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Residual effect of Mulberry Powdery mildew suppressive bio agents on growth and development of Silkworm (*Bombyx mori* L.), cocoon and silk quality

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Study on assessing the residual toxic effect of fungal and bacterial bio agents used against powdery mildew of mulberry on silkworms was conducted. The results of the toxicological studies on growth, development and cocoon parameters revealed that, *Trichoderma harzianum* had positive effect on both larval weight and its length with increase of 21.46 g and 5.18 cm respectively. Similarly, cocoon, pupal and shell weight of 14.99g, 11.86 g and 3.17 g respectively, shell ratio of 21.17 per cent and cocoon yield of 524.66 g/dfl was higher compared to other treatments. The filament length of 769.05 m, filament weight of 0.16 g and denier of 1.80 per cent fineness were found to be superior in *Trichoderma harzianum* treated mulberry leaves fed to silkworm. The present investigation clearly revealed that mulberry powdery mildew effective bio agents, *Trichoderma harzianum and Trichoderma viridae* at 15 per cent concentration, *Bacillus subtilis* and *Pseudomonas fluorescens* at 10, 15 per cent concentration were found to be safe to silkworms when leaves fed to them three days after treatment with culture filtrate of bio agents.

Key words: Powdery mildew, *Trichoderma harzianum, Bacillus subtilis, Pseudomonas fluorescens* , toxicity, cocoon, denier

INTRODUCTION

Mulberry (*Morus alba*) falls under the category of perennial crops belong to the family Moraceae. Mulberry foliage is the only food for the silkworm (*Bombyx mori* L.) and is grown under varied climatic conditions ranging from temperate to tropical. Mulberry leaf is a major economic component in sericulture since the quality and quantity of leaf produced per unit area have a direct bearing on cocoon harvest. In India, most states have taken up sericulture as an important agro-industry with excellent results. The total area of mulberry cultivation in India is around 2,82,244 ha and in Karnataka it is 1,66,000 ha. (Dutta, 2011). Leaf yield and quality is drastically reduced by powdery mildew disease caused by an obligate biotrophic ascomycete fungus, *Phyllactinia corylea* throughout the world (Chattopadhyay *et al.* 2011). The disease is characterized by white dust-like mycelia that develop over abaxial (lower) leaf surfaces. The heavily infected tissues develop chlorosis on the adaxial (upper) surface of leaves and senescence prematurely (Gupta, 2001). The disease is predominantly managed by spraying of systemic fungicides at different intervals. Fungicidal sprays residues considerably affect the silkworm health, cocoon quality and weight. To overcome such problems, an alternate method of control of disease needs to be explored. The bio agents can be used as an alternative strategy to manage the mulberry powdery mildew as it considered safe and eco friendly. Since the work on the residual toxicity of bio agents on silkworms is not done before, a first of its kind investigation was undertaken to study the same.

MATERIALS AND METHODS

An experiment was conducted during 2016 at College of Agriculture, V.C. Farm, Mandya to study the toxic effect of bio control agents which were found effective in the management of mulberry powdery mildew disease, on silkworms.

Preparation of culture filtrate of bio agents

The cultures of fungal bio control agents *Tricho-*

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derma viride and *Trichoderma harzianum* and bacterial bio agents *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from UASB, GKVK, Bengaluru. The fungal and bacterial bio agents were multiplied on potato dextrose and nutrient broth respectively contained in 2 litre capacity flat bottom round flasks incubated at room temperature by regular shaking. Ten day old fungal bio agent and two days old bacterial bio agent cultures from the broth were filtered through Whatman no.1 filter paper. The filtrate obtained served as a stock solution of 100 per cent concentration and were further diluted to 5, 10 and 15 percent concentrations by adding sterilized distilled water.

The mulberry leaves were immersed in respective concentration of bio-agent solutions for 2-3 minutes to absorb the solution uniformly. Later, the treated leaves were shade dried for 30 seconds and were fed once to 3rd instar, 1st day silkworm larvae. After treatment imposition, fresh untreated leaves were offered until final day of 5th instar. Control treatment was maintained by using the leaves sprayed with water. Treatments were replicated thrice by using twenty worms with the statistical design of CRD (Fisher and Yates, 1967).

RESULTS AND DISCUSSION

Residual effect mulberry suppressive bioagents on silkworm

The results from the present study revealed no larval mortality when silkworms were fed with leaves one day after treatment with *Trichoderma harzianum* even at higher concentration (15%) indicating it non toxic nature. It was followed by *Trichoderma viridae* (10 %), *Pseudomonas fluorescens* (13.6%) and *Bacillus subtilis* (14.5%) when fed to 3rd instar 1st day larva. The larval mortality was completely absent in all the treatments when 3rd instar larvae was fed with leaves 3 days after bioagent application (Table 1).

The growth parameters of silkworm viz: larval weight and larval length were recorded at 5th instar and it was found that the highest larval weight of 21.46 g and larval length of 5.18 cm was recorded in *Trichoderma harzianum* treated leaves fed to silkworm which was higher than untreated control treatment (18.41g and 5.02cm) and it was followed by *Trichoderma viridae* (18.15g and 4.32cm) treated leaves fed to silkworm at 15% concentration. At same concentration, least larval weight (15.09 g) and larval length (4.14 cm) was observed in *B. subtilis* treatment (Table 2).

Residual effect mulberry suppressive bioagents on cocoon and silk parameter

The cocoon weight, pupal weight, shell weight, shell ratio and cocoon yield was recorded and found that the maximum cocoon weight (14.99g) was recorded in *Trichoderma harzianum* at 15 per cent concentration and untreated control followed by (13.86g) with *Trichoderma viridae* at 15 per cent, *Pseudomonas fluorescens* at 10 per cent concentration (Table 3). The highest pupal weight was recorded in control treatment (11.96g), *Trichoderma harzianum* (11.86g) and *Trichoderma viridae* (11.81g) at 15 per cent concentration, *Pseudomonas fluorescens* (11.19g) and *Bacillus subtilis* (11.15g) at 10 per cent concentration. The shell weight was maximum (3.17g) in case of *Trichoderma harzianum* followed by control treatment (2.99g) and *Trichoderma viridae* (2.78g). The shell ratio was highest (21.17%) in *Trichoderma harzianum* at 15 per cent concentration followed by *Trichoderma viridae* with (20.05%) at 15 per cent concentration and the next best was recorded on *Bacillus subtilis* at 10 per cent concentration with (19.43%) after considering that the *Trichoderma harzianum* (19.52%) is best at 10 per cent concentration. Maximum cocoon yield (524.66g/dfl) was recorded in silkworm larva fed with *Trichoderma harzianum* at 15 per cent concentration administered mulberry leaves and water immersed leaves which indicate that there was no toxic effect by these two treatments on silkworm and also on cocoon parameters during larval period.

The filament length was found to be highest in control treatment (786.18m) followed by *Trichoderma harzianum* (769.05m) and *Trichoderma viridae* (603.68m) at 15 per cent concentration. The filament weight was maximum (0.16g) in case of *Trichoderma harzianum* at 15 per cent concentration which is in par with control treatment. The finer denier (1.80%) was observed in *Trichoderma harzianum* at 15 per cent concentration followed by control treatment (1.83%) (Table 4). Since, the reports on the effects of *Trichoderma harzianum*, *Trichoderma viridae*, *Bacillus subtilis* and *Pseudomonas fluorescens* filtrates supplemented with mulberry leaves and fed to silkworms not available for comparison. Hence, the reports on other

Table 1: Effect of biocontrol agents on larval mortality of silkworm (*Bombyx mori* L.)

Treat-ment	Bio control agents	Conc. (%)	Larval mortality (%)		
			1 DAT	2 DAT	3 DAT
T_1	*Trichoderma viridae*	5	4.80 (12.65)	0.00 (0.00)	0.00 (0.00)
T_2	*Trichoderma viridae*	10	9.60 (18.05)	3.32 (10.50)	0.00 (0.00)
T_3	*Trichoderma viridae*	15	10.00 (18.43)	5.36 (13.38)	0.00 (0.00)
T_4	*Trichoderma harzianum*	5	0.00(0.00)	0.00(0.00)	0.00 (0.00)
T_5	*Trichoderma harzianum*	10	0.00 (0.00)	0.00(0.00)	0.00 (0.00)
T_6	*Trichoderma harzianum*	15	0.00 (0.00)	0.00(0.00)	0.00 (0.00)
T_7	*Pseudomonas fluorescens*	5	9.10 (17.56)	4.92 (12.81)	0.00 (0.00)
T_8	*Pseudomonas fluorescens*	10	10.00 (18.43)	5.42 (13.46)	0.00 (0.00)
T_9	*Pseudomonas fluorescens*	15	13.6 (21.64)	9.59 (18.04)	0.00 (0.00)
T_{10}	*Bacillus subtilis*	5	5.00 (12.92)	4.37(12.06)	0.00 (0.00)
T_{11}	*Bacillus subtilis*	10	10.30 (18.73)	4.78(12.63)	0.00 (0.00)
T_{12}	*Bacillus subtilis*	15	14.50(22.38)	5.71 (13.82)	0.00 (0.00)
T_{13}	Control (Water spray)	-	0.00 (0.00)	0.00(0.00)	0.00(0.00)
		S.Em±	0.23	0.11	-
		CD @ P=0.05	0.67	0.31	-

DAT= Days after treatment; Tr = Treatment; Figures in the parenthesis are arc sine transformed values

Table 2: Effect of biocontrol agents on larval weight of silkworm (*Bombyx mori* L.)

Treat-ment	Bio control agents	Conc. (%)	Average larval weight (g) at 5th instar			Average larval length (cm) at 5th instar		
			5th day	6th day	7th day	5th day	6th day	7th day
T_1	*Trichoderma viridae*	5	10.84	14.04	15.56	3.77	4.15	4.19
T_2	*Trichoderma viridae*	10	10.49	14.26	17.67	3.97	4.19	4.20
T_3	*Trichoderma viridae*	15	11.91	14.57	18.15	4.11	4.30	4.32
T_4	*Trichoderma harzianum*	5	13.04	14.59	16.90	3.97	4.26	4.31
T_5	*Trichoderma harzianum*	10	13.18	16.45	19.00	4.17	4.36	4.49
T_6	*Trichoderma harzianum*	15	14.64	16.75	21.46	4.38	4.71	5.18
T_7	*Pseudomonas fluorescens*	5	11.44	12.84	15.47	3.90	4.13	4.19
T_8	*Pseudomonas fluorescens*	10	11.91	14.57	18.15	3.77	4.19	4.23
T_9	*Pseudomonas fluorescens*	15	10.38	13.89	17.67	4.04	4.28	4.30
T_{10}	*Bacillus subtilis*	5	10.46	13.87	17.10	3.81	4.10	4.24
T_{11}	*Bacillus subtilis*	10	10.62	12.48	17.37	4.06	4.18	4.27
T_{12}	*Bacillus subtilis*	15	10.07	12.25	15.01	3.75	4.09	4.14
T_{13}	Control (Water spray)	-	11.77	15.03	18.41	4.40	4.73	5.02
	S.Em±		0.46	0.58	0.56	0.16	0.11	0.10
	CD @ P=0.05		1.33	1.67	1.63	0.45	0.33	0.30

Table 3: Effect of biocontrol agents on cocoon parameters of silkworm (*Bombyx mori* L.)

Treat-ment	Bio control agents	Conc. (%)	Average Pupal weight (g)	Average cocoon weight(g)	Average shell weight(g)	Average shell ratio (%)	Cocoon yield (g)/1dfl
T_1	*Trichoderma viridae*	5	10.24	12.66	2.42	17.94 (25.06)	443.43
T_2	*Trichoderma viridae*	10	10.70	13.27	2.56	19.29 (26.05)	464.45
T_3	*Trichoderma viridae*	15	11.81	13.86	2.78	20.05 (26.60)	485.10
T_4	*Trichoderma harzianum*	5	11.25	13.76	2.51	18.24 (25.28)	481.60
T_5	*Trichoderma harzianum*	10	11.54	14.34	2.80	19.52 (26.22)	501.90
T_6	*Trichoderma harzianum*	15	11.86	14.99	3.17	21.17 (27.39)	524.66
T_7	*Pseudomonas fluorescens*	5	10.20	12.49	2.29	18.33 (25.35)	437.15
T_8	*Pseudomonas fluorescens*	10	11.19	13.86	2.61	18.83 (25.74)	485.10
T_9	*Pseudomonas fluorescens*	15	10.37	13.67	2.53	18.50 (25.52)	478.45
T_{10}	*Bacillus subtilis*	5	10.09	12.29	2.20	17.90 (25.03)	428.72
T_{11}	*Bacillus subtilis*	10	11.15	13.84	2.69	19.43 (26.15)	484.40
T_{12}	*Bacillus subtilis*	15	10.83	12.82	2.31	18.01 (25.11)	448.40
T_{13}	Control (Water spray)	-	11.96	14.99	2.99	19.94 (26.52)	524.66
	S.Em±		0.30	0.36	0.08	0.59	0.36
	CD @ P=0.05		0.88	1.03	0.23	1.73	1.05

dfl= disease free laying; Figures in the parenthesis are arc sine transformed values

Table 4. Effect of biocontrol agents on silk quality traits of silkworm (*Bombyx mori* L.)

Treat-ment	Bio control agents	Conc. (%)	Average filament length (m)	Average filament weight (g)	Denier (%)
T_1	*Trichoderma viridae*	5	581.18	0.13	2.01
T_2	*Trichoderma viridae*	10	584.10	0.13	2.00
T_3	*Trichoderma viridae*	15	603.68	0.13	1.93
T_4	*Trichoderma harzianum*	5	601.65	0.14	2.09
T_5	*Trichoderma harzianum*	10	724.06	0.16	1.98
T_6	*Trichoderma harzianum*	15	769.05	0.16	1.80
T_7	*Pseudomonas fluorescens*	5	457.88	0.12	2.35
T_8	*Pseudomonas fluorescens*	10	460.58	0.12	2.34
T_9	*Pseudomonas fluorescens*	15	525.60	0.14	2.39
T_{10}	*Bacillus subtilis*	5	570.38	0.13	2.04
T_{11}	*Bacillus subtilis*	10	572.38	0.13	2.04
T_{12}	*Bacillus subtilis*	15	580.50	0.14	2.17
T_{13}	Control (Water spray)	-	786.18	0.16	1.83
	S.Em±		15.38	0.01	0.13
	CD @ P=0.05		44.71	0.03	0.37

bio control agents were used for comparison. Kumar *et al.* (2009) reported that the effect of blue green algae (*Spirulina*) on silkworm gave positive results on both cocoon and silk characters. i.e., cocoon weight, shell weight, pupal weight, shell percentage and silk filament length. Stanley Raja and Kumar (2016) also reported the same results when they supplemented silkworm diet with algal extracts of *Chlorella* and *Scenedesmus* at different concentrations to improve growth and economic traits. The dual inoculation of *Glomus mosseae* (VAM) and bacterial bio fertilizers proved beneficial and improved the leaf quality, silkworm growth and cocoon characters (Rama Rao *et al.* 2007). Ranjitha and Savithri (2015) observed significant reduction of matured larval weight (2.08 g), cocoon weight (0.72 g), shell weight (0.09 g), shell ratio (12.80%), filament length (478.9 m), non-breakable filament length (120.8 m) and more number of breaks (4.1) and higher denier (2.62 d) in silkworms infected with *Beauveria bassiana*. The laboratory result indicated that a small number of conidia of *Metarhizium anisopliae* (Metsch.) Sorokin caused mortality and was found hazardous to silkworms (Pokhrel *et al.* 2014).

The present study clearly reveals the efficacy of *Trichoderma harzianum* and *Trichoderma viridae* at 15 per cent concentration, *Bacillus subtilis* and *Pseudomonas fluorescens* at 10, 15 per cent concentration are good in reducing powdery mildew disease of mulberry. Further it resulted in significant increase the larval growth and cocoon yield as compared to unsprayed leaves.

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