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In vivo Efficacy of some Antibiotics against Bacterial Blight of Pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*

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Abstract

A field trail was conducted in a farmer field at Madhugiri dist. Karnataka state to study the in vivo Efficacy of some Antibiotics Against Bacterial Blight of Pomegranate caused by Xanthomonas axonopodis pv. punicae. Five treatments consisting of antibiotics viz., Streptocycline (500ppm) +COC (0.3%), K-cycline (500ppm) + COC (0.3%), Ampiclox (500ppm) + COC (0.3%), Streptocycline (300ppm) + Ampiclox (500ppm) and Control were sprayed on five plants which form a replication. Among the different antibiotics tested a combination of Streptocycline (300ppm) + Ampiclox (500ppm) was proved to be more effective in reducing the disease incidence and severity with low percentage of leaves infected (3.70), less no of lesions per leaf (0.47), per cent leaf area affected (1.90), number of twigs infected (0.50) and low per cent fruit infection (0.70) followed by Streptocycline (500ppm) +COC (0.3%).

Keywords: *In vivo, a*ntibiotics, bacterial blight, *xanthomonas axonopodis* pv. *Punicae.*

Introduction

Pomegranate (*Punica granatum* L.) is known as the 'fruit of paradise' belongs to the family Punicaceae. It was grown since ancient times for its fruit, ornamental and medicinal purposes. Though it's native is Iran cultivated extensively in Spain, Morocco, Egypt, Iran, Afghanistan, Arabia and other Mediterranean countries. In India, pomegranate is commercially cultivated in Maharashtra, Karnataka, Andhra Pradesh, Himachal Pradesh[1]. In India it is grown in an area of 1.25 lakh hectares with 60.64 lakh tones of production. In Karnataka, it is grown in an area of 14,000 hectares with production of 1.5 lakh tonnes and productivity of 10 tonnes per hectare. Pomegranate occupies sixth place in the fruit export market of India, where it accounts for 50 per cent of world pomegranate production and earns foreign exchange to the tune of Rs. 270 crores[2].

Pomegranate is affected by various diseases caused by fungi, bacteria and nematodes. Among them bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* assumed epidemic form occurs in all pomegranate growing states resulting in abandoning of crop by the farmers. The disease at the initial stages cause small spots or chlortic lesions on the leaves later led to defoliation and on the fruits pathogen produces star shaped marks and cankerous lesions on stem and in severe cases plants dry leading to death of plants.

In recent years, due to severe/epidemic outbreak of the disease, many farmers have started uprooting the plants and destroying orchards and have incurred heavy losses. Thus, there is an urgent need to develop suitable management measures to mitigate the suffering of the farmers. Though, bacteriophages are discovered as early as 1917[3]. But, their potential as biocontrol agents has not been exploited so far except in epidemiology and detection of the disease. The use of bacteriophages for disease control is a quickly expanding area of plant protection with great potential to replace the recently wide spread chemical control measures in a number of plant diseases. Phages can be used effectively as a part of integrated disease management strategies. The relative case of preparing phage treatments and the low cost of production of these agents makes them a good candidate for widespread use in developing countries as well.

The bacteriophage to control bacterial diseases is quickly expanding area in management practices. The potential of bacteriophages to control bacterial blight of pomegranate was investigated and compared with antibiotics under field condition. Therefore under this scenario, bacteriophage seems to be ideal candidates to be exploited in management of bacterial diseases in view of the safety, renewable nature, cost effective and high target specificity. Hence, the present work was initiated to develop a potential biocontrol agent which is very effective, economical, eco-friendly and highly specific in the context of bacterial blight of pomegranate.

Material and Methods

The plants were sprayed with antibiotics at 15 days interval and observations were recorded on disease incidence and severity a day before spray and fourteen days after application of treatment. Field trail was carried out during Rabi season of 2007

in the farmer field in Madhugiri dist, Karnatka state. The following treatments were imposed on of five plants which constitute a replication. T1: Streptocycline (500ppm) +COC (0.3%) T2 : K-cycline (500ppm) + COC (0.3%) T3: Ampiclox (500ppm) + COC (0.3%) T4 : Streptocycline (300ppm) + Ampiclox (500ppm) T5: Control.

The plants were sprayed with antibiotics observations were recorded on disease incidence and severity *viz.,* as per cent leaves infected, number of lesions per leaf, per cent leaf area affected, number of twigs infected per plant, lesion size on the twigs, number lesions per fruit and per cent fruit infection were recorded a day before spray and fourteen days after application of treatments.

Results and Discussion

The percentage of leaves infected was low in Streptocycline (300ppm) + Ampiclox (500ppm) and Streptocycline (500ppm) + COC (0.3%) sprayed plants, which recorded 3.70 and 3.93 per cent leaf infection respectively after the 5th spray as compared to unsprayed control plants (58.00 per cent of leaf infection). Ampiclox (500ppm) + COC (0.3%) also recorded low percentage of leaf infection 4.53 per cent (table 1). While, K-cycline (500ppm) + COC (0.3%) recorded 6.00 per cent leaf infection. The data recorded on the number of lesions per leaf are presented in the table 2. After fifth spray, the mean number of lesions produced in Streptocycline (300ppm) + Ampiclox (500ppm) sprayed plants was 0.47 as compared to control (unsprayed plants) 8.33, while, Streptocycline (500ppm) + COC (0.3%) and Ampiclox (500ppm) + COC (0.3%) recorded 0.53 and 0.67 spots per leaf. K- cycline (500ppm) + COC (0.3%) recorded 0.87 spots per leaf. The per cent leaf area damaged was found to be low in plants sprayed with Streptocycline (300ppm) + Ampiclox (500ppm), Streptocycline (500ppm) + COC (0.3%) and Ampiclox (500ppm) + COC (0.3%) which recorded 1.90, 2.17 and 2.33 per cent of leaf infection respectively (table 3). Whereas, the plants sprayed with K- cycline (500ppm) + COC (0.3%), 2.47 per cent of leaf area infection. However, 45.33 per cent of leaf area was affected in unsprayed (control) plants after fifth spray.

The number of twigs infected per plant was found to be least (0.57 and 0.50) in Streptocycline (300ppm) + Ampiclox (500ppm) and Streptocycline (500ppm) + COC (0.3%) sprayed plants, after the fifth spray. Followed by Ampiclox (500ppm) + COC (0.3%) sprayed plants recorded 1.03 twig per plant (84.55 % reduction). While, 1.17 infected twigs in K- cycline (500ppm) + COC (0.3%) sprayed plants and 6.67 twigs in unsprayed plants after fifth spray (table 4). The data recorded infection on twigs is presented in the table 5. The lesion size on twig after fifth spray was found to be 0.70 cm in Streptocycline (300ppm) + Ampiclox (500ppm) and 0.83cm in Streptocycline (500ppm) + COC (0.3%) sprayed plants compared to unsprayed control plants which recorded 6.50 cm. Whereas, Ampiclox (500ppm) + COC (0.3%) sprayed plants recorded 1.03cm. While, 1.20cm of lesion size was recorded in plants sprayed with K- cycline (500ppm) + COC (0.3%) sprayed plants. Streptocycline (300ppm) + Ampiclox (500ppm) and Ampiclox (500ppm) + COC (0.3%) sprayed plants 0.67 and 0.87 lesions per fruit respectively. While, Streptocycline (500ppm) + COC (0.3%) sprayed plants recorded 0.90 lesions per fruit. K- cycline (500ppm) + COC (0.3%) also recorded less number of lesions per fruit (1.20) after fifth spray compared to control 3.57 lesions per fruit (table 6). The per cent fruit infection was found to be lowest in Streptocycline (300ppm) + Ampiclox (500ppm) and Ampiclox (500ppm) + COC (0.3%) sprayed plants after fifth spray which recorded 8.77 and 10.90 respectively. While, Streptocycline (500ppm) + COC (0.3%) recorded 12.82 per cent fruit infection. K- cycline (500ppm) + COC (0.3%) sprayed plants recorded 18.60 of per cent of fruit infection and 56 per cent fruit infection unsprayed plants after fifth spray (table 7).

Table-1
Effect of antibiotics on development of bacterial blight of pomegranate: Per cent leaves affected per plant

Treatments	Before spray	After 1st spray	After 2nd spray	After 3rd spray	After 4th spray	After 5th spray	Per cent reduction over control
Streptocycline (500ppm) + COC (0.3%)	38.00	33.00	25.00	15.00	9.25	3.93	93.22
K-cycline (500ppm) + COC (0.3%)	39.00	33.67	26.67	17.33	9.77	6.00	89.65
Ampiclox (500ppm) + COC (0.3%)	38.33	33.00	26.00	15.67	9.60	4.53	92.18
Streptocycline (300ppm) + Ampiclox (500ppm)	37.33	31.67	22.00	13.33	8.33	3.70	93.62
Control	38.50	41.00	45.33	50.33	52.00	58.00	-
SEm±		0.9661	0.9006	0.7601	0.4875	0.4875	
CD (5%)		2.2278	2.0769	1.7529	1.2410	1.1242	

Mean of 20 leaves (even numbered) from tip of branch in each plant. First spray was given three weeks after Etheral spray followed by fortnightly interval during month of November 2007.

Table-2
Effect of antibiotics on development of bacterial blight of pomegranate: Number of lesion per leaf

Treatments	Before spray	After 1st spray	After 2nd spray	After 3rd spray	After 4th spray	After 5th spray	Per cent reduction over control
Streptocycline (500ppm) + COC (0.3%)	3.30	3.00	2.50	1.80	1.23	0.53	93.63
K-cycline (500ppm) + COC (0.3%)	3.37	3.13	2.83	2.00	1.40	0.87	89.55
Ampiclox (500ppm) + COC (0.3%)	3.17	3.03	2.70	1.93	1.30	0.67	91.25
Streptocycline (300ppm) + Ampiclox (500ppm)	3.47	3.20	2.27	1.73	0.90	0.47	94.35
Control	3.37	4.43	5.33	5.93	6.90	8.33	-
SEm±		0.1140	0.1535	0.1033	0.1606	0.2787	
CD (5%)		0.2629	0.3539	0.2382	0.3702	0.6427	

Mean of 20 leaves (even numbered) from tip of branch in each plant. First spray was given three weeks after Etheral spray followed by fortnightly interval during month of November 2007.

Table-3
Effect of antibiotics on development of bacterial blight of pomegranate: Per cent leaf area affected

Treatments	Before spray	After 1st spray	After 2nd spray	After 3rd spray	After 4th spray	After 5th spray	Per cent reduction over control
Streptocycline (500ppm) + COC (0.3%)	26.43	22.67	16.67	12.50	5.67	2.17	95.21
K-cycline (500ppm) + COC (0.3%)	25.60	22.67	18.00	13.83	6.67	2.47	94.55
Ampiclox (500ppm) + COC (0.3%)	25.50	22.83	17.83	13.33	6.17	2.33	94.85
Streptocycline (300ppm) + Ampiclox (500ppm)	24.50	21.33	16.00	11.17	5.00	1.90	95.80
Control	25.00	27.00	31.67	37.00	40.00	45.33	-
SEm±		0.6368	1.1279	0.8882	0.7583	0.9266	
CD (5%)		1.4686	2.6010	2.0482	1.7486	2.1367	

Mean of 20 leaves (even numbered) from tip of branch in each plant. First spray was given three weeks after Etheral spray followed by fortnightly interval during month of November 2007.

Table-4
Effect of antibiotics on development of bacterial blight of pomegranate: Number of twigs infected per plant

Treatments	Before spray	After 1st spray	After 2nd spray	After 3rd spray	After 4th spray	After 5th spray	Per cent reduction over control
Streptocycline (500ppm) + COC (0.3%)	3.47	3.13	2.80	2.03	1.13	0.57	91.45
K-cycline (500ppm) +COC (0.3%)	3.93	3.73	3.33	2.63	1.70	1.17	82.45
Ampiclox (500ppm) + COC (0.3%)	3.97	3.63	3.23	2.53	1.40	1.03	84.55
Streptocycline (300ppm) + Ampiclox (500ppm)	4.00	3.40	2.93	1.93	1.03	0.50	92.50
Control	3.97	4.27	4.53	5.50	6.17	6.67	-
SEm±		0.1145	0.0972	0.2216	0.2234	02.2036	
CD (5%)		0.2640	0.2241	0.5110	0.5151	0.4695	

First spray was given three weeks after Etheral spray followed by fortnightly interval during month of November 2007.

Table-5
Effect of antibiotics on development of bacterial blight of pomegranate: Lesion size on twig (in cm)

Treatments	Before spray	After 1st spray	After 2nd spray	After 3rd spray	After 4th spray	After 5th spray	Per cent reduction over control
Streptocycline (500ppm) + COC (0.3%)	4.07	3.83	2.50	2.10	1.70	0.83	87.23
K-cycline (500ppm) + COC (0.3%)	4.20	3.97	3.57	3.23	2.03	1.20	81.53
Ampiclox (500ppm) + COC (0.3%)	3.97	3.77	2.67	2.37	1.80	1.03	84.15
Streptocycline (300ppm) + Ampiclox (500ppm)	4.10	3.70	2.33	2.07	1.47	0.70	89.23
Control	4.03	4.33	4.70	5.10	5.93	6.50	-
SEm±		0.1234	0.1738	0.1524	0.2014	0.1844	
CD (5%)		0.2845	0.4009	0.3514	0.4644	0.4252	

First spray was given three weeks after Etheral spray followed by fortnightly interval during month of November 2007.

Table-6
Effect of antibiotics on development of bacterial blight of pomegranate: Number of lesion per fruit

Treatments	After 3rd spray	After 4th spray	After 5th spray	Per cent reduction over control
Streptocycline (500ppm) + COC (0.3%)	2.37	1.93	0.90	74.78
K-cycline (500ppm) + COC (0.3%)	2.47	2.23	1.20	66.38
Ampiclox (500ppm) + COC (0.3%)	2.40	1.87	0.87	75.63
Streptocycline (300ppm) + Ampiclox (500ppm)	2.20	1.40	0.67	81.23
Control	2.40	3.10	3.57	-
SEm±	0.0753	0.0907	0.1033	
CD (5%)	0.1736	0.2091	0.2382	

First spray was given three weeks after Etheral spray followed by fortnightly interval during month of November 2007.

Table-7
Effect of antibiotics on the development of bacterial blight of pomegranate: Per cent fruit infection

Treatments	After 3rd spray			After 4th spray			After 5th spray			
	No. of fruits infected	Total No. of fruits per plants	% fruits infected	No. of fruits infected	Total No. of fruits per plants	% fruits infected	No. of fruits infected	Total No. of fruits per plants	% fruits infected	Per cent reduction over control
Streptocycline (500ppm) + COC (0.3%)	11.00	29.00	37.93	8.00	34.00	23.52	5.00	39.00	12.82	77.10
K-cycline (500ppm) + COC (0.3%)	12.00	35.00	34.28	9.00	40.00	22.50	8.00	43.00	18.60	66.78
Ampiclox (500ppm) + COC (0.3%)	16.00	38.00	42.10	9.00	54.00	16.66	6.00	55.00	10.90	80.53
Streptocycline (300ppm) + Ampiclox (500ppm)	15.00	37.00	40.54	8.00	56.00	14.28	5.00	57.00	8.77	84.33
Control	12.00	38.00	31.57	19.00	45.00	42.22	28.00	50.00	56.00	-
SEm±	0.6583	0.7746		0.8563	0.7149		1.0646	0.6912		
CD (5%)	1.5180	1.7862		1.9748	1.6486		2.4550	1.5940		

First spray was given three weeks after Etheral spray followed by fortnightly interval during month of November 2007

Similar trends were observed by spraying of antibiotics and botanicals under field conditions during *summer-kharif* season of 2002, Paushamycin, Streptocycline and K-cycline sprayed (500ppm) were very effective followed by Bactrinol in controlling the bacterial blight of pomegranate[4]. Similarly, spraying of streptocycline (500ppm) + Sufoof-E-Suneez 0.2 per cent and streptocycline (500ppm) + COC (0.3 per cent) followed with Ampiclox (500ppm) + Streptocycline (300ppm)

were found to be effective in reducing the disease incidence and disease severity of bacterial blight of pomegranate[5].

Conclusion

The combination of Streptocycline (300ppm) + Ampiclox (500ppm) was found to be highly effective followed by Streptocycline (500ppm) + COC (0.3%) Whereas, Ampiclox (500ppm) + COC (0.3%) and K- cycline (500ppm) + COC (0.3%) were found to next best in reducing the disease incidence and severity of bacterial blight of pomegranate, sprayed five times at fortnightly interval under field condition.

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