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COMPARATIVE EFFICACY OF GARLIC EXTRACT AND STREPTOMYCINE SULPHATE AGAINST *XANTHOMONAS CAMPESTRIS* PV. *CITRI* (HASSE) DYE *IN VITRO*, AND ON THE CONTROL OF CITRUS CANKER IN GREEN HOUSE

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Abstract

Streptomycine sulphate and garlic (*Allium sativum*) extract were tested at different concentrations against the multiplication of *Xanthomonas campestris* pv. *citri* on nutrient agar using dual culture assays. The effective concentration and their combination was then applied on the greenhouse grown citrus plants inoculated with the strain of bacterium tested in lab. The antibiotic and garlic extract inhibited the bacterial multiplication at all doses significantly ($P<0.05$) compared to control. Streptomycine sulphate at 1 % was most effective followed by 0.2 % and 0.1 % concentration against the multiplication *X. camp.* pv. *citri*. Garlic extract used at standard dose (S), S/5 and S/10 significantly inhibited the multiplication of *X. camp.* pv. *citri* compared to control but it was more effective when used with 0.1 % concentration of streptomycine sulphate. Thus streptomycine sulphate + *Allium sativum* at 0.1 % + S concentration, were the best in reducing the growth of bacterium followed by 0.1 % and S/5 and 0.1 % + S/10 combinations. Garlic extract proved effective *in vitro* but was not so effective on the inoculated plants grown in green house. However, its application at S/10 concentration plus 0.1 % concentration of streptomycine sulphate reduced 51.3 % canker disease over control compared to 60 % reduction in disease by the application of streptomycine sulphate alone (at 1 % concentration).

Introduction

Citrus canker caused by *Xanthomonas campestris* pv. *citri* (Hasse) Dye is a wide spread disease in the citrus growing areas of the world, reducing yield and market value of the fruits (Rangaswamy, 1962; Singh, 1998). This disease occurs commonly in citrus growing regions of the Punjab affecting leaves, twigs and fruits of plants (Hafiz & Sattar, 1952). In Punjab, citrus is mainly grown in the districts of Sargodha, Faisalabad, Sheikhupura, Multan, Sahiwal and Khushab (Khan, 1987). A survey conducted in three Tehsils of Faisalabad revealed 10-12 % incidence of citrus canker (Khan *et al.*, 1992). The incidence of canker disease has been recorded 8.78 % and 10.2 % at Postgraduate Agricultural Research Station and University of Agriculture, Faisalabad, respectively (Rehman, 1999).

To manage citrus canker, use of resistant rootstock is the best method. But durable host resistance is scarce in local/exotic citrus varieties, hence the chemical and biological control is the best alternative to manage disease. The use of chemicals and biocontrol agents to manage citrus canker has been reported (Pal & Chand, 1983; Leite *et al.*, 1987; Moses & Chandramohan, 1993; Masroor & Chaudra, 1995). Application of streptomycine sulphate and Agrimycin-100 (@ 0.2 % conc.) on inoculated citrus plants resulted in 41 and 31 % decrease in canker disease over control after 45 days (Khan *et*

al., 1992). Studies were carried out to compare various concentrations of garlic extract and streptomycine sulphate *in vitro* and on the citrus plants inoculated with *Xanthomonas campestris* pv. *citri*.

Materials and Methods

***In-vitro* evaluation of different concentrations of streptomycine sulphate and garlic extract against *X. campestris*:** Streptomycine sulphate at 1, 0.1 and 0.2 % and garlic extract (75 mg of garlic extract + 25 mL water), S/5 and S/10 were used. Sterilized Petri plates containing one mL suspension (having 10^8 cfu/mL) of *X. camp.* pv. *citri* were poured with 25 mL of sterilized luke-warm nutrient agar. The Petri plates were gently shaken to mix the bacterial suspension with nutrient agar and allowed to solidify. With the help of a sterilized cork borer, 1 cm diam. wells were made in the center of each Petri plate and the treatments were applied with the help of sterilized syringes. For each treatment there were three replications. These Petri plates were placed in refrigerator at 4 °C for 24 hours and then transferred to an incubator at 28±2 °C for 48 hours. The inhibition zones were recorded at 24, 36 and 48 hours interval after inoculation. Control was similarly treated except sterilized water poured in the wells. The data recorded on the inhibition zones were statistically analysed and the treatment means compared by Least Significant Difference Test (Steel & Torrie, 1980).

Evaluation of effective concentrations of antibiotic and plant extract against *X. camp.* pv. *citri* on greenhouse grown citrus plants: One year old healthy citrus plants were purchased from local nursery dealer and transplanted in pots (one plant/pot). These pots were placed outside in the greenhouse of Department of Plant Pathology, University of Agriculture, Faisalabad. After 15 days, streptomycin sulphate at 1 %, garlic extract at standard conc. and their combination (0.1 streptomycine sulphate + S/10 garlic extract) were sprayed on the adaxial surface of citrus plants. There were three replications for each treatment (2 pots/replication). After 24 hours of these treatments the plants were well irrigated and covered with polyethylene bags for two hours' humidity. Aqueous suspension of the bacterium prepared from actively growing culture of *X. camp.* pv. *citri* was inoculated with the help of spraying machine with a pressure of 1.1 kg/cm^2. The plants inoculated with pathogen only served as control. Data on the disease severity were recorded at 5 days interval according to a scale described by Croxall *et al.* (1952) and statistically analyzed.

Results and Discussion

Streptomycine alone or in combination with garlic extract significantly ($P \leq 0.05$) reduced the multiplication of *Xanthomonas campestris* pv. *citri* compared to untreated control (Table 1). Streptomycine sulphate at three concentrations produced almost similar effect (nonsignificant inhibition zones), however, garlic produced statistically different inhibition zones at standard, S/5, S/10 concentrations respectively. Garlic extract at the three concentrations produced significant inhibition zones compared to control. Garlic extract @ S/5 and S/10 when combined with 0.1 % concentration of streptomycine sulphate was significantly effective compared to its combination at standard dose with 0.1 % concentration of streptomycine sulphate (Table 1).

Table 1. *In vitro* evaluation of different concentrations of garlic extract and streptomycine sulphate against *Xanthomonas campestris* pv. *citri*.

Sr. No	Treatments	Conc.	Inhibition Zones (cm)	Percent decrease over control
1.	Streptomycine sulphate	1 %	4.03 a	44.78
2.	Streptomycine sulphate	0.2 %	3.70 ab	41.11
3.	Streptomycine sulphate	0.1 %	3.70 ab	41.11
4.	Streptomycine sulphate + garlic	0.1 + *S/10	3.43 ab	38.11
5.	Streptomycine sulphate + garlic	0.1 + S/5	3.30 ab	36.67
6.	Garlic	S	2.90 b	32.20
7.	Garlic	S/5	1.73 c	19.22
8.	Streptomycine sulphate + garlic	0.1 + S/5	1.57 c	17.44
9.	Garlic	S/10	1.57 c	17.44
10.	Control		0.00 d	----
	LSD		0.7749	

*Indicates standard concentration

Mean values sharing similar letters in a column do not differ significantly at $P < 0.05$

The bactericidal properties of streptomycine sulphate against *X. campestris* pv. *citri* have been reported by several research workers (Nirvan, 1960; Balaraman & Purusotman, 1981; Khan *et al.*, 1992). However, Leite *et al.* (1987) and El-Goorani, (1989) reported the ineffectiveness of this antibiotic against citrus canker disease. Pereira *et al.* (1981) used streptomycine + dihydrostreptomycin at 200 g/ha and achieved best results in controlling citrus canker disease. Khan *et al.* (1992) found that streptomycine sulphate reduced the multiplication of *X. camp.* pv. *citri in vitro* and gave 31.7 % decrease in disease severity on greenhouse grown citrus plants.

The efficacy of *Allium sativum* against citrus canker bacterium has been reported by Moses & Chandramohan (1993). After testing the garlic and streptomycine sulphate at different concentrations when applied singly or in combination on the inoculated citrus plants, there was a significant reduction in canker disease severity after 45 days in plants sprayed with streptomycine sulphate (1 %) or with combined application of the antibiotic (0.1 %) and garlic extract (S/10, Table 2). However, garlic extract alone, although proved effective *in vitro* was statistically ineffective in controlling citrus canker disease on green house grown plants. Streptomycine applied at 1 % concentration controlled 59.4 % disease severity over control. When this antibiotic was applied at 0.1% concentration alongwith S/10 garlic extract, canker disease was reduced to 51 % over control. This indicated fairly good compatibility of streptomycine sulphate with garlic extract in controlling citrus canker. To find out proper ratio of these combinations, further studies are required by including a suitable insecticide (to control citrus leaf minor) under field conditions.

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Table 2. Effect of *Allium sativum* and streptomycine sulphate on citrus canker disease development after inoculation with *Xanthomonas campestris* pv. *citri*.

S. No.	Treatments	Days after inoculation								Percent decrease over control
		10	15	20	25	30	35	40	45	
1.	*Allium sativum*	2.6 ijk	2.73 hij	3.0 fghi	3.40 defg	3.67 cde	4.07 bc	4.47 ab	4.60 ab	6.7
2.	Streptomycine sulphate (1 % conc.)	1.13 q	1.2 pq	1.33 opq	1.47 nopq	1.53 mnopq	1.73 klmnopq	1.93 klmno	2.00 klmno	59.4
3.	Streptomycine sulphate (0.1 %) + *Allium sativum*	1.6 mnopq	1.73 lmnop	1.87 lmnop	2.00 klmno	2.13 jklmn	2.20 jklm	2.40 ijkl	2.40 ijkl	51.3
4.	Control	2.87 ghi	2.93 ghi	3.33 efgh	3.60 cdef	4.00 bcd	4.47 ab	4.73 a	4.93 a	
	LSD									0.7121

*Mean values sharing similar letters do not differ significantly at 5 % level of significance at P <0.05

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