

BIOLOGICAL CONTROL OF BACTERIAL BLIGHT OF COTTON USING SOME PLANT EXTRACTS

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Leaf extract of *Datura alba*, seed oil of neem (*Azadirachta indica*), neemseed bitter and nimbokil 60 EC were evaluated at 1, 2 and 3% concentrations on the growth of *Xanthomonas campestris* pv. *malvacearum* in vitro and on the greenhouse grown cotton varieties/lines. At 3% concentration *Datura alba* significantly retarded the growth of bacterium followed by nimbokil, neemseed bitter and neemseed oil respectively. None of the plant extracts showed effectiveness at 1% concentration. There was significantly less number of leaf shedding, less number of bare nodes and more number of bolls, increased boll weight and yield of seed cotton of varieties sprayed with standard concentrations of *Datura alba* and nimbokil 60 EC as compared to untreated control. Disease severity was less in treated varieties as compared to untreated varieties.

Key words: bacterial blight, biological control, plant extracts, *Xanthomonas campestris* pv. *malvacearum*

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important cash crop of Pakistan sharing 60% earning of foreign exchange for the country (Anonymous, 1998-99). It is grown over 12% of the total cultivated area of Pakistan (Ahmad, 1999). Bacterial blight caused by *Xanthomonas campestris* pv. *malvacearum* can reduce the yield of the crop up to 50% under favourable conditions of the disease development. Its incidence was recorded 20-37% in Faisalabad district (Bhutta, and Bhatti, 1983). The use of resistant varieties is the most economical method of disease management but currently none of the available high yielding commercial varieties has durable resistance against this disease (Hussain et al., 1985; Khan and Rashid, 1996; Rashid and Khan, 1999). The use of chemicals for the control of this disease is advocated as an alternative method of disease management but has very limited success (Khan and Ilyas, 1989; Hussain and Tahir, 1993; Khan, 1995) due to systemic nature of the bacterium. Disease management through plant extracts has been reported by different workers in different crops (Mukhtar et al., 1994; Aziz et al., 1995; Mughal et al., 1996; Khan et al., 1998; Mukhtar and Chohan, 1999) but very little is known about the antibacterial effects of plant extracts against bacterial blight of cotton. The objective of these studies was to evaluate some plant extracts against *Xanthomonas campestris* pv. *malvacearum* in vitro and on greenhouse grown cotton plants.

MATERIALS AND METHODS

Preparation of Plant Extract: Fresh leaves of *Datura* were thoroughly washed, chopped and 25 g were macerated in an electric grinder with 100 ml of distilled water. These were then centrifuged and filtered through Whatman No. 1. This extract was arbitrarily designed as standard (S). Other dilutions viz. S/5 and S/10 were prepared by adding requisite

amount of distilled water. Neemseed bitter (NSB) was obtained by grinding washed neemseeds. Five percent solution of NSB was prepared by taking 100 g of NSB in a thin cotton cloth and suspending it into 200 ml of water for 16 hr. Neem oil (100%) was first sieved using a thin cotton cloth to remove solid matter and then emulsified by mixing 0.2 g/25 ml of "Surf" (a powdered detergent) and serial dilutions of 1%, 2% and 3% concentrations were made from emulsified solution. Nimbokil 60 EC (a neem product) commercially available was also used (1% concentrations of 60, 30 and 15% by adding requisite amount of distilled water. The suspension of culture of *Xanthomonas campestris* pv. *malvacearum* already available in the plant diagnostic lab. was mixed in the lukewarm nutrient agar (autoclaved at 15 Psi for 15 minutes). After solidification of media, using inhibition zone technique, wells (1 cm dia) were made in the centre of each petri plate. Plant extracts were poured in the well with the help of a sterilized syringe. The experiment was conducted in completely randomized design with three replications and having nine petri dishes in each treatment. All the petri dishes were placed in a refrigerator at 4°C for 24 hr and then incubated at 30°C. The data recorded on the inhibition zones after 12, 24, 36 and 48 hr were subjected to analysis of variance and treatment means were compared by LSD test.

RESULTS AND DISCUSSION

Datura alba significantly retarded the growth of *Xanthomonas campestris* pv. *malvacearum* by producing inhibition zone of 2.02 cm followed by nimbokil, neemseed bitter and neemseed oil at 3% concentration with 0.80 cm, 0.39 cm and 0.28 cm inhibition zones respectively. None of the plant extracts showed efficacy at 1% concentration and at 12 hr after inoculation. Concentration played a crucial

role in retarding the growth of the pathogenic bacterium, since with an increase in the concentration, there was a significant decrease in the colony diameter of the pathogen. Siddiqui and Alam (1987) reported that neem leaves possess some broad spectrum compounds which have more potential for use against nematode than other pathogens and less efficacy of the neem extracts (seed oil, seed bitter or formulated nimbokil 60 EC) can be viewed in the light of this fact. According to Hussain et al. (1984), the effective nematocidal activity of neem extract seems to be due to the action of its principles viz. Nimbine, Nimbinine, Nimbidine and Thionemone. These compounds might be least effective against this pathogenic bacterium. The efficacy of *Datura alba* leaf extract may be due to an elicitor/activator reaction initiated by some factor present in the extract (Bell, 1981; Giebel, 1982). Nematicidal effect of *Datura alba* and insecticidal effect of nimbokil 60 EC are well documented but antibacterial activities of these plant extracts need further investigation in this regard.

When the plants of four cultivars were sprayed with plant extracts, the height of the plants was not significantly different as compared to inoculated control (Table 2). Reduction in number of leaves per plant in untreated plants (14.50 per plant) was significantly different as compared to treated plants (20.75 and 19.85 in T₁ and T₂ respectively (Table 2). Thus the plant extract of *Datura alba* played an evident role in restricting the leaf shedding by depressing the activities of the pathogenic bacterium. Pathogenic effect of the bacterium on the number of branches was non-significant in all the treatments compared to untreated control. Inoculated control plants showed necrosis of the infected tissues, extensive leaf falling and reduced supply of water and nutrients to the apical growing parts possibly due to the blockage of xylem vessels by the pathogen as reported earlier by Bhagwat and Bhide (1962). Extensive leaf shedding led to greater number of bare nodes (14) as compared to those having application of plant extracts (10-11). Significant reduction in the yield of seed cotton of plants was observed in untreated plants. Effect of *Datura alba* was more profound and increased the yield per plant as indicated by boll weight and greater number of bolls per plant which may be due to delay in the establishment of pathogen in these plants as evident by mild symptoms of disease. Difference in varietal response to infection and recovery by the plant extracts may be due to the multiplication of the pathogen at a higher rate in a susceptible variety than in a resistant or an immune variety. This was more noticeable in B-284 (moderately resistant) compared to CIM-109, CIM-1100, CIM-435 (moderately susceptible) as reported by Khan and Rashid (1996). The varietal response (moderately resistant to moderately susceptible) was also evident in the inoculated control plants. However, for conclusive results large scale trials on field grown cotton plants are suggested.

Table 1. Effect of plant extracts against *Xanthomonas campestris* pv. *malvacearum*, the incitant of bacterial blight of cotton

Cultivar	Treatments	No. of plants	No. of leaves	No. of branches	No. of bolls	Boll weight (g)	Seed weight (g)	Seed yield (kg/ha)
B-284	Untreated	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14
	Extract	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14
CIM-109	Untreated	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14
	Extract	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14
CIM-1100	Untreated	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14
	Extract	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14
CIM-435	Untreated	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14
	Extract	14	14.50	14	14	14	14	14
	Control	14	14.50	14	14	14	14	14

* Mean values sharing similar letters do not differ significantly as determined by LSD test at 0.15.

Table 2. Effect of treatments on the various characters of yield of cotton plant infested by *Xanthomonas campestris* pv. *malvacearum*, the main inciting agent for bacterial blight

Treatments	Plant height (cm)	Number of leaves per plant	Number of branches per plant	Number of bare nodes per plant	Number of bolls per plant	Boll weight (g)	Yield of seed cotton per plant (g)
T ₁ = <i>Datura alba</i>	47.42 b	20.75 a	4.91 a	9.92 b	2.58 b	1.67 b	5.85 a
T ₂ = Nimbokil 50 EC	48.31 b	19.88 a	4.98 a	10.02 b	2.33 b	1.92 a	5.22 a
T ₃ = Untreated control	57.58 a	14.50 b	4.58 a	14.33 a	1.08 a	1.01 c	1.29 b

Table 3. Effect of plant extracts on the yield characters of different varieties of cotton plant inoculated with *Xanthomonas campestris* pv. *malvacearum*

Treatments	Plant height (cm)	Number of leaves per plant	Number of branches per plant	Number of bare nodes per plant	Number of bolls per plant	Boll weight (g)	Yield of seed cotton per plant (g)
V ₁ = B-284	50.40	16.29 b	4.97	11.57	3.10 a	1.90	6.02 a
V ₂ = C ₁ - 100	54.14	20.60 a	4.87	10.92	3.37 ab	1.84	5.91 ab
V ₃ = C ₁ - 435	52.14	18.14 b	4.80	10.80	2.47 c	1.86	4.66 c
V ₄ = C ₁ - 100	50.62	20.30 a	5.20	10.97	2.60 bc	1.87	4.98 bc
V = 100% control	57.35	14.50 c	4.53	14.33	1.08 d	1.01	1.29 d
UNE	---	2078	---	---	0.476	---	0.887

* Mean values sharing similar letters do not differ significantly as determined by LSD test at 0.05.

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