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RESEARCH ARTICLE

In vitro evaluation of chemicals, botanicals and bioagents against the bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*

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ABSTRACT

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* has become potentially destructive disease. An investigation was carried out to screen the different bactericides, bioagents and botanicals to inhibit the pathogen. Among the different chemicals, Streptocycline + COC with an inhibition zone of 3.3cm exhibited superior efficacy followed by Streptocycline (2.80cm) and COC (2.65cm). From the botanicals Tulsi leaves followed by Neem seed oil, Garlic bulb extract and Patchouli leaves were found effective. From the different antagonists, it was observed that *Bacillus subtilis* and *Pseudomonas fluorescens* were found significantly superior over other antagonists in inhibiting the growth of the pathogen.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family Punicaceae, which is the native of Iran. It is regarded as the "Fruit of paradise". It is one of the most adaptable subtropical minor fruit crops and its cultivation is increasing very rapidly. In India, it is regarded as a "vital cash crop". Successful cultivation of pomegranate in recent years has met with different traumas such as pests and diseases. Among various diseases, bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* (Vauterin *et al*., 1995) is a major threat of pomegranate that reduces fruit quality to a greater extent.

MATERIALS AND METHODS

***Inhibition zone assay method* :**

The bacterium was multiplied by inoculating the culture into 20 ml of nutrient broth taken in 'Erleyenmayers' flask. The inoculated flasks were incubated at 30^{0}C for 72 hours. The bacterial suspension was then seeded to the lukewarm Nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petriplates and plates were allowed to solidify. The filter paper discs (Whatman No. 42) measuring 5 mm in diameter were soaked in the respective chemical solution for 5 minutes and transferred onto the surface of the seeded medium in Petriplates. The inoculated plates were kept in the refrigerator at 5^{0}C for 4 hours to allow the diffusion of chemical into the medium. Then plates were incubated at 30^{0}C for 72 hours and observed for the production of inhibition zone around the filter paper discs.

Four biocontrol agents *viz., Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Bacillus subtilis* were evaluated for their efficacy against the growth of *X. axonopodis.* pv. *punicae* by inhibition zone assay

method. The cultures / formulations of these biocontrol agents were obtained from Department of Plant Pathology, University of Agricultural Sciences, Dharwad and Institute of Organic Farming, Dharwad. A loopful culture of each of the antagonistic organism was placed in the centre of Petriplates containing the seeded medium. The inoculated plates were then incubated at 30^0C for 72 hours. Observations were recorded for the zone of inhibition produced by the antagonistic micro organisms around the growth of the pathogen.

For botanicals, fresh plant materials were collected and washed first in tap water and then in distilled water. 100 g of fresh sample was chopped and macerated in a surface sterilized pestle and mortar by adding 100 ml of sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth; filtrate thus, obtained was used as a stock solution. Ten, twenty, thirty and forty per cent each of plant extract was prepared by mixing 10, 20, 30 and 40 ml of stock solution with 90, 80, 70 and 60 ml of sterilized distilled water, respectively. To study the antibacterial mechanism of plant extracts, inhibition zone assay method was followed.

RESULTS AND DISCUSSION

Among different chemicals and their combinations, Streptocycline + copper oxychloride showed the highest inhibition (2.8 cm) followed by copper oxychloride (2.62 cm) which were on par with each other. All other chemicals *viz*., Streptocycline + copper hydroxide, copper hydroxide were found to be moderately effective but were significantly different from each other, Bacterinol and Kasagumycin were less effective and were on par with each other (Table 1). Between the concentrations of each chemical, efficacy was

Table 1: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis* pv. *Punicae,* causal agent of bacterial blight of pomegranate

Sr. No.	Trade name of the chemical	Concentration (ppm)	Mean diameter of inhibition zone (cm)
1.	Bacterinol	250	0.67 (1.29)*
		500	0.72 (1.31)
		750	0.82 (1.34)
2.	Copper oxychloride (coc)	1500	2.62 (1.90)
		2000	2.62 (1.90)
		2500	2.65 (1.91)
3.	Copper hydroxide	1500	1.77 (1.66)
		2000	1.82 (1.67)
		2500	1.82 (1.67)
4.	Kasagumycin	250	0.55 (1.24)
		500	0.55 (1.24)
		750	0.65 (1.28)
5.	Streptocycline	250	2.37 (1.83)
		500	2.60 (1.89)
		750	2.80 (1.94)
6.	Streptocycline + Copper oxychloride	250 +1500	2.40 (1.84)
		500 +2000	2.75 (1.93)
		750 +2500	3.30 (2.07)
7.	Streptocycline + Copper hydroxide	250 +1500	2.10 (1.76)
		500 +2000	2.17 (1.78)
		750 +2500	2.40 (1.84)
8.	Pathonil	2500	1.15 (1.46)
		5000	1.42 (1.55)
		7500	1.60 (1.61)
9.	Untreated control		0.00 (1.00)
		S.E.±	CD at 1%
		0.02	0.08

* Figures in parenthesis are transformed values $\sqrt{x+1}$

significant from lower to higher concentration with greater efficacy at higher concentrations.

Interaction effect among the chemicals and concentration indicated that, Streptocycline (750 ppm) + COC (2500 ppm) and Streptocycline at 750 ppm were highly effective with an inhibition zone of 3.3 cm and 2.8 cm, respectively followed by COC 2500 ppm. The moderately effective treatments were Streptocycline (750 ppm) + copper hydroxide at 2500 ppm (2.4 cm), copper hydroxide at 2000 ppm (1.82), Pathonil at 7500 ppm (1.6cm), Kasagumycin at 750 ppm (0.65cm), of which Pathonil and Kasagumycin were on par with each other. Bacterinol at 250 ppm (0.67cm) and Kasagumycin at 250 ppm (0.55cm) were also on par with each other. The present findings are in agreement with Sharma *et al.* (1981), who reported that the combination of Streptocycline and copper sulphate was most effective in inhibiting the growth of *Xanthomonas vesicatoria* as assessed by *in vitro* paper disc method. Manjula *et al.* (2002) also recorded the highest inhibition zone produced by Paushamycin (0.05%) against the growth of *Xanthomonas axonopodis* pv. *punicae*. Bactrinol (0.05%) and Bacteriomycin were the next best effective chemicals and Kasugamycin @ 500 ppm concentration was least effective.

Out of seven botanicals evaluated against *Xanthomonas axonopodis* pv. *punicae*, Tulsi leaf extract at 40 per cent concentration showed maximum inhibition (1.76 cm) followed by Neem seed oil (1.50cm) (Table 2). Garlic bulb, Meswak stem and leaves of patchouli extracts were found to be on par at 30 per cent concentration. However, custard apple seed extract and leaves and stem extract of adathoda showed no effect on *Xanthomonas axonopodis pv. punicae* at all concentration tested.

Interaction effect among the botanicals and concentration indicated that, Tulsi leaves was found to be most effective at 40 per cent with (1.76 cm) inhibition zone and the next best botanicals were Neem seed oil at 40 per cent concentration (1.50 cm) followed by Garlic bulb (1.10 cm). The moderately effective treatments were patchouli at 40 per cent concentration (0.88cm) and Meswak powder at 40% (0.89cm), where as Adathoda and custard apple were found ineffective. Srinivasachary (1995) reported Ocimum plant extract as most effective botanical against the growth of *Xanthomonas campestris* pv. *mori* isolated from mulberry. Similar results were found in the present investigation.

Table 2: *In vitro* evaluation of botanicals against *Xanthomonas axonopodis* pv. *Punicae*, causal agent of bacterial blight of pomegranate

Sr. No	Name of the botanical	Plant part used	Mean diameter of inhibition zone (cm) Concentration (%)			
			10	20	30	40
1.	*Adathoda vasica* (Adathoda)	Leaves and stem	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
2.	*Annanosa squamosa* (Custard apple)	Seeds	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
3.	*Alium sativum* (Garlic)	Bulb	0.80 (1.34)	0.82 (1.34)	0.85 (1.36)	1.10 (1.44)
4.	*Azadirachta indica* (Neem)	Seed oil	0.80 (1.34)	1.00 (1.41)	1.22 (1.48)	1.50 (1.58)
5.	*Salvadora persica* (Meswak)	Stem	0.63 (1.27)	0.70 (1.30)	0.84 (1.35)	0.89 (1.37)
6.	*Pogostemon cablin* (Patchuoli)	Leaves	0.67 (1.29)	0.72 (1.31)	0.82 (1.34)	0.88 (1.37)
7.	*Ocimum sanctum* (Tulsi)	Leaves	0.70 (1.30)	0.70 (1.30)	1.62 (1.61)	1.76 (1.66)
8.	Control		0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	Factors	S.E.±	C.D. at 1%			
	Botanicals	0.03	0.13			
	Concentration	0.02	0.09			
	Interaction	0.07	0.26			

* Figures in parenthesis are transformed values $\sqrt{x+1}$

Table 3 : *In vitro* evaluation of antagonists against the growth of *Xanthomonas axonopodis* pv. *Punicae*

Sr. No.	Antagonistic organism	Mean diameter of inhibition zone (cm)
1.	*Bacillus subtilis*	0.67 (1.29)
2.	*Psuedomonas flourescens*	1.77 (1.66)
3.	*Trichoderma viride*	0.00 (1.00)
4.	*Trichoderma harzianum*	0.00 (1.00)
5.	Control	0.00 (1.00)
	S.E.±	0.02
	C.D. at 1%	0.10

* Figures in parenthesis are transformed values $\sqrt{x+1}$

Study conducted on effect of antagonistic agent on growth of *Xanthomonas axonopodis* pv.*punicae* (Table 3) indicated that among the four antagonistic agents tested, *Pseudomonas flourescens* was found significantly superior in inhibiting the growth of organism (1.77 cm) followed by *Bacillus subtilis* (0.67 cm). However, *Trichoderm viride* and *Trichoderma harzianum* were found ineffective as they failed to inhibit the growth of *Xanthomonas axonopodis* pv. *punicae*. These findings are confirmed by the results of Unnamalai and Gnanamanickam (1984) who reported the inhibiting effect of *Pseudomonas fluorescens* on the growth of *Xanthomonas citri*.

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