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EVALUATION OF ANTAGONISTIC ORGANISMS AGAINST *XANTHOMONAS CAMPESTRIS* PV. *MALVACEARUM* IN VITRO AND ON THE INOCULATED COTTON PLANT FOR THE CONTROL OF BACTERIAL BLIGHT DISEASE

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Verticillium chlamydosporium, Paecilomyces lilacinus, Aspergillus flavus, Arachniotus sp.. *Bacillus subtilis* and *Pseudomonas aeruginosa* were evaluated against actively multiplying culture (10^7 cfu/ml) of *Xanthomonas campestris* pv. *maivacearum* isolated from bacterial blight affected cotton leaves. Data recorded after 12, 2~. 36 and 48 hr revealed that all the fungal antagonists significantly retarded the multiplication of the pathogenic bacterium. *Verticillium chlamydosporium* and *Paecilomyces lilacinus* were found effective by producing 3.23 and 3.23 cm inhibition zones followed by *Aspergillus flavus* (2.53 cm), *Arachniotus sp.* (1.87 cm), *Bacillus subtilis* (0.22 cm) and *Pseudomonas aeruginosa* (0.15 cm) in that order. Fungal antagonists were more effective than bacterial antagonists. Spray of culture filtrates of the most effective antagonistic fungi on four commercially grown cotton varieties significantly reduced leaf shedding and number of bare nodes and increased number of boils, boil weight, number of healthy leaves and ultimately yield of cotton plant as compared to untreated control.

Key words: antagonistic organisms, bacterial blight, *Xanthomonas campestris* pv. *malvacearum*

INTRODUCTION

Bacterial blight of cotton first recorded near Multan in 1965 is one of the destructive diseases of this crop in Pakistan (Evans, 1976). It can reduce the yield of cotton crop up to 50% under favourable conditions of disease development (Hussain and Ali, 1975). Khan and Ilyas (1990) have reported 41.7% reduction in the yield of seed cotton under controlled conditions. The use of resistant varieties is the valid option in any of the disease management strategies 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. It may not be economical and may cause health hazards. Use of antibiotics has developed resistant mutant and resistant transfer factor in *Xanthomonas malvacearum* (Rangaswami, 1957; Verma et al., 1980). Disease management through biological agents has promising results in wheat. peanut. cotton and other crops (Verma et al., 1986: Weller and Cook, 1986; Randhawa et al., 1987: Stephen and AI-Din, 1987: Backman and Turner. 1989: Tabraiz and Hussain, 1989).

The objective of these studies was to evaluate some antagonistic microorganisms *against Xanthomonas campestris* pv. *malvacearum* which may be used to manage the disease in future.

MATERIALS AND METHODS

Cultures of *Verticillium chlamydosporium. Paecilomyces lilacinus* and *Pseudomonas aeruginosa* were obtained from the National Nematological Research Centre. Karachi (Pakistan) and were maintained at 25-30°C. The cultures of these fungi and bacteria were multiplied on corn meal agar (corn meal 20 g, dextrose 20 g. agar agar 20 g, and distilled water 1L) and on PDA (peeled potato 20 g, dextrose 20 g. agar agar 20 g and distilled water IL (Zaki and Maqbool. 1[illegible]) and on nutrient agar. The cultures of *Bacillus subtilis* .. *irachniotus* sp. and *Aspergillus flavus* were obtained from the Department of Plant Pathology. University of Agriculture . Faisalabad. Cotton leaves showing typical blight symptoms were washed with distilled water, surface sterilized with $HgCl_2$ (0.1 %) and then given three washings with distilled water and with sterilized cork borer. The discs obtained were ground in sterilized pestle and mortar From stock solution (10^7 cfu/ml. different dilutions up to 10^1. 10^1 and 10^2 were prepared by dilution plate technique. The suspension of culture of *Xanthomonas campestris* pv. *malvacearum* (isolated, purified and multiplied on nutrient agar medium) was mixed in the luke warm nutrient agar and 0.5 cm dia agar discs of actively multiplying cultures of each of the antagonistic organisms was placed in the centre of each petri plate (disc

sensitivity method or inhibition zone technique). All the petri plates were placed in a refrigerator for 24 hours and then incubated at 30°C. The experiment was conducted in completely randomized design with three replications and there were nine petri dishes in each' treatment. The data recorded on the inhibition zones after 12. 24. 36 and 48 hr was subjected to analysis of variance and LSD test was used to compare the treatment means. Treatments were designed as shown in Table I.

Preparation of Culture Filtrates: Cultures of *Verticillium chlamydosporium*, *Paecilomyces lilacinus*, *Aspergillus flavus* were purified and multiplied on corn meal agar and on PDA. The medium PD broth (100 ml in 250 m flask) was autoclaved at 15 psi for 15 minutes. Each flask was inoculated with 4 plugs/scoops of 5 mm diameter of the fungus from actively growing culture of the fungus under sterilized conditions and incubated at 25°C for 15 days. Some flasks were inoculated with the plugs of the medium only. At the end of the incubation period. the cultures were passed through sieve and were used for further experimentation. Four cotton varieties viz. 8-284. CIM-IIOO. CIM-435 and ClM-Uj() (moderately resistant. moderately susceptible and susceptible) (Khan and Rashid. 1999) were sown in earthen pots (25 cm dia) (cl) 5 seeds per. pot and were thinned to two plants per pot after germination under greenhouse conditions.

Leaves of the plants of each treatment were inoculated by spraying the abaxial surface with aqueous suspension of the bacterium (10^8 cfu/ml). At appearance of disease symptoms on 3-5 leaves of each plant. culture filtrates of each antagonistic microbe were sprayed with automizer. Data on growth parameters of the plant were recorded twice a week and analysed statistically.

RESULTS AND DISCUSSION

All the four fungi i.e. *Verticillium chlamydosporium*, *Paecilomyces lilacinus*, *Aspergillusflavus* and *Arachniotus* sp. significantly retarded the growth of the pathogenic bacterium as compared to *Bacillus subtilis* and *pseudomonas aeruginosa* which poorly inhibited the growth. Inhibition zones produced by *Verticillium chlamydosporium* and *Paecilomycess lilacinus* were 3.23 cm. followed by *Aspergillusflavus* (2.53 cm). *Arachniotus* sp. (1.87 cm). *Bacillus subtilis* (0.22 cm) and *Pseudomonas aeruginosa* (0.15 cm) in that order. *V chlamydosporium*, *P lilacinus* (Stephen and AI-Din. 1987) have been reported as nematophagous fungi. while *Archniotus* sp. (Ansar et al.. 1996) and *B. subtilis* (Backman and Turner.. 1989) *Pseudomonas* sp. (Weller and Cook. 1986) were reported as antagonistic against bacteria and fungi. Data revealed that the tested fungi gave significant results against the pathogenic bacterium. but at the same time antagonistic bacteria non-significantly retarded the growth of pathogen. Data revealed that maxi mum growth of the antagonistic fungi and bacteria was observed after 48 hr and there was no significant difference in the growth of fungi and bacteria before and after 48 hr. *Bacillus subtilis* appeared as a common contaminant during isolation and purification of the cultures of *Xanthomonas campestris* pv. *tnalvaccarum* but its anLlgonistic capability against *cantpestris* pv. *rnalvacearurn* was very poor. Effectiveness of fungal antagonists may be due to the toxins secreted by *V chlamydosporium* (Verticillin A. 8. C; Vcrmicillin and , Vermiculin), by *Paecilomyces lilacinus* (Paccilotoxins such as Leucinostain and Iilacin) and *Archniotus* sp. decompose the organic matter in the soil and arc known to produce antibiotics in the field/soil (Ansar et al.. 1)96). In another experiment carried out to determine the pathogenic effects of *Xanthomonas campcstris*. pv. *malvacearum* on various growth parameters and yield of cotton plant.. data revealed that when the plants of four cultivars were given different t reauncnts with fungal antagonistic microbes. the height of the plants was significantly different. 50.37. *49.7'1* and 5o(,O CIII in T_1. T_2 and T_3 as compared to control ('i7.'iX CIII) where none of the antagonistic microbe was applied and pathogenic bacterium caused necrosis of the leaf tissues. reduced leaf area. restricted supply of water and other nutrients.

Reduction in number. of leaves per plant in untreated plants (14.50 per plant) was significantb different as compared to treated plants (19.17. IX(,7 and 19,42 in T_1. T_2 and T_3 respectively. which were of course not significantly different from each other). I: *chlamydosporium*, *P lilacinus* and .I. *flavus* played an evident role in restricting the leaves to shed by antagonizing and lessening the activities ofthe pathogenic bacterium. In untreated control. reduct ion in number of leaves may be due to the damage bv the pathogen which after invasion. harbours the parenchymatous tissues resulting in disintegration of cells (Masscv, 19\ I: Casson et al., 1977: Safiyozov and Fattah, I97X) As disease progressed. this effect was also intcnsi fied by loss of photosynthetic area due to yellowing. necrosis and shedding of leaves. Pathogenic effect of the bacterium on the number of branches was non-significant in all the treatments and untreated control. This may be due to the systemic nature of the pathogen which led to loss of photosynthetic area' due 10 necrosis of the tissues. extensive leaf falling and reduced supply of water and nutrients to the apical growing parts possibly due to the blockage of xylem vessels b~

the parhogen in treated and untreated plants of all the four cultivars. Extensive leaf shedding supported greater number of bare nodes (14.33) as compared to those of treated plants (9.25. 10.08. and 9.58 in T\. T_2 and T) respectively).

Significant reduction in the yield of seed cotton of different cult ivars was observed in treated and untreated plants which was the outcome of development of severe systemic infection leading to the loss of photosynthetic area. necrosis. extensive leaf shedding. due to the supply of less water and nutrients. less retention of boils. shrinkage of boils and ultimately reduction in yield of untreated plants as compared to treated plants. In treated plants. there was greater number of boils per plant attaining more boil weight. This may be due to delayed establishment of pat hogcn in '\yleIII vessels. blocking it and enabling the plant to produce secondary leaves further away from the point of infect ion and ultimately increasing the yield of the plant The effectiveness of the antagonistic microbes mav be due to the toxin secreted by the respective fungal microbe as mentioned earlier. Difference in varicta I response to infection and recovery was also significantly different since the pathogen multiplied at a higher rate in a susceptible variety than in a resistant or an immune variety. This was also evident in 8-2x4 (moderately resistant). CIM-109 (susceptible). CIM-IIOO and CIM-435 (moderately susceptible) as reported by Khan and Rashid (1996).

Table 1. Effect of antagonistic microbes ;:,n the growth of *Xanthomonas cllmpestris* pv. *mllllllcellrum*

Treatments	Inhibition zones (cm) Time				
	12 he	24 he	36 hr	48 he	Mean
T1 = *Bacillus subti/is*	0.00 e	Q.oI1*e	0.20 e	0.22 e	0.13 e
T_2 = *Verticillium chlamydosporium*	0.00 c	1.83 e	3.07 e	3.23 a	2.03 a
T3 = *Psuedomonas aeruginosa*	0.00 e	0.00 e	0.12 e	0,15 e	0.07 e
T_4 = *Arachniotus* sp.	0.00 e	0.77 d	1,53 c	1.87 c	1.04 d
Ts = *Paecilomyces lilacinus*	0.00 e	0.87 d	2,50 b	3.23 a	1.65 b
T6 = *Aspergillus flavus*	0.00 e	0.67 d	1.97 c	2.53 b	1.29 c
To = *Untreated control*	0.00 e	0.00 e	0.00 e	0.00 e	0.00 e
LSD for time = 0.157					
LSD for treatments = 0.200					
LSD 'for time x treatment = 0:417					

* Mean values sharing the same letter do not differ significantly at P = 0.05.

Table 2. Effect of culture filtrate spray on the growth parameters of cotton plant [illegible] 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)
[illegible] = *Verticillium chlamydosporium*	50.37 b	19.17 a	4.91	9.25 b	3.67 a	2.04 a	7.48 a
T_2 = *Paecilomyces lilacinus*	49.73 b	18.67 a	4.50	[illegible]	3.50 a	1.96 ab	6.87 a
T_3 = *Aspergillus flavus*	50.60 b	19.42 a	4.75	9.58 b	3.33 a	1.69 bc	5.86 b
[illegible] = *Untreated control*	57.58 a	[illegible]	[illegible]	14.33 a	[illegible]	1.01 d	1.29 d
[illegible]	2.167	2.323	---	[illegible]	[illegible]	0.276	0.941

* Mean values sharing similar [illegible] do not differ significantly as determined by LSD test at [illegible].

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

[illegible]	[illegible] (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] = [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] = [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] = [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] = [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] = [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	---	[illegible]	---	---	[illegible]	---	[illegible]

* [illegible]

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