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Studies on the management of *Xanthomonas campestris* pv. *moricola* of mulberry

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In the last five years, bacterial blight of mulberry, caused by *Xanthomonas campestris* pv. *moricola* has become severe in the major mulberry growing areas of Karnataka, India. Since disease was new, not much information was available on the management of the disease. Therefore, we undertook *in-vitro* screening of some antibacterial chemicals, plant products and antagonistic bacteria on the growth of bacterium by inhibition zone assay technique. Paushamycin was found to be more effective (23.13 mm) followed by Bacterimycin (19.5 mm) both at 500 ppm and Streptomycin sulphate was least effective. Among the plant products tested, Ocimum oil was more effective (28.67 mm) followed by Lemon oil and Garlic extract. Of the three antagonistic bacteria tested, *Pseudomonas fluorescences* was found to be superior (25.33 mm) followed by *P. aerogenosa* (21.67 mm).

Key words : Mulberry, bacterial blight, chemical, plant products, antagonistic bacteria

INTRODUCTION

Mulberry (*Morus alba L.*) suffers from several diseases incited by fungi, bacteria, viruses and mycoplasmas-like organisms and this has become a major constraint in augmenting the yields of mulberry and silk. Excessive use of chemical pesticides have detrimental effect on the environment and leave toxic chemical residues in plants and this evinced great interest among scientists and the public in biological and eco-friendly means of plant protection (Safiyazove *et al.*,1995). Thus, an eco-friendly approaches such as plant products and biological agents along with pesticides for the control of plant pathogens has increased worldwide (Saktivel *et al.*, 1986; Unnamalai and Gnanamanickam, 1984 and Safiyoz *et al.*, 1995).

In the recent years, a bacterial disease, incited by *Xanthomonas campestris* pv. *moricola* appeared in severe form in the major mulberry growing areas of Karnataka, India (Srinivasachary *et al.*, 1995). Since the disease was of recent origin, information on the management of this desease was lacking. Therefore, studies were under taken to know the effect of some anti-bacterial chemicals, plant extracts and biological agents under *in-vitro* conditions.

MATERIALS AND METHODS

Inhibitory effect of antibacterial chemicals

Antibacterial chemicals namely, Streptomycin sulphate, Paushamycin, Streptocyclin, Bacterimycin and Copper sulphate were evaluated to test their efficacy in inhibiting the growth of the bacterium at different concentrations (Table 1) by inhibition zone technique.

A heavy supension of 48 h old culture of *X. c.* pv. *moricola* (7×10^7 cfu/ml) was seeded with molten (50°C) nutrient agar contained in an Erleynmayer's flask, so as to get the thick growth of the bacterium. The seeded medium was poured into the sterilized petriplates and allowed to solidify. Antibacterial solutions were prepared at different concentrations and sterilized paper discs (Whatman No. 44) measuring 8 mm diameter were soaked in antibacterial solutions for 10 minutes.

Then the paper discs were placed on the surface of the seeded agar medium. These were incubated first at 5°C for 4 h, so as to allow the diffusion of chemicals into the medium then at 30°C for 48 h. Observations were recorded for the production of inhibition zone around the filter paper discs against the bacteria by measuring the diameter of the inhibition zone. Experiment was conducted in three replications with three plates in each replication and analyzed statistically.

Inhibitory effect of plant products

The inhibitory effect of essential oils *viz*, Ocimum (*Ocimum sanctum* L.), Lemon (*Citrus latifolia* L.) Neem (*Azadiricta indica* L.) and Garlic extract (*Allium cepa* L.) and extract of different parts of Adathoda (*Adathoda zeylenica*) were assayed as described earlier.

Inhibitory effect of antagonistic bacteria

Three antagonistic bacteria, namely, *Psedomonas fluorescens, P. aerrogenosa* and *Bacillus subtilis* were also tested for their inhibitory effect. To the seeded medium, a loopful of antagonistic bacterium was placed in two marked places on the nutrient agar medium, seeded with the test organism. The plates were incubated at 30°C for 48 h for the production of inhibition zone and average diameter of inhibition zone produced in each replication was statistically analyzed.

RESULTS AND DISCUSSION

Of the various chemicals tested for their efficacy in inhibiting the growth of the bacterium, Paushamycin was found to be more effective (27.13 mm) at 500 ppm followed by Bacterimycin (19.50 mm) at the same concentration followed by Streptocyclin (16.70 mm) at 500 ppm. (Table 1). The least effective antibiotic was Streptomycin sulphate. Krishnaprasad and Siddaramaiah (1978) evaluated six antibiotics against *P. s.* pv. *mori* at the concentration ranging from 5-100 ppm and stated that Streptocyclin followed by Streptomycin sulphate and Pencillin were effective. Sengupta (1980) obtained better control of leaf blight of mulberry with Streptomycin sulphate and Streptocyclin at 0.1%. Valluvapuram and Mariappan (1994) also reported that Streptomycin sulpahte and Streptocyclin at 500 ppm inhibited the growth of *X. c.* pv. *vesicatoria.*

Table 1. Effect of antibiotics and fungicides on the growth of *Xanthomonas campestris* pv. *moricola*, causing mulberry blight under *in-vitro* conditions

Name of the chemical	Concentration (ppm)	Inhibition zone (mean diameter in mm)
Streptomycin sulphate	300	8.27
	400	9.27
	500	13.73
Streptocyclin	300	12.37
	400	14.13
	500	16.70
Paushamycin	300	7.33
	400	14.83
	500	27.13
Bacterimycin	300	8.10
	400	9.13
	500	19.50
	2000	15.13
	3000	18.17
Control (without chemical)		0.00
SEM +/– 0.0416		
CD at 1% 2.879		

Among the different plant products tested, the Ocimum extract registered high (28.67 mm) inhibitory effect (Srinivasachary, 1995) followed by Lemon oil (27.67 mm) and Garlic extract (23.00 mm). Neem oil extract though showed some inhibitory effect but was less effective and Adathoda extract was also found to be inhibitory (Table 2). Cowpea seeds treated with leaf and root extracts of *Adathoda zeylanica* gave better control of bactrical blight of cowpea caused by *X. c.* pv. *vignicola* (Thammaiah, 1991). Similarly, Ocimum and *Azadirata indica* extracts was inhibitory to *R. solanacearum* (Karuna, 1993). Plant extracts of medical value were known to have inhibitory effect on a wide range of microorganisms (Sivasnakara Rao and Nigam, 1978). Essential oils of *Calendula officinalis* and *Thymus serphyllum* was highest against *Corynebacterium michiganense* pv. *michiganense* and *X. c.* pv. *phaseoli* (Mishenkova *et al.*, 1983). Essential oils from *Anacardium occidentale* was inhibitory against four Gram +ve and eighteen Gram -ve bacteria (Grang and Kesara, 1984). Garlic extract was highly inhibitory to *X. o.* pv. *oryzae* of rice (Grainage *et al.*, 1985) and *R. solanacearum*, causing wilt of solanacious crops (Khan, 1974).

Table 2. Effect of plant extracts on the growth of *Xanthomonas campestris* pv. *moricola* under *in-vitro* conditions.

Name of the plant extracts used	Mean inhibition zone (mm diameter)
Garlic extract	23.00
Ocimum oil	28.67
Lemon oil	27.67
Adathoda zelenica (whole plant)	14.33
Adathoda-2 (leaf)	16.33
Adathoda-3 (root)	18.33
Adathoda-4 (combined)	14.33
Neem oil	11.67
Control	0.00
SEm	2.79
CD at 0.05%	8.00

Several workers very well established the antagonistic nature of bio-control agents such as *P. fluorescens, P. areogenosa, Bacillus subtilis* and *Enterobacter. P. fluorescens* was found to be inhibitory to a large number of plant pathogenic bacteria (Unnamalai and Gnanamanickam, 1984; Saktival *et al.,* 1986; Sivamani *et al.,* 1987; Tzeng *et al.,* 1994) and *B. subtilis* was found inhibitory to many plant pathogenic bacteria (Chen *et al.,* 1990; Karuna, 1993 ; Safiyazov *et al.,* 1995). Investigations carried out in the present studies revealed that *P. fluorescens* was found to be inhibitory followed by *P. aerogenosa* and *Bacillus subtillis* (Table 3) and the results are in agreement with report of Unnamalai and Gnanamanickam (1984).

Table 3. Effect of antagonistic bacteria on the growth of *Xanthomonas campestris* pv. *moricola* mulberry under *in-vitro* conditions.

Antagonistic bacterium tested	Mean inhibition zone (mm diameter)
Bacillus subtilis	15.00
Pseudomonas aerogenosa	21.67
Pseudomonas fluoroscene	25.33
SED	1.81
CD at 0.05%	4.41

Saktival *et al.* (1986), Sivamani *et al.* (1987), Gallardo *et al.* (1989) and Tzeng *et al.* (1994), reported the inhibitory effect of *P. fluorescens* against a wide range of bacteria. Chen *et al.* (1990) and Safiyozov *et al.* (1993) reported that *B. subtilis* was inhibitory to *X. o.* pv. *oryzae and X. c.* pv. *malvacearum,* respectively. *P. fluorescens* and *B. subtilis* were effective against *R. solanacearum,* causing bacterial wilt of tomato (Karuna, 1993) under *in-vitro* and *in-vivo* conditions.

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