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RESEARCH **P**APER

Bioefficacy of mycopathogens *Verticillium lecanii* Zimmermen and *Metarhizium anisopliae* Metchnikoff against sucking pests of Bt cotton

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Entomopathogenic fungi *Verticillium lecanii* Zimmermen and *Metarhizium anisopliae* Metchnikoff have generated a great deal of interest in recent years because of their potential as bio-control component in integrated pest management of cotton. These two mycopathogens were evaluated against sucking pests of Bt cotton during *Kharif* 2011 and 2012 at Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri to find out the scope and potentiality of *V.lecanii* and *M.anisopliae* against sucking pests of Bt cotton in intra hirsutum Bt hybrid (RCH- 2Bt). Mycopathogens *V.lecanii* (2×10^8 cfu/gm and 2×10^{12}cfu/g) and *M.anisopliae* @ 2000 g/hectare were evaluated alone and in combination (*V.lecanii* + *M.anisopliae*). The observations were recorded on 3,5 and 10 days interval after each spray application. Pooled analysis of two years data revealed that *V.lecanii* (2×10^{12}cfu/g) @ 2000 g/hectare + *M.anisopliae* (2×10^{12}cfu/g) @ 2000 g/ha in combination was found more effective and significantly superior to its lower dose *V.lecanii* (2×10^8 cfu/g)@ 2000 g/ha + *M.anisopliae* (2×10^8 cfu/g) @ 2000 g/ha. It was proved superior over all other treatments in reducing aphids, jassids, thrips and whitefly population, however, it was at par with *V.lecanii* (2×10^{12}cfu/g) @ 2000 g/ha. Thus, *V.lecanii* (2×10^{12}cfu/g) @ 2000 g/ha was found to be the effective dose against all major sucking pests of cotton. Investigation on evaluation of entomopathogenic fungi against natural enemies of sucking pests in Bt cotton revealed that there was no significant adverse impact of *V. lecanii* and *M. anisopliae* on the activity of natural enemies *viz*., Chrysoperla and Coccinellids; when compared with the population of natural enemies in control. Sole and combine application of *V. lecanii* and *M.anisopliae* at all the evaluated doses were not found toxic to natural enemies.

Key words : Bt cotton, *Kharif*, Sucking pests, Mycopathogen, Entomopathogenic fungi *Verticillium lecanii*, *Metarhizium anisopliae*

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INTRODUCTION

Cotton is the most important commercial crop playing a significant role in Indian Economy. Though India has largest acrage of cotton in the world, productivity is low because of higher incidence of insect pests at all the stages of crop. Besides this, more than 90 per cent area is under Bt cotton and Bt cotton is highly susceptible to sucking pests. The problem of sap sucking pests has become more serious from seedling stage thereby resulting considerable reduction in yield. A reduction of 22.85 per cent seed cotton yield due to sucking pests has been reported by Satpute *et al*. (1990). The pest problem in cotton is so intricate that about 56 per cent of total insecticides are consumed in India by cotton; while area under this crop is only 5 per cent of the total cultivated area (Balasubramanian, 1985 and Pray *et al.*, 2001).

The repeated use of synthetic chemical insecticides as crop protectants against sucking pests has posed serious hazards for humans and the environment, caused deleterious effects to natural enemies and led to résistance in pests to insecticides (Perry *et al.*, 1998). The recent trends in pest management emphasis on non-chemical approaches and there is growing demand worldwide for organically grown fibre, which is increasing annually in export markets. As synthetic insecticides cannot be used in organic crop production systems, organic growers need alternative tools for controlling devastating pests in crops (Yussefi and Willer, 2003). Therefore, various attempts have been made to find out new insecticides derived from fungi, with preferably reduced human toxicity and less persistence in the environment (Ahmed *et al.*, 1984; Isman *et al.*, 2001). Entomopathogenic fungi are one group of alternative pest control agents that are generally more biodegradable and less toxic to humans and natural enemies (Isman, 1984).

Keeping in view, the present studies was undertaken to find out the scope and potentiality of *V. lecanii* and *M. anisopliae* against sucking pests of Bt cotton and to develop easily available effective alternatives to synthetic pesticides against cotton pests.

RESEARCH METHODOLOGY

The present study was carried out at Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri to screen the efficiency of mycoinsecticides (*V. lecanii* and *M. anisopliae*) for the control of sucking pests of Bt cotton.

The field experiment was laid out during *Kharif* 2011 and 2012 in a Randomized Block Design (RBD) with seven treatments including control, each replicated thrice. The plot size was 7.2 x 5.4 m^2 with 90 x 90 cm row to row and plant to plant distance, respectively. Treatment details are given in Table A.

Table A : Treatment details

Sr. No.	Treatments	cfu/g	Formulation dose (g/ha)
T_1	*Verticillium lecanii*	2 x 10^8	2000
T_2	*Verticillium lecanii*	2 x 10^{12}	2000
T_3	*Metarhizium anisopliae*	2 x 10^8	2000
T_4	*Metarhizium anisopliae*	2 x 10^{12}	2000
T_5	*V.lecanii + M.anisopliae*	2x10^8 + 2x10^8	2000 +2000
T_6	*V.lecanii + M.anisopliae*	2x10^{12} + 2x10^{12}	2000 +2000
T_7	Untreated control	----	-----

The crop was sown in the first week of June during both the years. Fertilizers and other cultural practices were followed as per the recommendations in the package of practices. The mycoinsecticides *viz.*, *Verticillium lecanii* and *Metarhizium anisopliae* each and in combination *V.lecanii + M.anisopliae* were evaluated. Based on economic threshold level, three sprays of these mycoinsecticides were given initiating the first at 50 days after sowing. The data on sucking pest population were recorded from randomly selected five tagged plants per plot. Pre-treatment count was taken just before the application of mycoinsecticides and post treatment count after 3, 5 and 10 days of application of mycoinsecticides. The values were then transformed to square root transformation for number. At harvest, seed cotton yield was recorded in kg/plot and then transformed to quintal/ha. The data were subjected to statistical analysis for interpretation.

Besides this, the observations on number of natural enemies *viz.*, *Coccinellids* and *Chrysoperla* were recorded on ten randomly selected plants in each plot. In case of phytotoxicity, the observations were recorded at 1, 3, 5, 7 and 10 days after spray on leaf injury on tips and leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty on 10 randomly selected plants. The total number of leaves and those showing phytotoxicity, if any, were counted and data converted into percentage and extent of phytotoxicity were recorded on the basis of score (Table 3).

RESEARCH FINDINGS AND ANALYSIS

It is revealed from Table 1 (Pooled 2011 to 2012) that all the mycoinsecticide treatments were significantly superior over untreated control. Among the evaluated mycoinsecticides, the treatment with *V.lecanii* (2 x 10^{12}cfu/g) @ 2000 g/ha + *M.anisopliae* (2 x 10^{12}cfu/g) @ 2000 g/ha in combination was most effective against sucking pests of Bt cotton by recording 21.42, 7.54, 12.81 and 9.98 aphids, jassids, thrips and whiteflies per three leaves/plant, respectively. This treatment was statistically at par with *V.lecanii* (2 x 10^{12}cfu/g) @ 2000 g/ha, in which aphids, jassids, thrips and whiteflies of 23.08, 9.78, 14.76 and 12.20 per three leaves/plant, respectively. This was followed by treatment with *V.lecanii* (2 x 10^8cfu/g) @ 2000 g/ha + *M.anisopliae* (2 x 10^8cfu/g) @ 2000 gm/ha, *V.lecanii* (2 x 10^8cfu/g) @ 2000 g/ha and *M.anisopliae* (2 x 10^8cfu/g) @ 2000 g/ha. The treatment

Table 1 : Bioefficacy of mycopathogens against major pests on Bt cotton (Pooled 2011 to 2012)

Sr. No.	Treatments	cfu/g	Formulation dose (g/ha)	Average sucking pest population/3 leaves				Predators/ 10 plants		Yield (q/ha)
				Aphids	Jassids	Thrips	Whitefly	*Coccinellids*	*Chrysoperla*	
T_1	*Verticillium*	2×10^8	2000	28.49	12.24	19.58	16.02	12.93	10.73	14.67
	lecanii			(5.38) *	(3.57)	(4.48)	(4.06)	(3.66)	(3.35)	
T_2	*Verticillium*	2×10^{12}	2000	23.08	9.78	14.76	12.20	12.73	10.01	16.49
	lecanii			(4.86)	(3.21)	(3.91)	(3.56)	(3.64)	(3.24)	
T_3	*Metarhizium*	2×10^8	2000	30.39	12.59	21.88	11.95	12.47	9.27	14.29
	anisopliae			(5.56)	(3.62)	(4.73)	(3.53)	(3.60)	(3.13)	
T_4	*Metarhizium*	2×10^{12}	2000	26.71	11.19	18.26	10.64	11.93	8.27	15.04
	anisopliae			(5.22)	(3.42)	(4.33)	(3.34)	(3.53)	(2.96)	
T_5	*V.lecanii* +	$2 \times 10^8 + 2 \times 10^8$	2000 +2000	25.46	10.65	17.38	11.42	8.33	5.40	15.38
	M.anisopliae			(5.10)	(3.34)	(4.23)	(3.45)	(2.97)	(2.43)	
T_6	*V.lecanii* +	$2 \times 10^{12} + 2 \times 10^{12}$	2000 +2000	21.42	7.54	12.81	9.98	10.73	7.07	17.82
	M.anisopliae			(4.68)	(2.84)	(3.65)	(3.24)	(3.35)	(2.75)	
T_7	Untreated	----	----	50.35	19.24	33.38	24.64	14.33	10.47	9.47
	control			(7.13)	(4.44)	(5.82)	(5.01)	(3.85)	(3.31)	
		S.E. ±	----	0.46	0.68	0.52	0.57	0.03	0.03	0.49
		C.D. (P=0.05)	----	1.42	2.02	1.59	1.76	0.08	0.10	1.48
		CV %	-----	10.28	12.64	10.98	9.85	11.31	11.82	11.74

*(Figures in parenthesis are $\sqrt{x+0.5}$ transformed values for numbers)

with *V.lecanii* (2×10^8cfu/g) @ 2000 g/ha recorded 28.49, 12.24, 19.58 and 16.02 aphids, jassids, thrips and whiteflies/3 leaves, respectively. The untreated control recorded higher population of sucking pests *viz.*, aphids (50.35), jassids (19.24), thrips (33.38) and whitefly (24.64) per three leaves/plant, respectively. Pooled analysis of two years data revealed that *V.lecanii* (2×10^{12}cfu/g) @ 2000 g/ha + *M.anisopliae* (2×10^{12}cfu/g) @ 2000 g/ha was found more effective and significantly superior to its lower dose of *V.lecanii* (2×10^8 cfu/g) @ 2000 g/ha + *M.anisopliae* (2×10^8 cfu/g) @ 2000 g/ha. It was proved to be superior over all other treatments in reducing aphids, jassids, thrips and whitefly population; however, it was at par with *V.lecanii* (2×10^{12} cfu/g) @ 2000 g/ha. Thus, *V.lecanii* (2×10^8 cfu/g) @ 2000 g/ha was found to be the effective dose against all major sucking pests of cotton. These results are in agreement with those of Rachappa *et al.* (2004) who reported that *V.lecanii* and *M.anisopliae* (2×10^{12} cfu/g) @ 2000 g/ha alone and in combination was found effective against sucking pests of cotton.

The results showed that seed cotton yield (Table 1) under all the treatments were significantly superior over control. The highest seed cotton yield of 17.82 q/ha was obtained from the plots treated with *V.lecanii* (2×10^{12}cfu/g) @ 2000 g/ha + *M.anisopliae* (2×10^{12}cfu/g) @ 2000 g/ha followed by *V.lecanii* (2×10^{12}cfu/g) @ 2000 g/ha and *V.lecanii* (2×10^8 cfu/g) @ 2000 g/ha + *M.anisopliae* (2×10^8 cfu/g) @ 2000 g/ha which gave 16.49 and 15.38 q/ha, respectively. The counts of *Coccinellids* and *Chrysoperla* in different treatments presented in Table 1, clearly indicated that the counts of natural enemies were more or less similar to those recorded in untreated control. This indicates that there was no adverse effect of these mycoinsecticides on natural enemies at evaluated doses. The studies on phytotoxicity effects of these botanicals (Table 3) revealed that none of the mycoinsecticides treatment showed phytotoxic symptoms like leaf injury on tips and leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty on cotton crop

Table 2 : Rating criteria for phytotoxicity symptoms

Score	Per cent crop health affected
0	No adverse effect
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

Table 3 : Phytotoxicity effect of mycopathogens on leaf injury on tips and leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty

Sr. No.	Treatments	cfu/g	Formulation dose (g/ha)	Observations before and after spray					
				Before spray	1 DAS*	3 DAS	5 DAS	7 DAS	10 DAS
T_1	*Verticillium lecanii*	2×10^8	2000	0	0	0	0	0	0
T_2	*Verticillium lecanii*	2×10^{12}	2000	0	0	0	0	0	0
T_3	*M. anisopliae*	2×10^8	2000	0	0	0	0	0	0
T_4	*M. anisopliae*	2×10^{12}	2000	0	0	0	0	0	0
T_5	*V.lecanii + M.anisopliae*	$2 \times 10^8 + 2 \times 10^8$	2000 +2000	0	0	0	0	0	0
T_6	*V.lecanii+ M.anisopliae*	$2 \times 10^{12} + 2 \times 10^{12}$	2000 +2000	0	0	0	0	0	0
T_7	Untreated control		----	0	0	0	0	0	0

*DAS= days after spraying

at evaluated doses. These results are in accordance with the findings of Gopalkrishnan and Narayanan (1998) who reported that increase in yield in different mycoinsecticide treatment over control against pests of cotton. The effectiveness of *M.anisopliae* under laboratory condition was reported by Nahar *et al.* (2004). Singha *et al.* (2006) evaluated *M.anisopliae* and *Beauveria bassiana* for potential use as biocontrol agents against termites and found effective under laboratory conditions. The insecticidal activity of *M.anisopliae* and *Beauveria bassiana* has been reported in tobacco worm, *M.sexta* and desert locust, *S.gregaria* (Kershaw *et al.,* 1999). The overall results on bioefficacy of mycoinsecticides revealed that the mycoinsecticides *V.lecanii* and *M.anisopliae* were proved to be effective against sucking pests of cotton and therefore, can be harmoniously incorporated in the development of integrated pest management modules for these pests.

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