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Sensitivity of *Agrobacterium tumefaciens* (Smith and Townsend) Conn strain to some antibiotics

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Eight antibiotics were screened against ampicillin resistant strain of *Agrobacterium tumefaciens*. Streptocycline, Streptomycin and terramycin were good inhibitors. Post inoculation treatment with these antibiotics prevented gall development on marigold.

Key words : *Agrobacterium tumefaciens*, Marigold, Antibiotics, Sensitivity Study

INTRODUCTION

Agrobacterium tumefaciens (Smith and Townsend) Conn. a ubiquitous soil borne pathogen infects a wide variety of plants (De Cleene and De Ley, 1976 Anderson and Moore, 1979) causing a disease known as crown gall. As the bacteriumis a wound pathogen, it has become a serious problem in nursery. Control of this disease is difficult due to its well soil survival ability (Dickey, 1961). Effective control was achieved by the employment of antagonistic bacteria (Htay and Kerr, 1974 ; Miller and Miller, 1976 ; Kerr, 1980). A little is known about chemical control of crown gall disease (Dye *et al.* 1951 ; Sidorovich, 1986).

Present study attempts to identify the effective antibiotics that inhibit the highly ampicillin resistant strain of *A. tumefaciens in vitro* and also the disease development *in vivo*.

MATERIALS AND METHODS

A. tumefaciens of marigold (*Tagetes erecta* L.) isolated as pure culture from the diseased plant tissues was made ampicillin resistant and this strain was

used as a test bacterium. Eight antibiotics, namely, streptocycline, terramycin, streptomycin, ledermycin, erythromycin, penicillin, chloramphenicol and ampicillin were screened against the bacterium in *vitro* at 250 ppm, 100 ppm, 50 ppm, 20 ppm and 10 ppm concentrations following agar diffusion technique on potato dextrose agar (PDA) medium (Mondal and Mukherjee, 1975). Same concentrations were also tested against the disease development on marigold plant. Two sets of plants were used for pre- and post- inoculation treatments (10 plants for each concentration). Wound was created by cutting the top portion of the growing stem. In pre-inoculation treatment, wounds were covered with cotton soaked in antibiotics solution and inoculated with fresh active culture of *A. tumefaciens* 24 hours after wounding. In post inoculation treatment, inoculation was done immediately after wounding and wounds were covered with antibiotics solutions soaked cotton 24 hours after inoculation. Control plants received only distilled water soaked cotton instead of antibiotics solution. The experiment was conducted repeatedly during August-September, 1991, when the atmospheric temperature ranged from 25-32°C and relative humidity of 85-90%, along with high rainfall, which are congenial for gall development and also for plant growth.

RESULTS AND DISCUSSION

Of the eight antibiotics tested *in vitro*, streptocycline, terramycin and streptomycin showed considerable zone of inhibition even at 10 ppm concentration and ledermycin produced inhibition zone upto 50 ppm concentration (Table 1) whereas, penicillin, chloramphenicl and erythromycin remained effective upto the test concentration of 250 ppm against the ampicillin resistant *A. tumefaciens* strain.

Table 1. *In vitro* sensitivity of ampicilline resistant *A tumefaciens* strain to antibiotics

Antibiotics	Trade name	Diameter of inhibition zone (mm)*				
		Concentration of antibiotics (ppm)				
		250	100	50	20	10
Streptomycin sulphate + Tetracycline hydrochloride	Streptocycline	35.8	32.2	30.7	20.7	12.4
Oxytetracycline hydrochloride	Terramycin	34.2	24.4	16.1	13.2	10.4
Streptomycin sulphate	Ambistryn-S	23.7	21.5	19.6	19.0	15.2
Demeclocycline hydrochloride	Ledermycin	20.9	11.0	10.4	0	0
Chloramphenicol	Enteromycetin	11.1	0	0	0	0
Erythromycin	Erythromycin	10.3	0	0	0	0
Potassium penicillin	Pentids-400	10.0	0	0	0	0
Ampicillin trihydrate	Aristocillin	0	0	0	0	0

* Values are average of 9 replications

At the highest concentration tested (250 ppm), the zone of inhibition was highest in case of streptocycline whereas at the lowest test concentration (10 ppm) maximum inhibition zone was obtained with streptomycin. These results are well in agreement with the results of Mattos (1986) where tetracycline group of antibiotics showed considerable inhibition. In addition, it was noticed that the strain was also sensitive against streptomycin alone or in combination with tetracycline group of antibiotics.

Table 2. Effect of antibiotics on the development of galls on marigold plant under field condition

Antibiotics	Trade name	Gall development									
		Pre-inoculation treatment					Post-inoculation treatment				
		Concentration of antibiotics									
		250	100	50	20	10	250	100	50	20	10
Streptomycin sulphate + Tetracycline hydrochloride	Streptocycline	+	+	+	+	+	—	—	—	—	—
Oxytetracycline hydrochloride	Terramycin	+	+	+	+	+	—	—	—	—	—
Streptomycin sulphate	Ambistryn-S	+	+	+	+	+	—	—	—	—	—
Demeclocycline hydrochoride	Ledermycin	+	+	+	+	+	+	+	+	+	+
Chloramphenicol	Enteromycetin	+	+	+	+	+	+	+	+	+	+
Erythromycin	Erythromycin	+	+	+	+	+	+	+	+	+	+
Potassium penicillin	Pentids-400	+	+	+	+	+	+	+	+	+	+
Ampicillin trihydrate	Aristocillin	+	+	+	+	+	+	+	+	+	+

+ Galls developed
— Galls not developed

Eight antibiotics were screened for testing their effect on the development of galls on marigold plant under field conditions. In post-inoculation treatment, no galls could be developed in streptocycline, terramycin and streptomycin treated plants at any of the concentration tested, but here also chloramphenicol, penicillin, erythromycin, ledermycin and ampicillin remained ineffective (Table 2). In case of pre-inoculation treatment, none of the antibiotics could inhibit the development of galls. Ananda and Heberlein (1975) obtained good control of the disease with rifampin (200 μg/ml) on post inoculation treatment. Inhibition of disease development on marigold plant by tetracycline or streptomycin group of antibiotics separately or in combination developed an opportunity to test their efficiency in control of the disease in field scale.

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