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Efficacy of talc-based formulation of *Beauveria bassiana* (Bals.) Vuill. (MZ749636) against two spotted spider mite, *Tetranychus urticae* Koch

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Abstract
Tetranychus urticae is one of the most important and destructive mite species infesting the vegetable crops, which is very important to control because of its wide host range. The damage caused by the two spotted spider mite can be eliminated by using entomopathogenic fungi, as the acaricidal control causes various problems such as resistance, resurgence and residue problems. Therefore, the present study was carried out with a view to evaluate the efficacy of the talc-based formulation of *Beauveria bassiana* (MZ749636) under pot-culture conditions against two spotted spider mite (TSSM), *T. urticae* in potted bhendi (*Abelmoschus esculentus*) plants, in comparison with fenazaquin 10 EC @ 1.5ml/l, azadirachtin 3000 ppm @ 2ml/l and crude formulation of *B. bassiana,* MZ749636 @ 1 x 10^8 conidia/ml. After two rounds of spraying at fortnight intervals, the talc-formulation of *B. bassiana*, MZ749636 resulted in 62.83 per cent cumulative reduction of TSSM over the control. However, fenazaquin 10 EC @1.5 ml/l recorded the maximum cumulative mortality of 80.07 per cent, followed by azadirachtin 3000 ppm @ 2ml/l, which recorded a cumulative mortality per cent of 71.06 per cent. Crude formulation of *B. bassiana* recorded 58.12 per cent reduction of TSSM over the control after two rounds of spraying. This was the first study that attempted to evaluate the efficacy of the talc formulation of the *B. bassiana* (MZ749636) isolate against TSSM.

Keywords: *Beauveria bassiana,* Efficacy, Formulation, Two-spotted spider mite

INTRODUCTION

The two spotted spider mite (TSSM), *Tetranychus* spp (Acari: Tetranychidae) is an economically important polyphagous, cosmopolitan pest of vegetables, fruits and ornamental crops. It causes damage to a wide range of crops, including tomato, bhendi, cucumber, beans, peppers, strawberries and roses. Tetranychid mites infest over 1200 species of plants (Zhang, 2003, Puspitarini *et al.,* 2021) as Tetranychid mites have a high multiplication rate, short developmental period, high fecundity and long adult survival (Maric *et al.,* 2021).

Rabindra and Janardan (2011) reported two-spotted spider mites, *Tetranychus urticae* Koch and red spider mite, *T. macfarlanei* Baker and Pritchard *T. ludeni* Zacher are the major species affecting horticultural and agricultural crops. Among these, *T. urticae* pose a major threat during the summer, spring and post-rainy seasons, leading to a severe yield loss of 7 to 48 per cent in important vegetable crops like bhendi (Ghosh, 2013; Tripathi *et al.*, 2014a), brinjal (Tripathi *et al.*, 2014b), cucumber (Tehri *et al.*, 2014; Abo-Elmaged *et al.*, 2021) and potato

Insect pathogens, especially entomopathogenic fungi

(EPF) such as *Beauveria bassiana, Metarhizium anisopliae, Hirsutella* spp and *Lecanicillium lecanii* have been used to manage a variety of insect pests, particularly mites with significant effectiveness (Chandler *et al.*, 2005). These EPF are known to infect the pests naturally, causing to death due to mycosis. About 700 EPF are known to infect the insects like thrips, aphids, whiteflies, termites and beetles (Dembilio *et al.*, 2010). *B. bassiana* is the most promising EPF, among the 700 species of EPF reported (Khachatourians *et al.*, 2001).

The highly virulent strains of acaropathogens were isolated and commercial formulations were developed for effective control of TSSM in Western countries (Hajek and Tobin, 2010; Wraight *et al.*, 2017). To make a wider application of EPF for the control of TSSM, the EPF culture are formulated for easy delivery in the field (Bateman *et al.,* 1993). The conidial viability and virulence were found to be more in the talc formulation (Ali, 2016). The aim of the present study was to evaluate the efficacy of the talc- based formulation of *B. bassiana* against *T. urticae*.

MATERIALS AND METHODS

Rearing of TSSM, *T. urticae*

The mites collected from the field were reared in the Acarology laboratory using freshly prepared mulberry leaf discs of 5cm dia. and maintained as a pure culture. The leaf discs were placed in Petri Plates with moistened sterile cotton. Field collected mites were released in the Petri Plate and allowed to multiply. The cotton pads were moistened daily and the mulberry leaves were changed whenever it is necessary (Hassan *et al.*, 2017). After the multiplication of the mites, they were transferred to the caged potted bhendi (Bhendi hybrid COBh H1) plants maintained at Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, for further multiplication.

Development of crude formulation

250 ml of Potato Dextrose broth (PDB) was poured into a 500 ml conical flask and autoclaved at 121ºC for 20 minutes. *B. bassiana,* MZ749636 was inoculated into the conical flask and shaken for three days by keeping them in an orbital shaker and after three days, they were incubated at 25ºC for 10 days (Bhadauria *et al.* 2012).

After sporulation, the mycelial mat of the fungus and broth was ground in a 500watt mixer and filtered through double-layered muslin cloth. The suspension obtained was shaken thoroughly in distilled water containing 0.25 ml of Tween 80. The conidial suspension was vortexed for 5 min to produce a homogenous conidial suspension (Maniania, 1993) and the conidial load of the fungal suspension was assessed using the improved Neubauer haemocytometer.

Development of talc-based formulation

The mycelial mat from the fully sporulated *B. bassiana,* MZ749636 was ground using a 500watt mixer along with the broth. After grinding, the conidial strength was assessed using the improved Neubaeur haemocytometer and then the broth was mixed with autoclaved talc at 1:2 ratio (500 ml:1 kg). To the mixture, 10 g of carboxy methyl cellulose (CMC), 15 g of calcium carbonate and 1 g of citric acid were added and dried in the shade for 72 hours (Sivasundaram *et al.*, 2008). The number of colony-forming units present in the talc formulation was determined using serial dilution method.

Testing the efficacy of the talc-based formulation against TSSM under glass house condition

Talc formulation of *B. bassiana* (MZ749636) isolate was evaluated for its efficacy in controlling the population of TSSM in pot-culture studies along with fenazaquin 10 EC @ 1.5 ml/l, azadirachtin 3000ppm @2ml/l, crude formulation of *B. bassiana* MZ749636 and untreated control. Bhendi plants were raised in pots in the glasshouse at Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-03, using a Completely Randomized Design with five treatments and five replications. Uniform aged TSSM were inoculated in the bhendi plants and allowed to multiplicate for two weeks. After the establishment of the mite population on bhendi plants, the following treatments with standardized doses were imparted and spraying was done using a hand sprayer. The treatments used were: T_1- Crude formulation of *B. bassiana*, T_2- Talc formulation of *B. bassiana,* T_3-Fenazaquin 10 EC (Magister), T_4- Azadirachtin 3000ppm (Neemole) and T_5- Control (Water spray).

Two rounds of spraying were done at an interval of 14 days. The initial population of the TSSM were counted from each plant in the replication. Three leaves, one each from top, middle and bottom of each plant, were observed for the live mite population on the surface of the leaves in an area of 4 cm^2.Observations were made at the place where the population of *T. urticae* was maximum randomly.

After treatment, the mite population was assessed at 3, 5, 7, 9, 11, 13 and 14 days after inoculation using a 10x hand lens. The mortality per cent and per cent reduction over control was calculated.

Statistical analysis

The TSSM population in pot culture experiments and field studies were subjected to square root transformation(X+0.5) and the mean comparison was made using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1983). The analysis of variance was carried out using SPSS software.

RESULTS AND DISCUSSION

Crude formulation of *B. bassiana*

To make a wider application of EPF for the control of TSSM, the EPF culture are formulated for easy delivery in the field. To get a greater efficacy of the entomopathogens, the formulation must be done (Prior *et al.*, 1988). The conidial viability and virulence were found to be more in the talc formulation (Ali, 2016). A crude formulation of *B. bassiana*, MZ749636 was prepared and the conidial concentration of the crude formulation was found to be 1 x 10^8 conidia/ml.

Talc formulation of *B. bassiana*

The conidial concentration of the mycelial spore suspension was found to be 1 x 10^8 conidia/ml. After mixing with talc, the number of colony-forming units present in the talc formulation was determined and it was found to be 2.1 x 10^8 cfu/g and its efficacy under pot culture was assessed.

Efficacy of talc-based formulation of *B. bassiana,* MZ749636 against TSSM under glass house condition

The present study revealed that there was a significant difference (4.71% when compared to the talc formulation with crude formulation, 17.24 % when compared to the talc formulation with fenazaquin and 8.23 % when compared the talc formulation with Azadirachtin) in the population of TSSM in all the different formulations tested (58.12 % crude formulation of *B. bassiana*, 62.83 in talc formulation of *B. bassiana,* 80.07 fenazaquin and 71.06 % in Azadirachtin). The results are furnished in Tables 1 and 2.

The pre-treatment count of *T. urticae* was in the range of 16.93 to 17.67 and was found to be non-significant. After the first spray, the population of mites started to reduce significantly. Highest per cent reduction of 71.13 per cent reduction over the control was recorded in the fenazaquin 10EC @1.5ml/l treatment, followed by 63.85 per cent reduction over the control by azadirachtin 3000ppm@2ml/l treatment. The talc formulation of *B. bassiana*, recorded a 57.93 per cent mite population reduction over the control after the first spray (Table- 1).

After 14 days of the first spray, a second spray was given. Fenazaquin 10EC @1.5ml/l treatment resulted in 90.27 per cent reduction over the control after the second spray with a cumulative reduction of 80.07 per cent *T. urticae* over the control. Azadirachtin 3000ppm@2ml/l treatment resulted in 78.26 per cent reduction over the control after the second spray with a cumulative reduction of 71.06 per cent *T. urticae* over the control. The talc formulation of *B. bassiana* resulted in 69.72 per cent reduction over the control after the second spray with a cumulative reduction of 62.83 per cent *T. urticae* over the control (Table -2). Crude formulation of *B. bassiana* recorded 58.12 per cent cumulative reduction of *T. urticae* over the control. The crude and talc formulation resulted in 63.75 and 69.72 per cent reduction over the control after two rounds of spraying (Table 2), which is similar to the results of Mohan *et al.* (2014) as the talc formulation of *Hirsutella thompsonii* recorded 70-80 per cent control of coconut eriophyid mite. The results of Singh and Joshi (2020) were also in accordance with the present study's results, as the talc formulation of *Lecanicillium lecanii* resulted in 61.6 per cent reduction of aphid in green house conditions. The results were also in agreement with the results of Kumar *et al.* (2019), as the *M. anisopliae* caused 71.00 per cent reduction of *Nilaparvata lugens*. Chelvi *et al.* (2010) reported similar results against sugarcane white grub, *Holotrichia serrata* F (Coleoptera: Scarabidae) by using *B. bassiana.* Similarly, Das *et al.* (2013) also reported similar results by using *B. bassiana* against rice hispa, *Dicladispa armigera* (Olivier). Kumbhar *et al.* (2019) evaluated the bio-efficacy of various formulations of biopesticides such as *B. bassiana* and *M. anisopliae* against white grub, *Leucopholis lepidophora* infesting sugarcane and reported similar results.

Conclusion

Mycoinsecticides were very effective in managing various mites when the use of acaricides is limited due to the development of rapid resistance, resurgence and residues of the acaricides in plants. *B. bassiana* (MZ749636) isolate used in the present study was isolated from the cadavers of TSSM. Formulating the Entomopathogenic fungi was done to increase the viability and shelf life. The talc formulation of *B. bassiana* (MZ749636) was very effective in controlling the populations of two-spotted spider mites as it caused a 62.83 per cent reduction over the control. This isolate can be effectively used to manage the mites TSSM by replacing the acaricides, as controlling TSSM using talc formulation of *B. bassiana* (MZ749636) is not yet attempted earlier. Further work can be done to increase the viability of the *B. bassiana* (MZ749636) isolate so that a more per cent reduction of TSSM can be achieved.

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Conflict of interest

The authors declare that they have no conflict of interest.

Table 1. Efficacy of talc-based formulation *B. bassiana* (MZ749636) against *T. urticae* on bhendi (First spray) under pot culture conditions

Treatments	Pre-treatment count (No. of mites/plant)	Number of mites per 4 cm^2 (days after spraying)							Mean number of mites per 4 cm^2	Per cent reduction over control
		3	5	7	9	11	13	14		
T_1- Crude formulation of *B. bassiana*, MZ749636@ 1x 10^8 conidia/ml	16.93	14.40 (3.79)^c	12.47 (3.53)^b	11.07 (3.33)^d	8.67 (2.94)^d	11.80 (3.43)^d	13.13 (3.62)^d	14.40 (3.79)^d	12.28	52.48
T_2- Talc formulation of *B. bassiana*, MZ749636 @ @ 2.1x 10^8 cfu/g	17.07	13.93 (3.73)^c	11.87 (3.44)^b	9.33 (3.05)^c	6.80 (2.60)^c	9.73 (3.12)^c	11.47 (3.38)^c	12.93 (3.59)^c	10.87	57.93
T_3- Fenazaquin 10 EC @ 1.5 ml/l	17.33	8.80 (2.97)^a	6.73 (2.59)^a	3.87 (1.96)^a	2.00 (1.41)^a	5.07 (2.25)^a	6.53 (2.55)^a	9.33 (3.05)^a	7.46	71.13
T_4- Azadirachtin 1000ppm @ 2ml/l	17.47	12.00 (3.46)^b	9.00 (2.99)^b	6.20 (2.48)^b	3.80 (1.94)^b	6.67 (2.58)^b	8.40 (2.89)^b	11.20 (3.35)^b	9.34	63.85
T_5- Control (Water spray)	17.67	19.87 (4.45)^d	22.80 (4.77)^c	25.13 (5.01)^e	27.40 (5.23)^e	29.67 (5.44)^e	31.34 (5.59)^e	32.87 (5.73)^e	25.84	52.48
S.Ed	NS	0.08	0.08	0.09	0.10	0.11	0.11	0.09		
CD(0.05)	-	0.17	0.17	0.19	0.21	0.24	0.23	0.19		

*Number of mites per 4 cm^2 is the average number of mites present in the top, middle and bottom leaf; *Values are subjected to square root transformation; *Mean values sharing same letter(s) are significantly different from each other at ANOVA

Table 2. Efficacy of talc-based formulation *B. bassiana* (MZ749636) against *T. urticae* on bhendi (Second spray) under pot culture conditions

Treatments	Number of mites per 4 cm^2 (days after spraying)							Mean no. of mites per 4 cm^2	Per cent reduction over control	Cumulative mean number of mites per 4 cm^2	Cumulative per cent reduction over the control
	3	5	7	9	11	13	14				
T_1- Crude formulation of *B. bassiana*, MZ749636@ 1x 10^8 conidia/ml	13.4 (3.63)^d	12.66 (3.53)^d	11.45 (3.38)^d	10.33 (3.20)^d	13.13 (3.63)^d	15.33 (3.94)^d	10.67 (3.83)^d	12.42	63.75	12.35	58.12
T_2- Talc formulation of *B. bassiana*, MZ749636 @ 2.1x 10^8 cfu/g	11.47 (3.38)^c	9.33 (3.08)^c	8.47 (2.95)^c	7.54 (2.77)^c	9.65 (3.09)^c	11.54 (3.42)^c	14.65 (3.27)^c	10.38	69.72	10.62	62.83
T_3- Fenazaquin 10 EC @ 1.5 ml/l	7.27 (2.69)^a	4.00 (1.99)^a	2.33 (1.52)^a	1.33 (1.15)^a	1.6 (1.26)^a	2.47 (1.57)^a	4.33 (2.08)^a	3.33	90.27	5.39	80.07
T_4- Azadirachtin 1000ppm @ 2ml/l	9.47 (3.08)^b	7.33 (2.71)^b	6.54 (2.56)^b	5.43 (2.33)^b	6.59 (2.62)^b	7.65 (2.79)^b	9.15 (3.10)^b	7.45	78.26	8.39	71.06
T_5- Control (Water spray)	32.87 (5.86)^e	33.2 (6.02)^e	33.63 (5.84)^e	34.13 (5.89)^e	34.6 (5.95)^e	35.13 (5.98)^e	36.33 (6.04)^e	34.38		30.31	
S.Ed	0.09	0.10	0.08	0.09	0.09	0.09	0.07				
CD (0.05)	0.20	0.22	0.16	0.19	0.19	0.21	0.16				

*Number of mites per 4 cm^2 is the average number of mites present in the top, middle and bottom leaf;*Values are subjected to square root transformation; *Mean values sharing same letter(s) are significantly different from each other at ANOVA

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