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In vitro Evaluation and Sensitivity of Antibiotics and Fungicides against *Xanthomonas axonopodis* pv. *punicae*

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A B S T R A C T

Cultivation of high yielding varieties of pomegranate with intensive care and management in the recent past under irrigated condition with early stage exploitation of plants has lead to certain severe pest and disease problems. Bacterial blight disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is one of the most destructive disease of pomegranate (*Punica granatum*) inflicting considerable quantitative and qualitative losses. Mostly the disease occurred on leaves, stems and fruits. Extensive agricultural practices along with large amount of pesticide as well as antibiotic are required to control this disease. The antibiotic sensitivity against eight isolates was studied by paper disk inhibition method. Streptomycine sulphate (250ppm) + COC (2500ppm) was significantly superior than all the treatments showing maximum inhibition zone in all eight isolates of *Xanthomonas axonopodis* pv. *punicae*.

Introduction

Pomegranate (*Punica granatum* L.) is a favourite table fruit in tropical and sub-tropical regions of the world which belongs to family *Punicae*. Among the major diseases leaf spot, fruit spot and will results in reduction of pomegranate fruit yield and put the growers in to hardship. Pomegranate grows very well on the moderately alkaline soils as well as slightly acidic soils. Bacterial blight infection results in appearance of water soaked oily spot symptoms on leaves, stems and fruits which consequently decreases fruit production and market value. The continuous presence of the pathogen in the orchard throughout the season has led to many fold speculations on possible survival of the pathogen on some alternate hosts grown in and around the garden. Severity of incidence

and losses varies among different isolates and influenced by existing climatic conditions and geographical distribution (Mondal and Sharma, 2009; Mondal and Singh, 2009; Petersen *et al.,* 2007; Mondal *et al.,* 2012).

Materials and Methods

Collection of diseased sample and isolation of pathogen

The bacterial blight diseased samples were collected from different districts of Maharashtra state. The isolate of pathogen was obtained from infected leaves of Pomegranate showing typical symptoms of bacterial blight by tissue isolation method. A bacterial suspension of each specimen was then cultured on Nutrient Sucrose Agar (NSA) medium. Following incubation, colonies similar to *Xanthomonas* were maintained on NSA medium at room temperature by adopting subsequent subculturing at periodical and regular intervals. Three days old cultures were used for further studies.

Pathogenicity test of the isolate

For this study the healthy seedling of Pomegranate cultivar 'Bhagwa' was obtained from the central nursery of state agriculture university P.D.K.V. Akola, Dist. - Akola, Maharashtra state. The seedling was grown under aseptic in vitro condition and veinlets of some leaves were injected with 48 hold bacterial suspension with the help of sterile syringe. Some plant leaves were injured by sharp needle and inoculated by spraying bacterial suspension. The inoculated plants were covered with polythene sheets and incubated for 10-12 days at 25 to 28^{o}C temperature. After 10– 12 days observations made for typical symptoms of bacterial blight on leaves, the organism was reisolated from artificially inoculated leaves and used for furtherantib1`acterial studies.

In vitro evaluation and sensitivity test of antibiotics & fungicides against *Xanthomonas axonopodi* spv. punice by Paper disc method

Different chemicals at concentration 100, 300, 500 ppm were evaluated in vitro applying inhibition zone technique (paper disc method) and using Nutrient Agar (N.A.) as basal culture medium. Fresh Nutrient Agar medium was prepared and dispersed in 100 ml quantities in conical flask (200 ml. Cap.), plugged and autoclaved at 15 lbs/cm2 pressure for 15-20 minute. The desired concentration of chemicals i.e. 100, 300, 500 ppm was prepared by using appropriate quantities of chemical required for 100, 300, 500 ppm concentration. In this desire concentration of chemicals 5 mm discs of Whatman No. 1 filter paper was dipped for few minutes. After sterilization of media, it was allowed to cool down to 35°C before pouring. Approximately 20 ml liquid media was poured in previously sterilized Petri plates and allowed them to solidify. Pouring of plates were always be done by using Laminar Air Flow cabinet under aseptic condition. After solidification of media of Petri plates, the bacterial suspension was spread on Nutrient Agar with glass spreader. After uniform spreading of bacterial suspension 5 mm disc of Whatman No.1 filter paper previously dipped in desired concentration of chemicals was placed in centre of medium. Three replication for each concentration of chemical was maintained. The paper disc soaked in sterile distilled water served as control Three replication for each concentration of chemical was maintained. The paper disc soaked in sterile distilled water served as control.

All these Petri plates after treatment were incubated at 28 ± 2°C for 48 hours. Observation on radial growth of test pathogen.

Result and Discussion

Isolation and pathogenicity test

Plates showing well separated, typical, yellow, mucoid, colonies of *Xanthomonas* bacterium were used to check pathogenicity on leaves of Pomegranate to confirm the isolate. After inoculation of pathogen in seedlings, seedlings were covered with plastic bags for maintain humidity for 3-4 days. The symptoms of the disease were developed within 10 to 18 days after inoculation on leaves small, water soaked, brown to black coloured lesions, which later on developed into angular to irregular shaped spots along the veins and veinlets of the leaf lamina. The re-isolation attempted from artificially infected / diseased plant tissues on Nutrient Agar consistently yielded *X. axonopodis* pv. punicae, thus fulfilling Koch's postulates and association of *X. axonopodis* pv. punicae with pomegranate was confirmed.

Pathogenic variability among different isolates of *Xanthomonas axonopodi* spv. punicae

Amongst eight isolates Xap-3 isolate had more lesion size i.e. 3.5 mm after 18 days of inoculation which showed water soaked circular to irregular dark brown spots with yellow halo symptoms followed by Xap-7 which showed 1.5 mm lesion size after 18 days after inoculation showing water soaked circular to irregular, light brown spots. The data is Presented in Table 1.

In vitro evaluation and sensitivity test of antibiotics & fungicides against *Xanthomonas axonopodispv. punice* isolates

The sensitivity of antibiotics and fungicides was tested against eight isolates of *Xanthomonas axonopodis pv.* punicae (Xap-1 to Xap-8) by Paper disc inhibition method and data is Presented in Table 2 and Fig. 2. The streptocycline used in different concentration viz; 100, 250, 500 ppm. The results (Table 2) revealed that lower concentration of streptocycline i.e. 100 ppm showed maximum growth inhibition in Xap-2 i.e 15.67 mm whereas the minimum growth inhibition was observed in Xap-1 i.e 6.00 mm. For the concentration 250 ppm, maximum inhibition was seen in Xap-8 (20.67mm) and minimum inhibition in Xap-7 (11.67mm). The higher concentration of streptocycline i.e. 500 ppm reported maximum inhibition in Xap-2 (23.67mm) and minimum inhibition in Xap-1 (18.67mm). The results showed that COC and Salicylic acid did not inhibit the growth which shows that isolates are not sensitive to COC and Salicylic acid. The results (Table 2, Fig 2) revealed that among the isolates Xap-6 (39mm) showed maximum growth inhibition whereas minimum growth inhibition was shown by xap-8 (28.67mm). Streptocycline 250ppm + COC 2500 ppm showed maximum growth inhibition in Xap-4 (24.67) and minimum inhibition in Xap-2 (21.67mm). The findings (Table 2, Fig. 2) revealed that Streptomycine sulphate 250ppm + COC 2500ppm (T8) was significantly superior over rest of the treatments showing maximum growth inhibition zone (39mm) in Xap-6, followed by Xap-2 (34.67mm). Whereas growth inhibition zone was minimum in Xap-8 (28.67mm).

Similar results observed by Abhang *et al.*, (2015). The efficacy of bioagents, botanicals and chemicals was studied by paper disc method. The Copper oxychloride (0.2%) + streptomycin sulphate (200 ppm) was found significantly effective in inhibiting growth of *Xanthomonas axonopodis pv.* citri. Raju *et al.*, (2012) carried out investigation to screen the different bactericides against *Xanthomonas axonopodis pv.* punicae. The inhibition zone of 3.3 cm was observed in the treatment of Streptocycline + Copper oxychloride.

Ambadkar *et al.*, (2015) studied in vitro efficacy of different antibiotics for management of bacterial blight and disease of pomegranate caused by *Xanthomonas axonopodis pv.* punicae. They found that antibiotic streptocycline showed maximum inhibition zone of 22.21 and 31.00 per cent at 250 and 500 ppm against *Xanthomonas axonopodispv.* punicae followed by tetracycline (18.26 and 27.53 %) and bacterinol (17.40 and 27.15 %). Abhang *et al.*, (2015), the efficacy of bioagents, botanicals and chemicals was studied by paper disc method. The Copper oxychloride (0.2%) + streptomycin sulphate (200 ppm) was found significantly effective in inhibiting growth of bacteria.

Table.1 List of different isolates collected from various villages of Maharashtra state

Sr. no	Name of isolate	Name of Village	Taluka	District	Pathogen	Affected plant part
1	Xap-1	Mardi	Man	Satara	*Xanthomonas axonopodispv. punicae*	Leaf
2	Xap-2	Anjangaon	Mhada	Solapur	*Xanthomonas axonopodispv. punicae*	Leaf
3	Xap-3	Agoti -1	Indapur	Pune	*Xanthomonas axonopodispv. punicae*	Leaf
4	Xap-4	Bherdapur	Newasa	Ahmednagar	*Xanthomonas axonopodispv. punicae*	Fruit
5	Xap-5	Satephal	Jafrabad	Jalna	*Xanthomonas axonopodispv. punicae*	Leaf
6	Xap-6	Kolara	Chikhali	Buldhana	*Xanthomonas axonopodispv. punicae*	Leaf
7	Xap-7	Ekamba	Malegaon	Washim	*Xanthomonas axonopodispv. punicae*	Leaf
8	Xap-8	Akola	Akola	Akola	*Xanthomonas auxonopodis*pv.punicae	Leaf

Sr. No	Treatment No	Name of chemicals	Concentration of chemicals (ppm)
1	**T1**	Streptomycin sulphate	100
2	**T2**	Streptomycin sulphate	250
3	**T3**	Streptomycin sulphate	500
4	**T4**	Streptocycline	100
5	**T5**	Streptocycline	250
6	**T6**	Streptocycline	500
7	**T7**	Copper Oxychlorite	2500
8	**T8**	Streptomycin sulphate + Copper oxychlorite	250 + 2500
9	**T9**	Streptocycline + Copper oxychlorite	250 + 2500
10	**T10**	Salicyclic acid	200
11	**Control**		

Table.2 Pathogenic variability among different isolates of *Xanthomonas axonopodis* pv. *punicae* on Pomegranate 45-60 day old seedlings

Sr. No	Isolates	Lesion size (mm)
		After 18 days
1	Xap1	3
2	Xap2	3
3	Xap3	3.5
4	Xap4	3
5	Xap5	2
6	Xap6	2
7	Xap7	1.5
8	Xap8	2

Table.3 *In vitro* evaluation and sensitivity test of antibiotics & fungicides against *Xanthomonas axonopodis* pv. *punice* isolates

Sr No	Name of treatment	Concentration (ppm)	Zone of inhibition (mm) * average of three replication							
			Xap1 (Satara)	Xap2 (Solapur)	Xap3 (Pune)	Xap4 (Ahmednagar)	Xap5 (Jalna)	Xap6 (Buldhana)	Xap7 (Washim)	Xap8 (Akola)
T1	**Streptomycin sulphate**	**100**	0.00	0.00	0.00	0.00	0.00	15.50	0.00	0.00
T2	**Streptomycin sulphate**	**250**	0.00	0.00	0.00	0.00	0.00	16.67	0.00	0.00
T3	**Streptomycin sulphate**	**500**	0.00	0.00	0.00	0.00	0.00	19.17	0.00	0.00
T4	**Streptocycline**	**100**	6.00	15.67	0.00	0.00	0.00	12.67	0.00	15.00
T5	**Streptocycline**	**250**	15.67	20.33	15.33	18.00	16.33	18.33	11.67	20.67
T6	**Streptocycline**	**500**	18.67	23.67	23.00	20.67	22.00	23.67	21.67	22.67
T7	**Copper Oxychlorite**	**2500**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	**Streptomycin sulphate + COC**	**250+2500**	31.33	34.67	31.00	34.00	33.00	39.00	30.67	28.67
T9	**Streptocycline + COC**	**250+2500**	24.00	22.33	21.67	24.67	24.00	24.00	23.00	23.33
T10	**Salicyclic acid**	**200**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T11	**Control**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F test			**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**
SE(M) ±			**0.95**	**0.97**	**0.57**	**0.68**	**0.37**	**0.66**	**0.64**	**0.31**
CD (P=0.1)			**3.80**	**3.88**	**2.29**	**2.74**	**1.48**	**2.63**	**2.57**	**1.26**

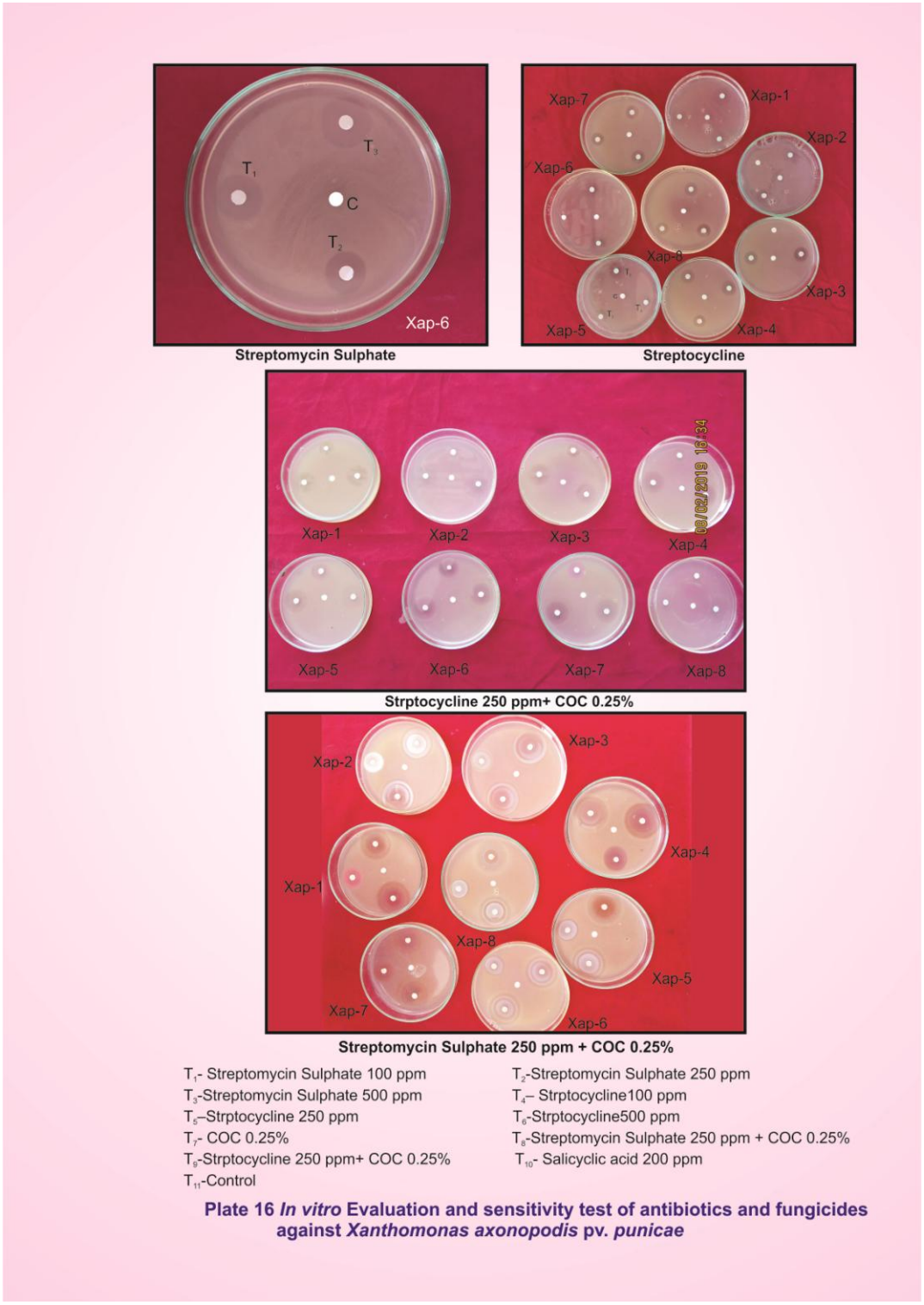
Fig.1 Pathogenicity test

Plate 8 Pathogenicity test of *Xanthomonas axonopodis* pv. *punicae* on Pomegranate seedlings

Plate 9 Pathogenicity test of *Xanthomonas axonopodis* pv. *punicae* on Fruit of pomegranate

Plate 10 Re-isolation of *Xanthomonas axonopodis* pv. *punicae*

Fig.2 *In vitro* evaluation and sensitivity test of antibiotics & fungicides against *Xanthomonas axonopodis* pv. *punice* isolates



T_1- Streptomycin Sulphate 100 ppm
T_2-Streptomycin Sulphate 250 ppm
T_3-Streptomycin Sulphate 500 ppm
T_4– Strptocycline100 ppm
T_5–Strptocycline 250 ppm
T_6-Strptocycline500 ppm
T_7- COC 0.25%
T_8-Streptomycin Sulphate 250 ppm + COC 0.25%
T_9-Strptocycline 250 ppm+ COC 0.25%
T_{10}- Salicyclic acid 200 ppm
T_{11}-Control

Plate 16 *In vitro* Evaluation and sensitivity test of antibiotics and fungicides against *Xanthomonas axonopodis* pv. *punicae*

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