INTERNATIONAL JOURNAL OF PLANT PROTECTION / VOLUME 6 | ISSUE 1 | APRIL, 2013 | 148-150

RESEARCH ARTICLE

Evaluation of bio-agents and antibiotics against *Xanthomonas axonopodis* pv. *punicae,* causal agent of bacterial blight of pomegranate

■ R.B. GAMANGATTI* AND M.B. PATIL

Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, RAICHUR (KARNATAKA) INDIA

ARITCLE INFO

Received : 14.12.2012
Revised : 03.04.2013
Accepted : 18.04.2013

Key **W**ords :
Xanthomoans axonopodis pv. *punicae*,
Bacterial blight,
Pomegranate,
Bioagents,
Antibiotics

*Corresponding author:
rajaniagri@gmail.com

ABSTRACT

In the present studies the antagonistic microorganisms and antibiotics were tested for their efficiency in inhibition of growth of *Xanthomonas axonopodis* pv. *punicae,* the incetant of bacterial blight of pomegranate. Among the different isolates of bioagents, *Pseudomonas florescens* (Pf4) was found significantly superior in inhibiting (15.73 mm) the growth of *X. a.* pv. *punicae* but remained on par with *Bacillus subtilis* isolates BS1 and BS2 with 12.1 and 13.66 mm, respectively. Whereas bioagents BS3 and Pf6 showed lower inhibitory zone of 0.76 and0.93 mm, respectively. The isolates of bioagents BS 4, Tv-16, Tv-R, Th-10 and Th-R recorded zero inhibition zone. Differences among the treatments and concentrations were found to be statistically significant except between streptocycline and streptocycline + $CuSO_4$. Among them, streptocycline was found to be the best and was significantly superior from the rest of the chemicals with mean inhibition zone of 25.75 mm in all the tested concentrations, followed by streptocycline + $CuSO_4$ (20.24) and bactinash-200 (11.74). Among different concentrations, 1000 ppm recorded the mean maximum inhibition zone (11.33 mm) followed by 750 and 500 ppm. Whereas the least inhibition was observed at 250 ppm (8.33 mm).

How to view point the article : Gamangatti, R.B. and Patil, M.B. (2013). Evaluation of bio-agents and antibiotics against *Xanthomonas axonopodis* pv. *punicae,* causal agent of bacterial blight of pomegranate . *Internat. J. Plant Protec.*, **6**(1) : 148-150.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a favourite table fruit in tropical and sub tropical regions of the world which belongs to family Punicaceae. In India, pomegranate is commercially cultivated in Maharashtra and small scale plantations are seen in Gujarat, Rajasthan, Karnataka, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Punjab and Haryana (Chadha, 2001). A plant with wider adaptability and benefits may also fall sick, which may be due to a pest or pathogen attack. Such sick plants grow and produce poorly. However, bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* is assuming serious proportion in view of the fact that the pathogen is present in a plant and translocates easily wherein the wilting of branches are seen one after another, ultimately the whole plant dries and dies. The disease causes spots on leaves leading to defoliation and fruit spots, and cankerous lesions on stem and in severe cases leading to death of plants. The disease has assumed its severity in all the growing areas of Maharashtra, Karnataka and Andhra Pradesh resulting in severe yield losses both in terms of quality and quantity. The information available on this disease, pathogen and management strategies are very meagre. In the present investigation, an attempt has been made to identify the best bioagent and antibiotic for the inhibition of growth of *Xanthomonas axonopodis* pv. *punicae* through inhibition zone method.

MATERIALS AND METHODS

The present investigation was carried out during 2008-2010 at the Department of Plant Pathology, College of Agriculture, Raichur University of Agricultural Sciences, Raichur. Antagonists isolated from rhizosphere soils of different crops were evaluated in the laboratory for their effectivity of inhibiting the growth of *X. a.* pv. *punicae* by using the inhibition zone method.

Collection of the samples :

The various parts of the plants such as leaves, fruits young branches and twigs showing symptoms were collected from the fields in pomegranate growing areas comprising Bellary, Koppal, and Raichur districts of Karnataka, for the purpose of isolation and identification of the pathogen. The inoculated plates were incubated at the 28^0C for 2 to 3 days. Observations were made for development of bacterial colonies on the plates.

Inhibition zone test :

A heavy suspension (3 days old) of *X. a.* pv. *punicae* multiplied in nutrient broth (20 ml) was mixed with lukewarm nutrient agar medium (1000 ml) contained in Erlenmayer's flask. Fifteen to twenty ml of seeded medium was poured into the sterilized Petriplates and allowed to solidify. A loopful culture of each of the antagonistic organism was placed in the centre of Petridishes containing the seeded medium. In case of fungal antagonists, mycelial discs of 5 mm size taken from actively growing culture were placed in the centre of the plates. The inoculated plates were then incubated at 30^0 C for 72 hours. Observations were recorded for the zone of inhibition produced by antagonistic microorganisms around the growth of the pathogen. A heavy suspension of *X. a.* pv. *punicae* 7×10^8cfu / ml was mixed with molten NA contained in 500 ml Erlenmeyer flask, so as to get a thick growth of bacterium on the medium. The seeded medium was poured in sterilized Petri plates and allowed to solidify. Previously sterilized filter paper discs (Whatman no.42) measuring five mm in diameter were soaked in different chemical solutions for five min and transferred on to the surface of the seeded medium contained in the Petriplates. Then plates were incubate at 30^0 C for 72 hours and observed for the production of inhibition zone around the filter discs.

RESULTS AND DISCUSSION

Among the different isolates of bioagents *Pseudomonas florescens* (Pf 4) was found significantly superior in inhibiting (15.73 mm) the growth *of X. a.* pv. *punicae* but remained on par with *Bacillus subtilis* isolate BS1 and BS 2 with 12.1 and 13.66 mm, respectively (Table 1). Whereas bioagents BS3 and Pf6 showed lower inhibitory zone of 0.76 and 0.93 mm, respectively. The isolates BS 4, Tv-16, Tv-R, Th-10 and Th-R recorded zero inhibition zone (Table 1). Among the different anatagonists tried as biocontrol agents in the present study, *Bacillus subtilis* and *Pseudomonas fluorescens* (both exhibiting on par efficacy with each other) were found significantly superior over other antagonists in inhibiting the growth of the pathogen. The fungal biocontrol agents *viz.*, *Trichoderma viride* and *Trichoderma harzianum* were found totally ineffective. In contrast to the report by earlier workers and findings of present investigation, Manjula (2002) examined the effective mechanism of *Pseudomonas flourescens* and *Bacillus subtilis* against *Xanthomonas axonopodis* pv. *punicae*.

Differences among the treatments and concentrations were found to be statistically significant except between

Table 1: *In vitro* efficacy of antagonistic organisms on the growth of *Xanthomonas axonopodis* pv. *punicae*

Sr. No	Bioagents	Inhibition zone (mm)
1.	*Bacillus subtilis* (BS1) (Maize rhizosphere)	12.1
2.	*Bacillus subtilis* (BS2) (Rice rhizosphere)	13.66
3.	*Bacillus subtilis* (BS3) (Indigenous)	0.76
4.	*Bacillus subtilis* (BS4) (Papaya rhizosphere)	0.00
5.	*Pseudomonas fluorescens (*Pf-4	15.73
6.	*Pseudomonas fluorescens (*Pf-6)	0.93
7.	*Trichoderma viride*(Tv-16)	0.00
8.	*Trichoderma viride* (Tv-R)	0.00
9.	*Trichoderma harzianum*(Th-10)	0.00
10.	*Trichoderma harzianum* (Th-R)	0.00
11.	Control	0.00
	S.E.±	0.3932
	C.D. at 1%	1.582

Table 2: *In vitro* evaluation of antibiotics and chemicals against *Xanthomonas axonopodis* pv. *punicae*

Sr.No.	Chemicals	Inhibition zone (mm) Concentration (ppm)				
		250	500	750	1000	Mean
1.	Streptocycline	24.00	25.30	26.00	27.33	25.75
2.	Streptomycin sulphate	0.00	0.00	0.00	0.00	0.00
3.	Streptocycline+$CuSO_4$*	18.00	18.33	20.66	24.00	20.24
4.	Copper oxychloride(COC)	0.00	0.00	0.00	0.00	0.00
5.	Bactinash -200	8.00	10.66	11.66	16.66	11.74
6.	Control	0.00	0.00	0.00	0.00	0.00
	Mean	8.33	9.21	9.72	11.33	9.62
Source			S.E.±		C.D. (0.01)	
Chemicals			0.120185		0.455887	
Concentration			0.098131		0.37223	
Chemical x Concentration			0.049065		0.186115	

* Both at same concentration

treatment streptocycline and streptocycline + $CuSO_4$ (Table 2). There was a significant difference between the chemical concentrations. Among them, streptocycline was found to be the best and was significantly superior from the rest of the chemicals with mean inhibition zone of 25.75 mm in all the tested concentrations, followed by streptocycline + $CuSO_4$ (20.24) and bactinash-200 (11.74). Among different concentrations, 1000 ppm recorded the mean maximum inhibition zone (11.33 mm) followed by 750 and 500 ppm. Whereas the least inhibition was observed at 250 ppm (8.33 mm) . *In vitro* evaluation of *Xanthomonas axonopodis* pv. *punicae* with different chemicals and antibiotics and combination of antibiotics and fungicides revealed that excellent control of *Xanthomonas axonopodis* pv. *punicae* was provided by the streptocycline alone and in combination with $CuSO_4$ followed by bactinash- 200. Among different concentrations of streptocycline, 1000 ppm provided the maximum inhibition zone. Combination of streptocycline and $CuSO_4$ provided the maximum inhibition. This indicated that copper enhanced the activity of antibiotics in reducing the disease. The inhibition of *Xanthomonas axonopodis* pv. *punicae* by the antibiotics was also supported by various workers (Namasivayam and Hedge, 1971: Verma *et al*., 1976: Wet and De, 1982; Bose and Singh, 1984, Balaraman, 1987) against species of *Xanthomonas.*

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 (2002) also recorded the highest inhibition zone produced by Paushamycin (0.05%) and K-cycline (0.05%) against the growth of *Xanthomonas axonopodis* pv. *punicae.* bactrinol (0.05%) and bacteriomycin were the least effective.

REFERENCES

Balaraman, K. (1987). New bactericides for citrus cancker control. *The Hindu,* August 19, 1987.

Bose, P.C. and Singh (1984). Chemical control of bacterial canker of mango. *Pesticides*, **14**: 30-31.

Chadha, K.L. (2001). Pomegranate. In: *Handbook of horticulture* (Eds.): Shashi A Verma and Somdutt, pp.297, Directorate of Information and Publications of Agriculture, ICAR, NEW DELHI, INDIA.1031pp.

Mahto, B.N., Singh, R.N and Singh,G.P. (1988). Response of rice bacterial blight (bb) pathogen *in vitro* to antibitics and fungitoxicants. *Internat. Rice Res. Notes*, **15**(1): 23.

Manjula, C. (2002). Studies on bacterial blight of pomegranate (*Punica granatum* L.) caused by *Xanthomonas axonopodis* pv. *punicae*. M.Sc. Thesis, University of Agricultural Sciences, Bengaluru, KARNATAKA (INDIA).

Namasivayam, L. and Hegde, R.K. (1971). Studies on the black rot of crucifers caused by *X.campestris. Mysore J. Agric. Sci.,* **5**:322-334.

Sharma, R.R.,Thind, B.S. and Singh, N. (1981).*In vitro* and *in vivo* evaluation of chemicals against *Xanthomonas vesicatoria*, the casual agent of bacterial leaf spot of chilli. *Indian J. Mycol. Pl. Pathol.*, **11**(2):178-181.

Verma, J.P., Singh, R.P. and Nayak, M.L. (1976). Laboratory evaluation of chemicals against *X. malvacearum* the incitant of bacterial blight if cotton. *Indian Phytopathol.*, **28**: 177-174.

Wet, T.H. and De (1982). Bacterial spot and blossom malformation. *Mango Growers' Association*, **2**:18-22.

****