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# Isolation and evaluation of bacterial biocontrol agents for management of leaf spot (*Myrothecium roridum*) in mulberry

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Biocontrol agents (BCAs) are promising alternatives to chemicals in crop protection. Watery extracts of various composts and associated bacteria are found success to control various diseases of agricultural crops. Studies were conducted to identify potential BCAs from various compost extracts to control leaf spot caused by *Myrothecium roridum* in mulberry (*Morus* spp.). Twelve numbers of bacteria isolated from watery extracts of various composted materials were tested for their ability to suppress the disease and compared with the efficacy of carbendazim (0.1%), a recommended fungicide for management of the disease. *In vitro* studies showed significant ($P < 0.05$) suppression of mycelia growth and sporulation of the pathogen by six bacterial isolates in potato dextrose broth. To test their *in vivo* efficacy, experiments were conducted on mulberry plants (cv. $S_1$) raised in earthen pots spraying with 48 h old bacterial suspension ($1 \times 10^8$ CFU/ml) at 30 days after pruning. Disease severity recorded 7, 14 and 21 day after treatment revealed significant ($P < 0.01$) influence of three *Bacillus* isolates *viz. B. lentimorbus, B. pumilus* and *B. cerceus* reducing the disease severity comparable to 0.1% carbendazim. No significant interaction was noticed between bacterial isolates and days after application. Bioassay by feeding silkworms with the leaves 10 days after treatment revealed BCAs are safe to silkworms.

**Key words :** Biological control agents, leaf spot, mulberry, *Myrothecium roridum*

## INTRODUCTION

Leaf spot caused by *Myrothecium roridum* (Tode) Ex Fr. is an important disease of mulberry in mulberry growing areas West Bengal. The disease is more frequent during July-October, with maximum severity in August (Prathess Kumar, 2002 ; Pratheesh Kumar and Chattopadhyay, 2002). The disease causes disruption in the metabolic activities of the host leading to reduction in leaf yield and nutritive value (Umesh Kumar, 1991 ; Shree and Nataraja, 1993 ; Pratheesh Kumar *et al.,* 2002). Besides consumption of diseased leaves lead to prolonged larval period, poor cocoon formation, reduced silk content and slik filament length (Qadri *et al.,* 1999). Although the disease can be partially controlled by application of chemical fungicides (Govindaiah *et al.,* 1988 ; Philip *et al.,* 1990 ; Teotia *et al.,* 1994), this is not economically viable besides environmental considerations. In addition, repeated use of fungicides may cause development of resistant pathogen strains. Alternative methods are therefore needed for its management.

There are reports on use of aqueous extracts of compost for foliar disease control (Weltzine, 1991 ; Trankner, 1992 ; Jongebloed *et al.,* 1993 ; Elad and Steinberg, 1994). Weltzien observed good control of gray mould (*Botrytis cinerea*) on strawberries, various powdery mildews, and late blight of potato (*Phytophthota infestans*) upon treating composted plant extracts. The mechanism of phytosanitary effect of decomposed plant extracts is attributed to biological activity of microorganisms available in the extracts. Similar experiments resulted control of powdery mildew in mulberry by watery extracts of various composts (Pratheesh Kumar *et al.,* 2000). Present experiments were conducted to assess the bacteria isolated from various composted organic materials, which are found effective for the control of leaf spot caused by *Myrothecium roridum* in mulberry.

## MATERIALS & METHODS

Twelve bacterial strains were isolated from compost of silkwrom rearing waste, *Eichhornia,* and compost of common weeds, which are used as organic manure in mulberry cultivation. The potato dextrose broth was prepared and poured in 250 ml conical flask. All the twelve bacterial isolates after 24 h growth in nutrient medium (peptone, 5 g ; beef extract, 3 g ; NaCl, 5 g ; agar, 5 g distilled water, 1l) were inoculated separately in the conical flask containing broth. A 5 mm viable disc of mycelia obtained from 7 day old culture of test fungi was transferred in each conical flask. Broth with carbendazim 0.1% was kept for comparison. Similarly, the broth without bacterial isolate was also kept as check. The treatments and control was replicated thrice. The whole set was incubated at 28 ± 2°C. Fourteen days after incubation the spores were harvested and enumerated using a hemocytometer. Similarly, the mycelia were harvested dried at 60°C for 72 h and weighed.

Two sets of experiments were conducted for *in vivo* evaluation of antagonistic bacterial isolates. The bacterial isolates were grown on soild nutrient medium after 24 h of growth ; the bacterial suspension was prepared in sterilized distilled water and adjusted to an absorbance of 0.1 at 600 nm ($10^8$ CFU/ml). These suspensions were then sprayed separately on 30 day old mulberry plants (cv. $S_1$) raised in earthen pots following the recommended package of practices (Subba Rao, 1989) with a hand held sprayer. Recommended fungicides carbendazim (0.1%) was sprayed separately. A control row, which was sprayed with sterilized distilled water, was maintained for comparison. Conidial suspension ($1\times 10^6$ / ml) was prepared in distilled water using one-week-old culture of *Myrothecium roridum* grown in potato dextrose agar. The suspension was sprayed on the mulberry plants 24 h after treatment. Three replications were kept for each treatment and control in random. The data on the severity of leaf spot was recorded in a 0-5 scale where, 0 = no infection, 1 = 1-5 %, 2 = 6-15 %, 3 = 16-30 %, 4 = 31-50 % and 5 = > 50 % leaf area infected with leaf spot. Severity (%) was then calculated using formula

$$\text{Disease severity (\%)} = \frac{\Sigma \text{ Numerical values}}{\text{Total number of leaves observed} \times \text{maximum score (5)}} \times 100$$

A third set of experiment was conducted by using only four identified effective bacterial isolates *viz. Arthrobacter ilicis, Bacillus cereus. B. lentimorbus* and *B. pumilus.*, identified from the previous experiment. Experimental procedures were followed as in the previous experiments except disease severity was recorded 7, 14 and 21 days after treatment.

Bioassay was conducted following the method (Jolly, 1987). Multivoltine silkworms (Nistari × Nistari) were fed with leaves harvested 10 days after spray of the bacterial strains of 30 days old mulberry plants (cv. S1). Single cocoon weight, single shell weight, effective rearing rate, 10 mature larval weight, silk ratio and total silk filament length were calculated following standard methods.

The data were subjected for ANOVA, *F* test were conducted and the values were compared for least significant difference.

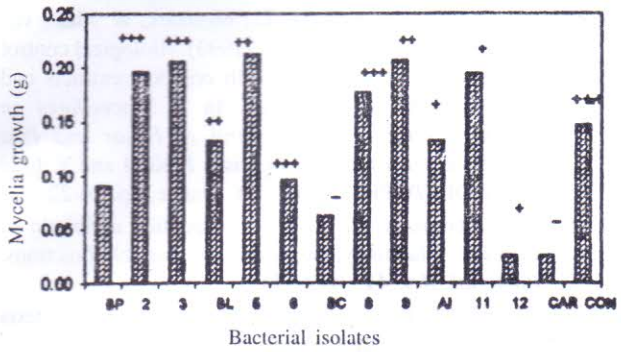
## RESULTS AND DISCUSSION

Bacterial isolates significantly influenced on the mycelia production. Six bacterial isolates and carbendazim showed significant reduction in fungal growth. Interestingly, except two bacterial isolates, spore production was significantly decreased in all other treatments (Fig. 1). In the first set of *in vivo* experiment with all the bacterial isolates, four isolates significantly reduced the disease severity > 40 % compared with control. However, carbendazim more effectively controlled the disease. The effective four bacterial strains were identified as *Arthobacter ilicis, Bacillus lentomorbus, B. cercus,* and *B. pumilus* which reduced the disease severity from 14.7 % to 8.2 %, 6.8, 7.8 % (Fig. 2), respectively controlling 44.1 %, 53.8 %, 50.7 % and 47.2 % fourteen days after treatment.

In the second set of experiment, all bacterial isolates reduced disease severity. In all the treatment maximum reduction in disease severity was obtained 21 days after treatment. Here also

carbendazim reduced the severity maximum (66.7 %) followed by *B. lentimorbus* (53.5 %), *B. pumilus* (48.6 %), *B.cereus* (46.1 %) and *A. ilicis* (31.6 %).

*Myrothecium roridum*




Mean of three replications, BP—*B. pumilus*, BL—*B. lentimorbus*, BC—*B. cereus*, AI—*A. ilicis*, CAR—carbendazim, CON—control.
+++ high, ++ medium, + low and – no sporulation.

**Fig. 1 :** Effect of bacterial isolates on mycelia growth and sporulation of *Myrothecium roridum*

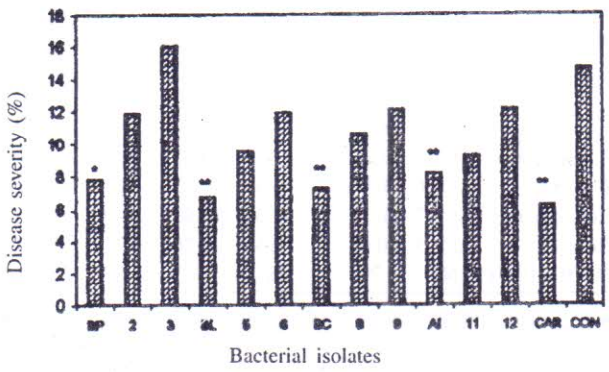
There was no significant decrease in mean disease severity after 7 days. However significant reductions were obtained after 14 and 21 days and were statistically at par. There was no significant interacton of bacterial isolate with days after treatment for reduction of disease severity (Table 1).

**Table 1 :** Influence of bacterial isolates on severity and control of *Myrothecium* leaf spot in mulberry

| Bacterial isolates | Diseases severity (%) | | | Mean |
|---|---|---|---|---|
| | 7 DAS | 14 DAS | 21DAS | |
| *Arthrobacter ilicis* | 17.28 | 13.99 | 13.17 | 14.81 |
| | (–9.85) | (11.95) | (31.61) | (12.67) |
| *Bacillus cereus* | 12.37 | 10.26 | 10.38 | 11.00** |
| | (21.36) | (35.43) | (46.10) | (35.00) |
| *Bacillus lentimorbus* | 13.76 | 8.53 | 8.96 | 10.41** |
| | (12.52) | (46.31) | (53.47) | (38.07) |
| *Bacillus pumilus* | 16.34 | 12.04 | 9.90 | 12.76* |
| | (–3.87) | (24.22) | (48.59) | (24.09) |
| Carbendazim (0.1%) | 12.98 | 10.47 | 6.41 | 9.95** |
| | (17.48) | (34.10) | (66.71) | (40.80) |
| Check | 15.73 | 15.89 | 19.26 | 16.96 |
| | (0.00) | (0.00) | (0.00) | (0.00) |
| Mean | 14.74 | 11.86** | 11.35** | |

* Significant P, 0.05, ** P < 0.01, DAS = days after spray, Figures in parenthesis are percent disease control.

Significant reduction in mean disease severity was obtained in plants treated with *B. cereus, B. lentimorbus* and *B. pumilus* as well as carbendazim lowering mean severity from 16.96 % to 11.89 %, 10.41 %, 12.76 % and 9.95 % respectively. The reduction in disease severity by *B. lentimorbus* and *B. cercus* was statistically as par with that of carbendazim. Compared to check, highest mean disease controlling efficacy showed by carbendazim (40.80 %). Except *A. ilicis*, all *Bacillus* isolates controlled the disease significantly with highest in *B. lentimorbus* (38.07 %) followed by *B. cereus* (34.56 %) and *B. pumilus* (24.09).




Mean of three replications, BP—*B. pumilus*, BL—*B. lentimorbus*, BC—*B. cereus*, AI—*A. ilicis*, CAR—carbendazim, CON—control.
*Significant p < 0.05, ** significant p < 0.01

**Fig. 2 :** Influence of bacterial isolates on severity of *Myrothecium roridum*

The rearing result showed no significant influence of bacterial treatment of the economic characters of silkworm (Table 2).

The results reported here indicate that bacterial isolates protect mulberry plants from *Myrothecium* leaf spot. The disease severity reduced significantly by treatment with *Bacillus isolates*, which was comparable to carbendazim. Several *Bacillus* strains are reported antagonistic to plant pathogens (Baker and Cooke, 1982 ; Ferreira *et al.*, 1991 ; Fravel and Spurr, (1977). The disease controlling efficiency of the bacterial isolates in the present study may be due to any one of the mechanisms such as the microbial parasitism, elicitation of phytoalexin, induction of systemic resistance, antibiotics produced by the bacterial isolates or a combined effect of these factors. Several workers (Thomashow and Waller, 1988 ; Kloepper, 1991 ;

Zhou *et al.*, 1994) proved production of bacterial metabolites such as siderophores, hydrogen cynide, antibiotics, or extracellular enzymes that account for antagonism against the pathogens as mechanism of biocontrol. About 66 different antibiotics are reported to produce different *Bacillus* spp. (Katz and Demin. 1977). Significant reduction of disease was obtained in 14 and 21 days after treatment may be due to the increase in population of the bacterial isolates on the phyllosphere.

**Table 2 :** Effect of feeding bacteria treated mulberry leaves to silkworm

| Bacterial isolates | SCW(g) | SSW(g) | SR(%) | Wt. of 10 mature larvae (g) | ERR No. | Filament Length (mt.) |
|---|---|---|---|---|---|---|
| *A. ilicis* | 0.9130 | 0.106 | 11.59 | 17.16 | 9800 | 369 |
| *B. cereus* | 0.9010 | 0.121 | 13.47 | 16.92 | 9400 | 360 |
| *B. lentimorbus* | 0.9047 | 0.1123 | 12.41 | 16.73 | 9667 | 367 |
| *B. pumilus* | 0.9540 | 0.1187 | 12.43 | 17.63 | 9670 | 362 |
| Carbendazim | 0.9517 | 0.117 | 12.33 | 17.03 | 9400 | 356 |
| Check | 0.8223 | 0.098 | 11.95 | 15.76 | 9733 | 343 |
| CD ($P < 0.05$) | NS | NS | NS | NS | NS | NS |

SCE : Single cocoon weight, SSW : Single shell weight, SR : Silk recovery (%), ERR : Effective rearing rate, NS : Not significant.

The studies are further required on the population dynamics of these isolates on the phyllosphere and the mechanism actually involved is whether induced resistance, micro-parasitism or a combined effect.

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