

Original Research Article

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In vitro Evaluation of *Trichoderma* Species for the Biological Control of *Beauveria bassiana* (Balls) Vuill Causing White Muscardine Disease of Silk Worm, *Bombyx mori* L. under Temperate Conditions

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ABSTRACT

Antagonistic potentiality of four isolates of *Trichoderma* species viz. *Trichoderma viride* isolate A, *Trichoderma viride* isolate M, *Trichoderma harzianum* isolate C and *Trichoderma harzianum* isolate L against *Beauveria bassiana* causing white muscardine disease of Silk worm, *Bombyx mori* L, was studied *invitro* by studying the mycelial interaction between them as well as by the effect of culture filtrate of biocontrol agents on sporulation and spore germination of pathogen. *Trichoderma viride* isolate A and *Trichoderma viride* isolate M produced the maximum inhibition zone of 68.00 and 44.25 mm area respectively, followed by *Trichoderma harzianum* isolate L with inhibition zone of 39.60 mm and *Trichoderma harzianum* isolate C with 33.50 mm. In dual culture all the isolates of *Trichoderma* spp. over grew the pathogen and covered the entire medium surface. Its mycelia coiled around the hyphae of test pathogen and finally disintegrated it. The inhibition in mycelial growth by the cultural filtrate of *Trichoderma harzianum* isolate L was 55.35 %. The cultural filtrate of *Trichoderma viride* isolate A has inhibited the spore production and germination by 63.65 and 83.70 % respectively. The mechanism of antagonism was found to be hyper parasitism and antibiosis. The studies conducted revealed that all the tested biocontrol agents possessed antagonistic properties against *Beauveria bassiana* *in vitro* and could be used in controlling the white muscardine disease of silkworm.

Keywords

Bombyx mori L,
White muscardine,
Beauveria bassiana,
Antagonism,
Trichoderma spp.

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Introduction

Mulberry Silkworm, *Bombyx mori* L is most susceptible to different diseases caused by various infecting pathogens viz., viruses, fungi, bacteria and microsporodians. The white muscardine disease caused by a pathogen *Beauveria bassiana* (Balls) vuill an

entomopathogenic fungi is prevalent in all the Sericulture countries of the world (Bulmer and fromtling, 1983). The cocoon production losses by this disease in different countries varies from 5-50% (Jayaramaiah *et al.*, 1986; Jayaramaiah and kuberappa, 1987) and is among the most common diseases of silkworm in India (Krishna swami,

1973). During 1920-1925 the entire sericulture industry in Italy and France was wiped out due to this disease (Govindan *et al.*, 1998)

In J&K state as well the outbreak of this disease caused the heavy loss to the silk industry every year (Chishti and Sahaf, 1989). Many successful attempts have been made to save the crop loss of silkworm *Bombyx mori*, L by application of chemicals and fungicides (Kawakami, 1973; Samson and Mummigutti, 1979; Samson *et al.*, 1986; Baig *et al.*, 1987; Balavenkatasubbaiah *et al.*, 1994; Pasha and Alam, 1999; Govindan *et al.*, 1998; Munshi *et al.*, 2004; Haroon *et al.*, 2002) However, farmers are reluctant to use these disinfectants directly on silkworms mainly because of their apprehension about their toxicity on silkworms or at least adverse effects on the health of worms and quality of cocoons formed.

This has focussed the attention of sericulturists on the use of an eco-friendly method through the use of biological agents for sustainable silkworm disease management. Thus in the present investigation four locally available isolates of *Trichoderma* spp. Viz *Trichoderma viride* isolate A, *Trichoderma viride* isolate M, *Trichoderma harzianum* isolate L and *Trichoderma harzianum* isolate C have been used invitro for their antagonistic effect on mycelia growth, sporulation and spore germination of *Beauveria bassiana* causing white muscardine disease of Silkworm, *Bombyx mori* L.

Materials and Methods

Isolation of causal organism

The pathogenic fungi (*Beauveria bassiana*) were isolated repeatedly from the dead cadavers of muscardine diseased silkworm which were collected from rearer's houses.

The freshly dead and mummified worms were surface sterilized with 0.1 percent Mercuric chloride solution for about 5 minutes and later rinsed with sterilized distilled water several times (Johnston and Booth, 1983). The cuticular tissue of the dead worms was then cut into 4mm square bits (Kirlay *et al.*, 1974) with the help of sterilized scalp/blade. These bits were again passed through 0.1percent solution of Mercuric chloride for 30 seconds. Finally with the help of sterilized inoculation needle these bits were placed under aseptic conditions on the petriplates containing Samsinakova's Corn Liquor Agar (SKLAM) media (Samsinakova, 1966) and incubated at 28±2⁰C and 85±5% relative humidity for about 10 days.

Isolation of antagonist (biocontrol)

All the isolates of the biocontrol agents viz., *Trichoderma viride* isolate-A, *Trichoderma viride* isolate-M, *Trichoderma harzianum* isolate-L and *Trichoderma harzianum* isolate -C were procured from Division of Pathology, S.K University of Agricultural sciences and technology Shalimar Kashmir (J&K). They were grown on Peptone yeast agar medium.

Interaction between biocontrol agent and pathogen (*Beauveria bassiana*) was studied by Agar plug and Dual culture methods and by the effect of Culture filtrate of biocontrol agents on pathogen growth and spore production.

Agar plug method

In agar plug method spore suspension of pathogen (*Beauveria bassiana*) was poured in sterilized 90mm petriplates containing PDA medium. The seeded plates were then stored in refrigerator at 4⁰C and used as and when required. A 7mm disc of biocontrol grown on Peptone yeast extract medium was kept at the

centre of seeded plates and Incubated at 28±2°C for 4days (Munshi *et al.*, 2004).

Diameter of the inhibition zone produced by biocontrol agents against pathogen was measured as clear distance between periphery of agar plug and growth of the Pathogen (Shivapratap *et al.*, 1996).

Dual culture method

In dual culture method 7mm plug of the test pathogen and biocontrol agent were placed 70 mm apart from each other on 90mm petridish. Whole set was incubated at 28±2°C for 15 days. The method described by Bell *et al.*, (1982) was used to test the antagonistic ability of biocontrol against the pathogen *Beauveria bassiana*.

Effect of cultural filtrate (metabolite) of bio control agents on pathogen growth

Metabolites of the bio control agents were obtained by growing them separately in peptone yeast extract liquid medium for 15 days and filtering the culture filtrate through Whatman filter paper No.42 and repeated centrifugation at 9000rpm (Shivapratap, 1996). Pathogen (*Beauveria bassiana*) was grown on samsinakova agar medium amended with 20% metabolite of biocontrol agent. The pathogen in medium without metabolite served as control. Each treatment and control was replicated five times. Growth of the fungus in both the sets were measured after 15 days of incubation and compared.

Effect of metabolites on spore production and germination

7mm mycelial disc of pathogen was cut from above experiment at different places in each treatment and macerated thoroughly in 20 ml sterilized distilled water to release the spores. The average number of Spores per ml spore

suspension per microscopic field (x600) was counted from 20 observations in treated and control set to determine the inhibition in spore production.

The effect of metabolites on spore germination of the pathogen was studied by the hanging drop method (Brian, 1960). The percentage inhibition in mycelia growth, spore production and spore germination were calculated by the formula of Vincent (1947).

Statistical analysis

Each experiment was performed twice with five replications with appropriate control batch. All the data was statistically analysis.

Results and Discussion

Interaction between bio control agents and Beauveria bassiana

Agar plug method

All the four isolates of *Trichoderma* spp. tested viz *T.viride* isolate-A, *T. viride* isolate-M, *T.harzianum* isolate-L and *T. harzianum* isolate-C (Table 1) produced the inhibition zone ranging from 33.50 to 68.00 mm against *B.bassiana*. Maximum inhibition zone (68.00mm) was produced by *T. viride* isolate - A followed by *T.viride* isolate -M (44.25 mm). *T.harzianum* isolate -L and *T.harzianum* isolate-C were also effective by producing an inhibition zoon of 39.60 and 33.50 mm respectively (plate-2 a-d).

Dual culture method

All the biocontrol agents used in the present study showed strong antagonism against the pathogen by covering the entire medium surface and completely overgrowing the pathogen and were grouped into class-1(plate 3 a-d).

Effect of culture filtrate (metabolites) on mycelia growth of *B.bassiana*

Metabolites of all the biocontrol agents inhibited the growth of pathogen in varying degree. Maximum inhibition was produced by culture filtrate of *T.harzianum* isolate-L (55.35%) followed by that of *T.harzianum* isolate-C (54.28%) and *T.viride* isolate-A (53.48%). Minimum inhibition zone was produced by *T.viride* isolate-M (50.00). However, they were statistically different from each other.

Effect of metabolites on spore production and germination

The metabolites of all bio-control agents have reduced the spore production and germination of the test pathogen (Table 2). The percentage decrease in spore production over control varied from 54.49 to 63.65%. *T.viride* isolate-A have produced the maximum reduction (63.65%) followed by *T.viride* isolate-M (61.70%) and *T.harzianum* isolate-L

(55.03%). Minimum inhibition (54.49%) was recorded in *T.harzianum* isolate –C. The inhibition in spore germination ranged from 73.78 to 83.70 % with *T.viride* isolate -A produced the maximum inhibition (83.70%) followed by *T.viride* isolate-M (81.80%). Minimum inhibition in spore germination (73.78%) was recorded in *T.harzianum* isolate-C.

Thus from the above study it was observed that all the isolates of *Trichoderma* spp. viz. *Trichoderma viride* isolate-A, *Trichoderma viride* isolate-M, *Trichoderma harzianum* Isolate-L and *Trichoderma harzianum* isolate-C evaluated under invitro conditions have inhibited the mycelia growth, sporulation and spore germination of the pathogen, *Beauveria bassiana*. *Trichoderma viride* isolate-A and M proved highly antagonistic followed by *Trichoderma harzianum* isolate –L and C against the tested pathogen. These antagonists have suppressed the growth of *Beauveria bassiana* by way of Mycelial interaction and as well as metabolite.

Table.1 Interaction between biocontrol agents and *Beauveria bassiana* in Agar plug, Dual culture and culture filtrate methods

Biocontrol agent	Method			
	Agar Plug	Dual Culture	Culture filtrate	
	Mean area of inhibition zone(mm)	Class	Colony diameter(mm)	% inhibition over control
<i>Trichoderma viride</i> isolate-A	68.00 a	1	5.21c	53.48(46.99)
<i>Trichoderma viride</i> isolate-M	44.25b	1	5.60d	50.00(45.00)
<i>Trichoderma harzianum</i> isolate-L	39.60c	1	5.00a	55.35(48.07)
<i>Trichoderma harzianum</i> isolate-C	33.50d	1	5.12b	54.28(47.45)
Control/(untreated)	-		11.20e	-
CDP=(0.05)	1.28		0.01	

Figures with in parenthesis are angular transformation values. Means followed by similar letters are statistically identical

Table.2 Effect of culture filtrates of bio-control agents on spore production and spore germination of *B. bassiana*

Culture filtrate	Spore production		Spore germination	
	No of spores per microscopic field	% Inhibition over control	% Spore germination	% Inhibition over control
<i>Trichoderma viride</i> isolate-A	14.00a	63.65(52.92)	16.30 (23.81) a	83.70(66.18)
<i>Trichoderma viride</i> isolate-M	14.75a	61.70(51.76)	18.20 (25.25)b	81.80(64.74)
<i>Trichoderma harzianum</i> isolate-L	17.32b	55.03(47.88)	22.12(28.05) c	77.88(61.94)
<i>Trichoderma harzianum</i> isolate-C	17.53b	54.49(47.57)	26.22(30.80)d	73.78(59.19)
Control/untreated)	38.52c	-	100.00(90.00)e	-
cDP=(0.05)	1.17		0.21	

Figures with in parenthesis are angular transformation values.
Means followed by similar letters are statistically identical

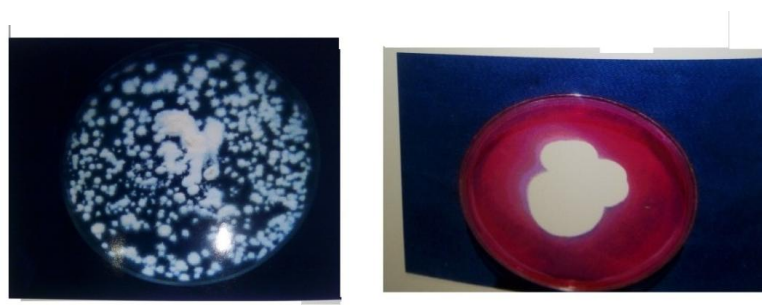


Plate.1 Isolation and growth of *Beauveria bassiana* in (a) Samsinakova's corn Liquor Agar Medium and (b) PDA medium

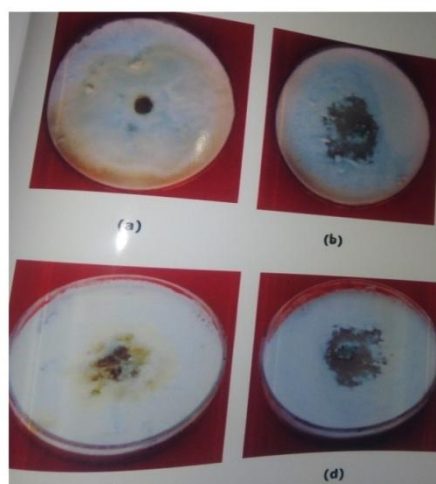


Plate.2 Mycelial interaction by Agar plug method
(Inhibition Zone produced by bio control agent against *Beauveria bassiana*)
a) *T. viride*-A b) *T. viride*-M c) *T. harzianum*-L d) *T. harzianum*-C



Plate.3 Mycelial interaction by dual culture method

- a) *T.viride-A* and *Beauveria bassiana* b) *T.viride-M* and *Beauveria bassiana*.
c) *T. harzianum-L* and *Beauveria bassiana* d) *T.harzianum-C* and *Beauveria bassiana*

This type of activity of *Trichoderma* spp. was previously reported against many plant pathogens (Chet *et al.*, 1979; Bell *et al.*, 1982; Elade *et al.*, 1983; Sivan *et al.*, 1984; Goodman and Burpee, 1991; Shivapratap *et al.*, 1996; Charati *et al.*, 1998; Biswas and Sen, 2000; Sridhar *et al.*, 2000; Munshi and Dar, 2004)

Since antagonists have inhibited the growth of pathogen at mycelia as well as metabolite level, the strong antagonistic action of above biocontrol agents in the present study is attributed due to the production of antimicrobial metabolites as well as by hyper parasitism.

Thus the results of the present study have shown that all the isolates of *Trichoderma* species possesses antagonistic properties against the *Beauveria bassiana* causing white muscardine disease in silkworm, *Bombyx mori* L, and could be used as efficient bio control agent for the management of this menace. However, further studies regarding their field efficacy and bioassay on silkworms should be carried out to find out their field applicability.

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