation. The median lethal dose and median lethal time were calculated and was found to be 1.55×10^6 conidia/ml and 111.01 hours respectively.

Keywords: *Beauveria bassiana*, *Tetranychus urticae*, Pathogenicity, LC50 value

Introduction

The two spotted spider mite, *T. urticae* is a polyphagous pest, reported in more than 900 host plants worldwide (Migeon *et al.*, 2010), causing a severe yield loss up to 50 per cent in Bhendi (Roseleen *et al.*, 2010). The management of two spotted spider mite mainly rely on acaricides (Sato *et al.*, 2009). Due to the development of rapid resistance, resurgence and residues of the acaricides in plants, acaricidal control of two spotted spider mite is limiting factor (Leeuwen *et al.*, 2010). Adoption of eco-friendly methods like biological control is the alternate way to mitigate the problems caused due to the use of pesticides. Entomo Pathogenic fungi (EPF) such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Hirsutiella*

spp and *Lecanicillium lecanii* were successfully used for the control of two spotted spider mite (Chandler *et al.*, 2005). Jeyarani *et al.*, (2011) reported that *B. bassiana* has been found to cause mycosis on two spotted spider mite and the pathogenicity increases as the conidial concentration increased. The present study aims to find the pathogenicity of *B. bassiana* isolate, MZ674187 isolated from the two spotted spider mite cadavers with different conidial concentrations.

Materials and Method

Fungal culture

B. bassiana, MZ674187 isolated from the infected ca-

daughters of two spotted spider mites were maintained in glycerol stock. A loopful of fungal spores from stock culture were transferred on potato dextrose agar (PDA) plates and incubated at $25 \pm 2^\circ\text{C}$ for 2 weeks until plates were fully grown.

Rearing of two spotted spider mite, *T. urticae* Koch

The two spotted spider mites collected from the field were reared in the Acarology Laboratory using freshly detached mulberry leaves. The mulberry leaves were placed in a 90 mm petri-plate with moistened cotton and the mites collected from the field were released in the petri plate and allowed to multiply.

Preparation of conidial suspension

For testing the pathogenicity of *B. bassiana*, fully sporulated (fifteen days old) culture was used (Malarvannan *et al.*, 2010). The fungal spores were harvested from the plates using a sterile scalpel and transferred to 10 ml of sterile water containing 0.01% Tween 80 as a wetting agent. The concentration of spores in the final suspension was determined using a Neubauer haemocytometer and a series of dilutions were made with the spore concentrations ranging from 1×10^3 to 1×10^9 conidia/ml.

Pathogenicity testing

Mulberry leaf discs were cut to the diameter of 5 cm and were dipped in the prepared *B. bassiana* suspension ranging from 1×10^3 to 1×10^9 conidia/ml and dried. Mulberry leaves dipped in the sterile water containing 0.01% Tween 80 served as control. After

drying, the leaves were placed in a petri plate with moist filter paper and 30 uniform aged adult mites were released to each petri plate (Lee *et al.*, 1997). The mortality of the mites was calculated at 24 hrs interval.

Statistical Analysis

Mortality days after inoculation (DAI) was adjusted using Abbott's formula (Abbott 1925). Analysis of variance was used to examine the differences in mortality between the fungal isolates and the control group (ANOVA). The statistical software SPSS 16.0 was used to perform all of the analyses.

Results and Discussion

Mortality of the two spotted spider mites

The mortality of the mites due to mycosis began at 48 hrs after inoculation. The mites that are infected by the entomopathogenic fungi, *B. bassiana* (MZ674187) are different from the uninfected mites in a way that they were sluggish and swollen before death. The sporulated cadavers were then re-isolated to confirm the Koch's postulate. The results of re-isolation confirmed that the mites died due to *B. bassiana*, MZ674187 infection.

Pathogenicity of the isolate MZ674187 (*B. bassiana*)

The mortality per cent of two spotted spider mite increased with increasing concentration of conidia. The isolate MZ674187 caused 82.22 per cent mortality of two spotted spider mite after 9 days of inoculation at the spore concentration of 1×10^9 spores/

Table 1. Pathogenicity of *B. bassiana* MZ674187 isolate against *T. urticae*

Treatment details	Per cent mortality (%)				
	48 HAI	3 DAI	5 DAI	7 DAI	9 DAI
T1 (1×10^3 conidia/ml)	0.00(2.87) ^d	1.11(5.42) ^{ef}	4.44(11.99) ^g	8.89(17.28) ^g	14.44(22.31) ^g
T2 (1×10^4 conidia/ml)	1.11(5.42) ^d	3.33(9.45) ^e	10.00(18.27) ^f	14.44(22.31) ^f	21.11(27.34) ^f
T3 (1×10^5 conidia/ml)	4.44(11.99) ^c	7.78(16.12) ^d	15.56(23.20) ^e	25.56(30.36) ^e	34.44(35.93) ^e
T4 (1×10^6 conidia/ml)	7.78(16.12) ^b	14.44(22.31) ^c	27.78(31.79) ^d	37.78(37.92) ^d	47.78(43.73) ^d
T5 (1×10^7 conidia/ml)	8.89(17.28) ^b	22.22(28.11) ^b	38.89(38.58) ^c	47.78(43.73) ^c	62.22(52.08) ^c
T6 (1×10^8 conidia/ml)	11.11(19.43) ^b	27.78(31.79) ^{ab}	45.56(42.45) ^b	57.78(49.48) ^b	74.44(59.64) ^b
T7 (1×10^9 conidia/ml)	17.78(24.92) ^a	34.44(35.93) ^a	51.11(45.64) ^a	67.78(55.42) ^a	82.22(65.08) ^a
T8 (Control)	0.00(2.87) ^d	0.00(2.87) ^f	0.00(2.87) ^h	0.00(2.87) ^h	0.00(2.87) ^h
CD(0.05)	3.83	5.05	3.04	2.22	2.09
S.Ed	1.82	2.40	1.44	1.06	0.99

* No. of insects per replication: 30.

*Values in bracket are subjected to square root transformation.

*Values sharing same alphabets in superscript are statistically on par based on ANOVA.

Table 2. Concentration and mortality response of *B. bassiana*,MZ674187against *T. urticae*

Heterogeneity	Regression equation	LC50	LT50	limit
11.07	$y = 0.5969x + 1.1877$	1.55×10^6 conidia/ml	-	9.3×10^4 - 2.56×10^7 conidia/ml
12.592	$y = 2.6507x - 0.3924$		111.01	82-150.29

ml. Least mortality of 14.44 per cent was reported in the conidial concentration of 1×10^3 conidia/ml after 9 days of inoculation (Table 1). The difference in mortality per cent between the two concentrations of 1×10^9 conidia/ml and 1×10^3 conidia/ml was found to be 67.78 per cent.

The median lethal concentration (LC 50) value of the isolate MZ674187 was calculated and it was found to be 1.55×10^6 conidia/ml (Table 2 & Fig-1). The median lethal time (LT50) was found to be 111.01 hours (Fig. 2).

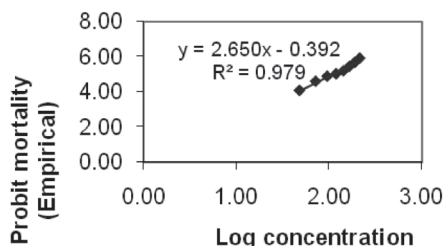


Fig. 1. Dose mortality response

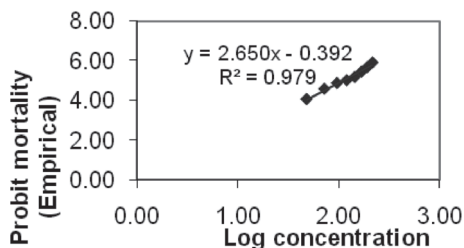


Fig. 2. Time mortality response

Discussion

The current study attempted to determine the pathogenicity of the *B. bassiana* isolate MZ674187 against two spotted spider mite, *T. urticae*. The *B. bassiana* isolate DEB1008 caused 74.68 per cent mortality of two spotted spider mite at the spore concentration of 1×10^8 conidia/ml on seventh day after the treatment (Seidey *et al.*, 2010) which is in confirmity with the present study as the isolate MZ674187 caused 74.40 per cent mortality at concentration of 1×10^8 conidia/ml. Similarly, Yanaret

al., (2018) found that the two isolates of F-12 and F-56 caused the maximum mortality of 78.3 and 76.7 per cent respectively with the conidial load of 1×10^8 conidia/ml which is in accordance with the present study. Wekesa *et al.*, (2005) found that the LC50 value of *B. bassiana* isolate GPK against *T. evansi* was 1.1×10^7 conidia/ml and it is in conformity with the present study. Similarly, Saranya *et al.*, 2013 calculated the LC50 value of the *B. bassiana* isolate Bb101 against *T. urticae* and it was found to be 1.2×10^5 spores/ml which is further in conformity with our results.

Conclusion

The *B. bassiana* isolate MZ674187 is found to be effective in controlling two spotted spider mite, *T. urticae* Koch under laboratory conditions and it can be utilized as the biocontrol agent for the control of two spotted spider mite, *T. urticae* in mite management programs.

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