Management of powdery mildew, *Phyllactinia corylea* (Pers.) Karst of Mulberry (*Morus* sp.) using chosen biocontrol agents

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ABSTRACT

Powdery mildew caused by *Phyllactinia corylea* (Pers.) Karst is a serious disease of mulberry inflicting considerable qualitative as well as quantitative losses. Owing to non eco-friendly nature, toxicity to silkworm, high cost and other side effects of chemical control measures, search for other management strategies devoid of such drawbacks becomes inevitable. In the present study, five biocontrol agents *viz., Trichoderma viride, Trichoderma harzianum, Gliocladium roseum, Trichothecium roseum* and *Chaetomium indicum* were evaluated for their efficacy against powdery mildew of mulberry. Culture filtrate of these biocontrol agents were tested *in vitro* for their fungitoxicant activity against conidial germination of *P. corylea*. Among the culture filtrate of biocontrol agents tested, both *T. viride* and *T. harzianum* @ 50.0% were highly effective and inhibited the conidial germination by 75.08% and 72.23%, respectively. *Gliocladium roseum*, *T. roseum* and *C. indicum* each at 6.25% concentration were the least effective treatments and did not provide more than 11.0% conidial germination inhibition. The *in vitro* treatments exhibiting conidial germination inhibition of more than 50.0% were also tested *in vivo* for the management of the disease. Culture filtrates of *T. viride* and *T. harzianum* both at 50.0% concentration were equally most effective and were at par with carbendazim 50 wp @ 0.05% by providing 65.14% — 68.68% disease control.

Key words: Biocontrol agents, culture filtrate, conidial germination, powdery mildew, mulberry

INTRODUCTION

Sericulture as an agro based industry is a high priority sector for resource allocation in developing countries. After China, India has emerged as a second largest producer of raw silk and contributes about 19 per cent to total global production (Datta *et al.*, 2001). In India, West Bengal and Jammu and Kashmir are the oldest silk rearing states. Jammu and Kashmir has been one of the pioneer states for the production of uni \ bi voltine silk of international quality. Mulberry which belongs to family *Moraceae*, genus *Morus* and is a perennial woody plant of considerable economic importance due to its foliage which constitutes the food for mulberry silkworm (*Bombyx mori* L.). About 1100 Kg of mulberry leaves are required for rearing 100 layings of hybrid eggs (Dar and Singh, 1998).

One of the major constraints in the production of required quantity and quality of mulberry leaf are mulberry diseases. Powdery mildew of mulberry caused by *Phyllactinia corylea* (Pers.) Karst. reduces leaf yield and adversely affects the feeding quality of the leaf due to luxuriant mycelial growth on lower surface (Noamani *et al.*, 1970). Upon feeding mulberry leaves infected with powdery mildew, the larvae of silkworm remain smaller in size, resulting in smaller sized cocoons as well as poor quality silk (Sullia and Padma, 1987).

Considerable work has been carried out by a number of workers on the chemical control of this disease. Multiple applications of chemicals are often required to keep the disease under check. Owing to non eco friendly nature, toxicity to silkworms, high cost of fungicides and reluctance by the farmers to their use and other side effects of chemicals, search for other alternative / eco friendly control strategies devoid of these drawbacks becomes unavoidable. Keeping in view the above aspects, present study was initiated for the first time in Kashmir valley with the objectives of screening some locally isolated bio-control such as agents against the disease / pathogen.

MATERIALS AND METHODS

Preparation of culture filtrate of biocontrol agents

Pure culture of five biocontrol agents *viz:* (*Trichoderma harzianum* Rifai, *Trichoderma viride* Pers. ex S. F. Gray, *Gliocladium roseum* Link.) Thom., *Chaetomium indicum* Corda. and *Trichothecium roseum*

Link). were obtained from silkworm and mulberry pathology section of Division of Sericulture SKUAST-K Mirgund. Mass culturing of each bio control agents was done on potato dextrose agar (PDA) medium in 9cm petri plates. Fifty ml of potato dextrose broth was taken in 250 Erlenmeyer flask and sterilized. A 4 mm dia. disk of 10 days old culture of each biocontrol agent was aseptically transferred to cooled broth and incubated at 28 ± 2 °C for 10 days. On the 10th day, culture filtrate of these biocontrol agents were harvested by filtering through Whatman filter paper No. 42 and repeatedly centrifuged at 9000 rpm to obtain a cell free culture filtrate (Shivapratap *et al.*, 1996). The supernatant served as a stock solution of 100% concentration and was further diluted to desired concentrations viz., 6.25%, 12.50%, 25.00% and 50.00%, by adding sterilized distilled water.

***In vitro* evaluation of culture filtrates**

Each concentration of culture filtrates were bio-assayed for their fungitoxicant activity on conidial germination of *Phyllactinia corylea* by employing detached leaf method of Sukumar and Ramalingam (1981) with some modifications. The leaves on fourth position (in descending order from the top) of a susceptible mulberry variety (Goshoerami) were detached and washed thoroughly with tap water and then with sterilized distilled water. The leaves were then dipped for five seconds in test concentrations of biocontrol agents (6.25%, 12.50%, 25.00% and 50.00%) and 0.05% carbendazim 50 wp and then air dried in shade to remove excess water droplets. The water treated leaves served as check. The leaves of each treatment were placed on moist blotting paper in 15mm diameter petridishes, keeping abaxial surface exposed. .The freshly developed conidia were obtained by shaking off the mature conidia from the highly infected leaves in the field, one day prior to their collection. These were evenly dusted over the abaxial surface of the treatment leaves. The inoculated leaves in the petridishes were incubated at ambient temperature (28 ± 2°C) for 24 hours under continuous fluorescence light of 400 lux. The conidia from incubated leaves were picked on sticky side of cellophane tape to observe the conidial germination. Cellophane tapes were placed on glass slides having a drop of cotton blue in lactophenol and were examined under microscope (Nayar and Wilson, 1973). Twenty random microscopic fields were scored for each of the three replications. Percent conidial germinations and percent conidial germination inhibition were calculated.

***In vivo* evaluation of culture filtrates**

The test concentrations of culture filtrate of biocontrol agents which inhibited the conidial germination of *P. corylea* by more than 50.0% were evaluated *in vivo*, along with check (water spray) and carbendazim 50 wp at recommended concentration of 0.05%, for their efficacy in controlling powdery mildew intensity. The experiment was conducted on two year old plants at mulberry plants. The experiment was laid down in a randomized block design with three replications, each comprising of three plants of a susceptible variety (Goshoerami). The treatments were applied three times at an interval of 15 days starting from the first appearance of the disease. A guard row was kept in between the treatments to prevent the drift while spraying. Observations were recorded 15 days after third spray to estimate disease intensity. All the leaves of three randomly selected plants, per replication, were examined and were grouped in to the following five grades (0, 1, 2, 3 and 4 grades for no infection, 0.1 - 25.0, 25.1 to 50.0, 50. 1 to 75.0 and > 75.0% infection respectively) for calculation of disease intensity (James, 1974). Percent disease intensity (PDI) and percent disease control (PDC) were calculated using standard method of plant disease assessment (FAO, 1967).

RESULTS AND DISCUSSION

Conidial germination

Culture filtrate of all the bio-control agents at each concentration as well as carbendazim 50 wp at recommended concentration of 0.05% significantly declined the conidial germination as compared to check (Table 1). Carbendazim 50 wp @ 0.05% was the most effective, exhibiting a mean conidial germination inhibition of 81.33%. However, *T. viride* and *T. harzianum* each at 50.0% concentration were equally most effective by inhibiting 75.08% and 72.23%. *T. roseum* @ 50.0% was the next best treatment (61.65% conidial germination inhibition). However, *T. harzianum* @ 25.0%, *G. roseum* @ 50.0%, *C. indicum* @ 50.0% and *T. viride* @ 25.0%, each divulging a conidial germination inhibition of more than 50.0% were statistically at par with *T. roseum* @ 50.0%. All remaining treatments inhibited the conidial germination by less than 50.0%. *G. roseum*, *T. roseum* and *C. indicum* each at 6.25% concentration were the least effective treatments and did not provide more than 11.0% conidial germination inhibition.

Powdery mildew intensity

The test concentrations of culture filtrate of bio-control agents which inhibited the conidial germination of

Table 1. Effect of culture filtrates of various bio-control agents on Inhibition of conidial germination of *Phyllactinia corylea*

Concentration (%) / Treatment	Conidial germination inhibition over check(%) 6.25	12.50	25.00	50.00	Means
Trichoderma harzianum	16.44*(23.78)^{fgh}	28.63(32.35)^{de}	57.89(49.55)^{c}	72.23(58.23)^{b}	43.80(40.98)^{B}
Trichoderma viride	12.84(20.77)^{gh}	22.95(28.56)^{ef}	51.89(46.08)^{c}	75.08(60.06)^{b}	40.69(38.87)^{B}
Gliocladium roseum	6.93(13.62)^{i}	17.72(24.72)^{fg}	37.55(37.77)^{d}	54.24(47.43)^{c}	29.11(30.88)^{C}
Chaetomium indicum	10.25(17.47)^{hi}	20.38(26.75)^{efg}	38.72(38.37)^{d}	54.08(47.35)^{c}	30.86(32.49)^{C}
Trichothecium roseum	10.23(17.96)^{hi}	19.70(26.34)^{efg}	37.93(38.00)^{d}	61.65(51.77)^{c}	32.38(33.52)^{C}
Carbendazim 50 wp	81.33 (64.40)^{a}	81.33(64.40)^{A}			
Means	23.00(26.33)^{Di}	31.79(33.85)^{Ci}	50.89(45.69)^{Bi}	66.44(54.87)^{Ai}	

*Figures in parentheses represent arc sine transformed values; ** Carbendazim 50 wp was used only at recommended concentration of 0.05%; Values superscripted with similar letter(s) are statistically at par with each other.

Phyllactinia corylea by more than 50.0% were evaluated *in vivo,* along with check (water spray) and carbendazim 50 wp at recommended concentration of 0.05%, for their efficacy in controlling powdery mildew intensity on mulberry plants. Three sprays were conducted at an interval of 15 days, starting from the first appearance of disease. Observations were recorded 15 days after the final spray. The data on percent disease intensity and percent disease control over check is presented in Table 2. Analysis of the data indicated that all the treatments significantly reduced the disease intensity as compared to check. Lowest disease intensity (20.89%) was observed in plants sprayed with carbendazim 50 wp. However, 50.0% culture filtrate of *T. viride* and 50.0% culture filtrate of *T. harzianum* exhibited a disease intensity of 22.84% and 23.42% respectively were statistically at par with one another as well as with carbendazim 50 wp. Maximum disease intensity of 67.02% was observed in case of check.

Analysis of data with regard to percent disease control over check revealed that all the treatments significantly reduced the disease intensity. Carbendazim 50 wp @ 0.05% was the most effective treatment exhibiting maximum disease control of 68.68%. However, culture filtrate of *T. viride* and *T. harzianum* @ 50.0% were statistically at par with carbendazim @ 0.05% and provided 65.88%, 65.14% disease control, respectively. Culture filtrate of *T. roseum* at 50.0% concentration was the next best treatment exhibiting a disease control of more than 62.0%.

Trichoderma spp. as well as some other bio-control agents have been reported to produce metabolites which inhibit the spore germination of number of fungi (Dennis and Webster 1971; Dennis and Webster 1971; Sokita *et al.* 1981; Biswas *et al.* 2000). Little information is available on inhibition of conidial germination of *P. corylea* by culture filtrate of *Trichoderma* spp. However, Kikkort *et al.* (2000) reported that chitinase from *T. harzianum* inhibit spore germination of *Uncinula necator* causing powdery mildew of grapes. Shivapratap *et al.* (1996) found that the culture filtrates of *T. harzi anum* isolate 1 and 2 and *T. viride* isolate 2 were highly

Table 2. Effect of culture filtrate of bio-control agents on powdery mildew intensity of mulberry

Culture filtrates	Concentration(%)	Disease intensity(%)(%)		Disease control over check	
Trichoderma harzianum	25.00	32.56	(34.78)^{ghij}	51.03	(45.59)^{fghi}
Trichoderma harzianum	50.00	23.42	(28.91)^{abc}	65.14	(53.83)^{ab}
Trichoderma viride	25.00	32.22	(34.58)^{ghij}	51.88	(46.08)^{fghi}
Trichoderma viride	50.00	22.84	(28.53)^{abc}	65.88	(54.27)^{ab}
Gliocladium roseum	50.00	30.68	(33.62)^{fgh}	54.27	(47.45)^{efg}
Chaetomium indicum	50.00	31.51	(34.14)^{fghi}	52.83	(46.63)^{efgh}
Trichothecium roseum	50.00	25.16	(30.10)^{bcd}	62.32	(52.15)^{bc}
Carbendazim 50 wp	0.05	20.89	(27.18)^{a}	68.68	(56.00)^{a}
Water (check)			67.02	(54.97)^{l}	

*Figures in parentheses represent arc sine transformed values; Values within columns superscripted with similar letter(s) are statistically at par with each other

antagonistic against mulberry leaf spot pathogen (*Cercospora moricola*) by recording more than 67% spore germination inhibition. Similarly Philip *et al.* (2000) reported that culture filtrates of *T. harzianum* isolate 1 and 2 and *T. viride* isolate 2 inhibited about 70.0% spore germination in *Colletotrichum gloeosporioides* (causing black spot in mulberry) and *Pestalotiopsis disseminata* (causing anthracnose on mulberry). Little information is available regarding the use of culture filtrates of various bio-control agents against powdery mildew of mulberry. However, effectiveness of bio-control agents like *T. harzianum*, *T. viride* etc. against powdery mildew of some other crops have been well documented. Elad *et al.* (1998) reported that *T. harzia num* T_{39} (sprayed as Trichodex) reduced cucumber powdery mildew (*Sphaerotheca fusca*) by 55% & 97%. While in culture filtrates of *T. viride* and *T. harzianum* provided about 90.06% disease control of powdery mildew (*Leveillula taurica*) on guar as recorded by Deore and Sawant (2000). Martinez (1999) reported that cellulase produced by *T. harzianum* induced systemic acquired resistance in melon plants against powdery mildew.

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