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Dry weather: A crucial constraint in the field efficacy of entomopathogenic fungus *Beauveria Bassiana* against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract
Field trials were conducted to evaluate the efficacy of the entomopathogenic fungus, *Beauveria bassiana* against the two spotted spider mite *Tetranychus urticae* Koch on okra during the crop seasons 2010, 2011 and 2014 at Research Farm, Department of Vegetable Science, CCS HAU, Hisar. Significant reduction in *T. urticae* population was recorded during crop seasons 2010 and 2011 subjected to *B. bassiana* treatments over control (P= 0.05). However, during the crop season 2014, the fungal treatments were found to be ineffective in controlling the pest population. This may be attributed to the dry environmental conditions prevalent in 2014 in the study area pertaining to which the fungal spores failed to undergo sporulation, effective host invasion and mycosis.

Keywords: okra, *Beauveria bassiana*, *Tetranychus urticae*, bioefficacy, field trials

1. Introduction

Tetranychids are polyphagous pests that seriously damage vegetables, ornamentals and agricultural crops. The damage caused by spider mites to agriculture has greatly increased during the past 60 years. Two species that have been given much attention worldwide are the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, and the European red mite, *Panonychus ulmi* Koch [1]. *T. urticae* is a ubiquitous and economically important agricultural pest throughout the world [2]. It is known to attack about 1200 species of plants, of which more than 150 are economically important, often causing 50-100 percent yield loss [3]. Okra is a popular vegetable crop in India and insect and mite pests are major factors limiting okra yield. Many of the pests occurring on cotton are found to ravage okra crop. Around 72 species of insects and mites have been recorded on okra, of which, the sucking pests comprising of aphids (*Aphis gossypii* Glover), leafhopper (*Amrasca biguttula biguttula* Ishida), whitefly (*Bemisia tabaci* Gennadius) and two spotted spider mite (*T. urticae*) cause significant damage to the crop [4]. Traditionally, TSSM has been controlled using synthetic chemical acaricides with a level of residuality and permanence that constitute a barrier to the commercialization of agricultural products and cause detrimental effects to environment and human health. Acaricide resistance is accelerated by its high fecundity, inbreeding, arrhenotokous reproduction, high mutation rate and very short life cycle resulting in many generations per year [5]. As a consequence, *T. urticae* has attained the dubious reputation to be “the most resistant species” in terms of the total number of pesticides to which it has become resistant [6]. Currently, great efforts are directed towards reduction in the use of traditional pesticides and increase in the use of Integrated Pest Management (IPM) techniques. One alternative pest control strategy involves the use of entomopathogenic fungi. Research and development of biological control options for spider mites has largely concentrated on the conservation of natural enemies and releases of predatory mites [7]. However, this is often not sufficient and supplementary sprays of acaricides are needed. 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 stand-alone solution in replacement of synthetic acaricides that are currently in use, or as a component of integrated mite management. The present study was focused in examining the effectiveness of entomopathogenic fungus *Beauveria bassiana* (strain Hb) against *T. urticae* on okra crop under field conditions.

2. Materials and Methods

2.1 *Beauveria bassiana* culture: The culture of *B. bassiana* (strain- Hyderabad) was raised on Potato Dextrose Agar media in 250 ml conical flasks for experimental purpose which is a standard procedure. Regular passaging and maintenance was done for further multiplication at 27±2ºC, > 90 % RH and 16: 8 (Light: Dark) photoperiod. Pure mother cultures in slants were stored under refrigerated conditions till further use.

2.2 Preparation of *B. bassiana* suspension: Aqueous conidial suspensions were made from conidia harvested from the slants prepared in conical flasks after 14 days of inoculation. Conidial spores were harvested by scrapping the surface of sporulating cultures and suspended in 100 ml distilled water containing Tween 80 (0.02%) which was used as surfactant to disperse the conidia. After the conidia came into the solution completely, it was filtered through a double layered muslin cloth. Desired concentrations of *B. bassiana* conidia were prepared using haemocytometer counts. Lower conidial concentration was obtained through serial dilution. Freshly prepared conidial suspensions were used for spray.

2.3 Field efficacy: Field trials were conducted during the crop seasons 2010, 2011 and 2014 at Research Farm, Department of Vegetable Science, CCS Haryana Agricultural University, Hisar to evaluate the efficacy of *B. bassiana* against mixed population of *T. urticae*. It was measured in terms of the population build up of the pest as affected by changes in the concentration of formulation and duration of exposure. For the present experiment, natural *T. urticae* infestation was used. *B. bassiana* treatments were compared with commercially available acaricides, Omite/ Ethion (as per availability), considered as standard check. The experimental units comprised of three plots each measuring 10 m × 40 m. The okra plants were grown following standard agronomic practices. The plants were irrigated as and when needed. A randomized block design with three replicates was used for experimentation. During application of fungal suspension, the whole plant was thoroughly covered by spray fluid and care was taken to maintain the distance around 25 cm between the nozzle and plant parts. To judge the potential of particular treatment, five plants in each treatment were selected at random. Observations on the number of *T. urticae* population (mobile stages) per sq. cm leaf were recorded using hand lens from both upper and lower surface of two leaves from each category (tender, grown up and older) per plant. Pre treatment count was done following which the number of mites was counted each day for a week. It was easy to distinguish live mites from dead individuals by observing their movement. The live mites were mobile whereas immobile mites failing to respond with leg movements after being tightly nudged with a fine bird feather pick were considered dead. For assessing the effectiveness of the treatments, mean numbers of *T. urticae* were pooled and analyzed statistically. The percent reduction in mite count as compared to pre- treatment count was calculated by the formula:

$$\text{Percent Reduction} = \frac{\text{(Pre- treatment count – Average number of live mites after treatment)}}{\text{Pre- treatment count}} \times 100$$

Meteorological data was obtained from the Department of Agricultural Meteorology, College of Agriculture, CCS HAU, Hisar.

2.4 Statistical analysis: The statistical significance of data was assessed by one factorial analysis of variance (ANOVA) under RBD using software 'OPSTAT' and Critical Difference (CD) was calculated to know the efficacy of different treatments in reducing *T. urticae* population. Means were then compared using Duncan's multiple range test (P= 0.05).

2.5 Results and Discussion

The results of the present investigation have been presented in Tables 1-3. During 2010, the highest reduction in *T. urticae* population was recorded in Ethion 50 EC (0.05%) treated plots (64.92%) followed by 59.64, 48.23 and 37.92 percent at 0.3×10^9, 0.3×10^8 and 0.3×10^7 conidia ml^{-1} of *B. bassiana*, respectively (Table 1). All the treatments showed significantly less number of mites as compared to controls. *B. bassiana* treatments $0.3\times10^9$and 0.3×10^8 conidia ml^{-1} did not differ significantly with each other. Both the controls were statistically comparable with each other. During 2011, two sprays of *B. bassiana* at the interval of seven days were done for the control of *T. urticae* (Table 2). *T. urticae* population was found significantly lower as compared to control in treated plots with mortality recorded as 65.18, 55.44 and 43.07 percent at 0.3×10^9, 0.3×10^8 and 0.3×10^7 conidia ml^{-1} of *B. bassiana*, respectively. In contrast, *B. bassiana* treatments proved highly ineffective against *T. urticae* on okra during the crop season 2014 (Table 3). Only the commercial acaricide, Omite was capable of providing significant control.

Table 1: Efficacy of *Beauveria bassiana* (strain Hb- Hyderabad) against *Tetranychus urticae* on Okra during 2010

Treatment	Average number of mites/ sq. cm. of leaf after days of treatment								Mean	Reduction after treatment (%)
	Pre – treatment count	1	2	3	4	5	6	7		
0.3×10^9 (conidia ml^{-1})	25.99	17.92	13.56	10.48	9.28	7.60	6.56	5.62	10.41 (40.05)^a	59.64
0.3×10^8 (conidia ml^{-1})	32.88	25.76	22.06	18.66	15.70	13.74	11.96	11.26	17.02 (51.76)^a	48.23
0.3×10^7 (conidia ml^{-1})	18.96	15.62	13.92	12.24	11.32	9.92	9.46	9.90	11.77 (62.07)	37.92
Ethion 50 EC (0.05%)	15.28	10.46	8.98	6.74	4.76	3.28	2.46	1.72	5.48 (35.40)	64.92
Control (water + Tween 80)	9.02	9.02	8.94	8.93	8.92	8.88	8.86	8.82	8.94^b	0.69
Control (No spray)	14.34	14.34	12.96	13.98	15.00	15.52	15.20	15.32	14.85^b	-3.43
Mean (Duration)		15.52	13.40^a	11.84^{a,b}	10.83^{b,c}	9.82^{b,c}	9.08^c	8.77^c		

CD (p= 0.05) for Treatment (T) = 2.06 (12.95); Duration (D) = 2.30; T x D = NS

Figures denoted by same superscript do not differ significantly Figures in parentheses represent percent reduction in mite count as compared to pre- treatment count

Table 2: Efficacy of *Beauveria bassiana* (strain Hb- Hyderabad) against *Tetranychus urticae* on Okra during 2011

Treatment	**1^{st} Spray**								**2^{nd} Spray**							**Mean**	**Reduction after treatment (%)**
	Average number of mites/leaf (sq. cm.) after days of treatment								**Average number of mites/leaf (sq. cm.) after days of treatment**								
	Pre – treatment count	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**		
0.3×10^{9} (conidia ml^{-1})	16.37	11.26	7.11	6.23	5.45	6.75	7.01	7.31	5.33	4.2	4.12	3.96	3.82	3.78	3.65	5.70 $(34.82)^{a}$	65.18
0.3×10^{8} (conidia ml^{-1})	24.44	18.22	15.43	13.12	10.11	10.32	12.26	13.31	8.95	7.57	6.29	5.76	5.68	5.54	5.43	10.89 $(44.55)^{a}$	55.44
0.3×10^{7} (conidia ml^{-1})	24.31	20.66	16.35	14.23	17.22	18.13	20.15	23.25	11.21	10.4	9.23	7.24	6.25	5.23	5.12	13.84 $(56.93)^{a}$	43.07
Ethion 50 EC (0.05%)	15.09	11.26	9.3	6.99	5.45	7.62	11.82	12.09	7.26	5.1	3.99	3.99	3.62	2.32	2.46	7.28 $(48.24)^{a}$	51.82
Control (water + Tween 80)	11.3	10.5	10.32	10.11	9.26	10.69	12.34	12.94	10.58	9.53	9.48	19.25	9.02	9.1	9.12	11.03	2.38
Control (No spray)	13.5	14.54	15.92	16.94	18.19	20.54	21.52	23.6	24.82	26.34	28.32	29.65	31.17	32.54	33.45	22.68	-55.98
Mean (Duration)		14.40	$12.40^{a,c}$	$11.27^{a,c}$	$10.98^{a,c}$	$12.13^{a,c}$	$14.18^{a,b}$	15.24^{b}	$11.35^{c,d}$	$10.52^{c,d}$	$10.23^{c,d}$	$11.64^{c,d}$	9.92^{d}	9.75^{d}	9.87^{d}		

CD (p= 0.05) for Treatment (T) = 2.49(24.11); Duration (D) = 2.19; T x D = 6.59
Figures denoted by same superscript do not differ significantly with each other
Figures in parentheses represent percent reduction in mite count as compared to pre- treatment count

Table 3: Efficacy of *Beauveria bassiana* (strain Hb) against *Tetranychus urticae* on Okra during 2014

Treatments	**Number of mites/ sq. cm leaf**									**Reduction after treatment (%)**
	Pre treatment count	**1 DAS**	**2 DAS**	**3 DAS**	**4 DAS**	**5 DAS**	**6 DAS**	**7 DAS**	**Mean (Treatment)**	
(*B. bassiana* - 10^{9} conidia/ ml)	3.82	3.71	3.48	3.54	3.55	3.67	4.75	4.88	$3.95^{a,c}$	-27.74
(*B. bassiana* -10^{10} conidia/ ml)	3.84	3.81	3.60	3.83	3.84	4.16	4.34	4.86	$4.05^{a,b}$	-26.57
(Omite 57 EC - 0.05%)	3.26	3.52	1.83	0.88	0.36	0.17	0.17	0.15	1.01	95.39
Control (Water +Tween80)	3.61	3.54	3.25	3.21	3.56	3.74	4.92	4.98	3.89^{c}	-40.67
Control (Untreated)	3.16	3.44	3.74	3.75	4.20	4.36	4.37	5.17	4.14^{b}	-63.61
Mean (Duration)		3.62^{c}	3.18^{b}	3.04^{a}	$3.10^{a,b}$	$3.16^{a,b}$	3.71^{c}	4.01		

CD (P= 0.05) for Treatment (T) =0.11; Duration (D) = 0.13; T x D = 0.28
Values with the same superscript do not differ significantly
Percent reduction in mite population is calculated over Pre treatment count

Relatively few field trials have been undertaken to evaluate entomopathogenic fungi against phytophagous mites. In the present study, *B. bassiana* proved to be an effective acaricide during the crop seasons 2010 and 2011. In conformity with our findings, spray applications of *B. bassiana* were reported to reduce *T. urticae* population in tomato [12], chrysanthemum [13], beans [14], eggplants [12] and cucumber [15]. However, during crop season 2014 no significant mortality in *T. urticae* population was recorded subjected to *B. bassiana* treatments. This variation may be explained by the fact that the environmental conditions for *B. bassiana* conidia germination and host mycosis were not suitable in the field. The year was recorded as dry monsoon period with high temperature and little rainfall during or around the study period (season 2014). Our observations were supported by the studies conducted by James *et al.* [16] who reported that difference in environmental conditions between the laboratory and greenhouse had a large effect on mycosis. Constant temperatures between 15 and 35°C had a significant effect on both germination rate and vegetative growth of *B. bassiana*, with the fastest germination occurring at 25-32°C and the fastest growth occurring at 30°C. They also suggested that temperature and humidity also affect physiological interactions between the host and pathogen. High temperature and low relative humidity during the crop season 2014 could thus be held responsible for the failure of fungus-induced mortality in *T. urticae* in our investigation as the meteorological data was recorded as average maximum temperature 39.4ºC, average minimum temperature 25.3ºC, average relative humidity 58% (morning) and 33% (evening) during the period when fungal sprays were conducted. Fargues and Luz [17] studied the effect of both moisture and temperature on the infective potential of *B. bassiana* to the Chaga's disease vector, *Rhodnius prolixus* and reported that daily high humidity as the most crucial climatic constraint for the entomopathogen potential to control *R. prolixus* population. Walstad *et al.* [18] investigated the principal environmental factors affecting the efficacy of the white and green muscardine fungi *B. bassiana* and *Metarhizium anisopliae* as biocontrol agents for pales weevil, *Hylobius pales*. As per their reports, the fungi required relative humidity above 92.5% and temperature between 15 to 35ºC for spore germination, mycelial growth and sporulation. Host death caused by these fungi normally occurs at night, when relative humidity is high, favorable for sporulation. At higher temperatures, the rate of pathogen germination and growth is faster but the arthropods may be able to resist infection because of increased metabolism rate or decreased stadium period leading to more rapid shedding of the cuticle where the fungus attaches and invades [16].

Entomopathogens are well characterized in respect to pathogenicity to several insects and have been used as myco-biocontrol agents for biological control of agricultural pests worldwide [8]. Use of fungi as bio-pesticides is considered an attractive strategy in inundation of biological control of mites/insects, where the fungal inoculums are applied directly to the crop or the target pest and control is achieved exclusively by the released propagules themselves [9]. Bugeme *et al*. [10] evaluated twenty-three isolates of *M. anisopliae* (Metschnikoff) Sokorin and three isolates of *B. bassiana* for their virulence against the two-spotted spider mite, *T. urtica*e Koch. At 1.0 ×10^7 conidia ml^{-1} concentration, the percent mortality varied from 95.2 to 99 and 36.5 to 100 with *B. bassiana* and *M. anisopliae*, respectively. LT_{50} values from 4.1 to 4.6 days were observed with *B. bassiana* and 2.6 to 8.2 days with *M. anisopliae.* The efficacy *B. bassiana* was tested by Gatarayiha *et al.* [11] against *T. urticae* on *Phaseolus vulgaris* under laboratory and greenhouse. The ovicidal action with 2. ×10^8 conidia ml^{-1} concentration 20.1 to 55.5 percent reduction in hatchability of eggs was noticed. In other stages of mite 66.5 to 95.6 percent and 3.7 to 85.7 percent mortality was recorded under laboratory and greenhouse, respectively.

Based on our results, it can be inferred that *B. bassiana* needs particular micro environmental conditions, such as moderate temperature and high humidity for being used as myco pesticide. The dry weather may be a crucial constraint against such otherwise efficient myco acaricides. The most difficult problem facing *T. urticae* control with entomopathogenic fungi is the occurrence of this mite in dry and hot environments [19], which are unfavourable for the development of the fungi. It may be thus stated that the ability of *B. bassiana* propagules to persist in an environment is an important factor in its success as a biological control agent (BCA) which is highly dependent on moisture conditions.

3. Conclusion

Entomopathogenic fungi can play an important role in IPM if used in conjunction with other strategies for sustainable pest control. To achieve this, the compatibility of the mycoacaricides with other mite biocontrol agents, especially phytoseiidae mites, as well as with synthetic pesticides, needs to be investigated. The further development of entomopathogenic fungi as control agents for spider mites needs considerable investment in multidisciplinary research by the public and private sectors. When commercial interests are absent, as in the development of classical biological control and conservation strategies, especially in developing countries, long-term government support is essential.

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