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Evaluation of mulberry phylloplane bacteria for biological control of powdery mildew of mulberry caused by *Phyllactinia corylea*

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In order to develop biological control of powdery mildew caused by *Phyllactinia corylea* (Pers) Karst, five mulberry phylloplane bacteria (MPB) viz., *Bacillus* sp., *Bacillus megaterium, Micrococcus* sp., *Brevibacterium lines* and *Serratia marcescens* were isolated on nutrient agar medim. Five MPB wer screened *in vitro* against *P. corylea* conidia by slide germination method. Analysis of variance revealed that *Bacillus* sp., *B. megaterium, Micrococcus* sp., and *B. linens* inhibited conidial germination significantly. *Phyllactinia* mildew control efficacy of five MPB was tested in potted mulberry plants cv. S_1 under natural condition. Twenty four hour old bacterial culture suspension ($1x10^8$ colony forming unit/ml) was sprayed 30 days after pruning. Four MPB *viz. Bacillus* spp., *B. megaterium, Micrococcus* sp., and *B. lines* reduced the disease severity significantly and was at par with 0.2% Thiovit (micronized wettable sulphur 80 WP). Feeding of silkworm (*Bombyx mori* L) with MPB sprayed leaves revealed no harmful effect on silkworm in respect to larval weight, cocoon production, silk content, filament length and egg laying capacity of the female moth. Overall results indicated that phylloplane bacterial strains have an appreciable potential of biological control *Phyllactinia* mildew and are also safe to silkworm.

Key Words : Biological control, mulberry, *Phyllactinia corylea,* phylloplane bacteria, powdery mildew

INTRODUCTION

Mulberry (*Morus* sp.), the sole food plant of silkworm (*Bombyx mori* L) is cultivated in large scale in the Gangetic plain of West Bengal for silkworm rearing since medieval period. Powdery mildew caused by *Phyllactinia corylea* (Pers) Karst is one of the major fungal diseases of mulberry. The disease regularly appears during winter, affecting leaf yield and quality. Chemical fungicides like Carbendazim 50 WP and Wettable sulphur effectively control the disease. Use of fungicides, has limitation due to their residual toxicity to silkworm, high cost, kill non-targeted beneficial phyllospheric and rhizospheric microorganisms, and may lead to development of resistant strains of pathogen. The protective action of leaf surface organisms against foliar pathogens has been reported by Fokkema *et al.* (1975) ; Kalita *et al.* (1996); Maji *et al.* (2001, 2002) ; Saikia and Chowdhury (1993) ; Spurr (1992) ; and Verma *et al.* (1983). Keeping this in view, phylloplane bacteria strains of S_1 mulberry cultivars are isolated and their efficacy are evaluated *in vitro* and *in vivo* avainst *P. corylea* and phylloplane sprayed leaves are fed to silkworm larvae to test its residual toxicity.

MATERIALS AND METHODS

Isolation of mulberry phylloplane bacteria

Mulberry cv. S_1 leaves were collected in sterile polythene bags from Central Sericultural Research and Training Institute, Berhampore farm. Five leaf discs of 10 mm dia were taken from each leaf by cutting out aseptically by a sterile cork borer. Fifty discs were transferred into 100 ml sterile dist. water in a clask and was shaken for 20 min. Aliquots of 1 ml, 0.5 ml and 0.1 ml suspensions were transferred into sterile petriplates, containing Nutrient Agar medium (45-50°C). Plates were incubated at 25°C in inverted position for seven days. Bacteria were isolated and purified by streak method. Mulberry phylloplane bacteria (MPB) were identified by comparing morphology, staining, cultural and biochemical characteristics on the guidelines of Bergey's Manual of Determinative Bacteriology.

In vitro screening of antagonistic MPB against P. corylea

MPB were screened against *P. corylea* by slide germination methods. An aliquot of 0.2 ml of 1 x 10^8 bacterial suspension was smeared on clean grease free microscopic slide and allowed to dry for 20 minutes at room temperature. Powdery mildew infected leaves were collected from green house and gently shacked on the treated slide to release mature conidia on the slide and incubated for 24 hrs and per cent in moist chamber. Conidial germination was recorded under 100X magnification and per cent of conidial germination was calculated by observing the number of conidia germinated in twenty microscopic fields. The per cent of conidial inhibition was calculated by following (Vincent, 1947) formula.

$$I = \frac{C-T}{C} \times 100$$

Where, I = Inhibition percentage, C = Conidial germination in control ; T = conidial germination in treatment.

Field evaluation of MPB for control of P. mildew

Five MPB strains were tested *in vivo* against mildew during peak season. Pot experiment was conducted in completely readomised design during November 2001 on mulberry cv. S_1. MPB strains (48 hr old) grown on nutrient glucose agar were suspended in sterile dist water and were adjusted to standard turbidity (0.1 OD at 600 nm) to contain approximately 10^8 cfu/ml. MPB suspension (30 ml/plant) was sprayed on mulberry foliage 30 days after pruning with hand sprayer (Baygon make) at 4-5 PM. Micronised wettable sulphur fungicide (Thiovit 80 WP) was sprayed at 0.2% concentration and control plants were sprayed with water. Each set of treatment was triplicated. Per cent disease index (PDI) data were recorded 20 and 30 days after spray (DAS).

Disease incidence was recorded by randomly selecting five branches from each plant. In each plant the total numbers of healthy and diseased leaves were graded into 0-5 scale [0-healthy leaf, 1 = 1-5% leaf area infected, 2 = 6-10% leaf area infected, 3 = 11-25% leaf area infected, 4 = 26-50% leaf area infected, 5 = 51 and above per cent leaf area infected]. per cent of disease index (PDI) was calculated according to FAO (1967) formula.

$$PDI = \frac{\text{Sum of all numerical rating}}{\text{Total no. of leaves observed x maximum grade (5)}} \times 100$$

Bioassay of MPB treated leaf

The effect of feeding of MPB treated leaves on silkworm rearing, cocoon production, silk quality and egg laying capacity was assessed through bioassay (silkworm rearing). Aqueous suspension of MPB (1×10^8 cfu/ml) was sprayed 30 days after pruning. Sprayed leaves were fed to silkworm (Nistari Marked) after 10 days of application from the date of hatching up to spinning. Each of the treatment was replicated three times. Fifty larvae were taken in each replication. Date of important rearing, reeling and grainage parameters were recorded.

RESULTS AND DISCUSSION

In vitro screening of MPB against P. corylea

Analysis of variance revealed that *Bacillus* sp., *Bacillus megaterium, Micrococcus* sp., *Brevibacterium lines* and *Serratia marcescens* inhibited *P. corylea* conidial germination significantly (Table 1). Among the bacterial strains *B. lines* inhibited 74.40% conidial germination followed by *Bacillus sp.* (62.20%). Antagonistic activity of several *Bacillus* app. against plant pathogenic fungi reported by Baker and Cook (1982) ; Broadbent *et al.,* (1971) ; Chang and Kommedahl, (1968) ; Fravel and Spurr, (1977) ; Gregory *et al., (1984) ;* Katz and Demain (1977) reported that *Bacillus* sp. produce at least 66 different antibiotics. The antagonistic activity in the present study may be attributed to the production of some antifungal chemical (s) against *P. corylea.*

Field evaluation of antagonistic MPB

In vivo study indicate that foliar spray of four phylloplane bacterial strains viz. *Bacillus* sp., *Bacillus megaterium, Micrococcus* sp., and B. *linens* significantly reduced mildew disease severity even 30 days after spray (Table 2). Among four bacterial strains, *Bacillus* sp. was found to be most effective and reduced disease severity (57.62%) followed by the *Micrococcus* sp. (50.76%). Disease controlling efficacy of these two bacterial strains was at par with fungicide Thiovit (0.2%). Several workers reported that *Bacillus* spp. controlled fungal diseases of plants (Hall *et al.,* 1986 ; Gregory *et al.,* 1984 ; Ferreira *et al.,* 1991). Similarly, use of *Serratia marcescens* in biocontrol of plant diseases was also reported (Kalita *et al.,* 1996 ; Liu *et al.,* 1995 ; Wei *et al.,* 1991). Brian *et al.* (1957), Deacon (1991) and Rothrock and Gottlieb (1991) reported that biological antagonists controled plant pathogen through antagonism. Recently, different workers demonstrated that *Bacillus* sp., *Pseudomonas* sp., and *Serratia* sp. induced systemic resistance in various plants (Kloepper, 1991 ; Wie *et al.,* 1991). Cook (1980) reported that the effect of epiphytes to control diseases was partly due to prior use of nutrient needed for pathogen. The decreases of mildew severity in our study may be due to production of antimicrobial substance by *Bacillus* spp., which induced systemic resistance or prior utilization of nutrient by *Micrococcus* sp.

Bioassay of MPB treated leaf

Silkworm rearing data indicated that larval wt., single cocoon wt., single shell wt., silk ratio, effective rate of rearing [ERR] (no. & wt.), filament length, non breakable filament length, Denier, egg laying capacity were at par with the unsprayed plants (Table 3). It is thus inferred from the bioassay study that feeding of the phylloplane treated leaves are safe to silkworm.

Table 1 : *In vitro* screening of mulberry phylloplane bacteria against *Phyllactinia corylea.*

Phylloplane Baccterial strains	Germination (%)	Inhibition (%)
Micrococcus sp.	42.40 (39.69)*	38.21
B. linens	17.57 (20.08)	38.21
Bacillus sp.	25.94 (27.28)	62.20
B. megaterium	42.38 (41.04)	38.24
S. marcescens	56.86 (52.16)	17.13
Control	68.62 (57.88)	
CD at 5%	12.52	

* Figures in parentheses are angular transformed values.

Table 2 : Evaluation of mulberry phylloplane bacteria against Powdery mildew

Phylloplane Baccterial strains	Conc. (cfu/ml)	Percent disease index 10 DAS	20 DAS
Micrococcus sp.	10^8	4.87 (64.79)*	6.82 (50.76)
B. linens	10^8	6.22 (55.02)	9.32 (32.70)
Bacillus sp.	10^8	4.28 (69.05)	5.87 (57.62)
B. megaterium	10^8	9.10 (34.20)	9.79 (29.31)
S. marcescens	10^8	5.10 (60.08)	5.98 (56.82)
Control		13.83	13.85
CD at 5%		5.11	3.57

* Figures in parentheses are percent disease control.

Overall results indicated that phylloplane bacterial strains have an appreciable potential for biological control of mildew and which are also non pathogenic to silk worm.

Table 3 : Effect of mulberry phylloplane bacteria treated leaves on silkworm rearing, reeling an grainage parameters.

Phylloplane Bactrial sp.	Wt. of 10 mat. Larvae (g)	Wt. of Single Cocoon (g)	Wt. of Single Shell (g)	Silk Ratio (%)	ERR (no)	Wt. of ERR (kg)	Length of Filament (mts)	Length of Non breakabl (mts)	Denier	Total egg (no)
Micrococcus sp.	16.97	0.982	0.125	12.73	9733	8.177	348	348	1.580	438
B. linens	17.11	0.941	0.114	12.16	9267	7.877	365	324	1.723	458
Bacillus sp.	17.47	0.898	0.120	12.07	9267	8.037	355	355	1.597	441
B. megaterium	17.66	0.922	0.116	12.60	9733	8.341	354	354	1.603	414
S. marcescens	17.53	0.953	0.120	12.60	9533	8.264	361	361	1.573	400
Control	17.51	0.968	0.118	12.18	9400	8.614	360	360	1.660	427
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

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