

In vitro* Evaluation of Antibiotics, Chemicals and Bioagents against *Xanthomons axonopodis* pv. *glycines

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Effect of different antibiotics, chemicals and bio-agents was assessed *in vitro* by paper disc method and the antibiotics revealed that Streptocycline (750 ppm) with the inhibition of 2.69 cm was more effective and bactrinashak was least effective. Among chemicals Bordeaux mixture at 1 per cent recorded maximum inhibitory zone. Among the combination COC (0.3%) + streptocycline (750 ppm) was found significantly very effective than rest of the treatments with maximum inhibition zone of 3.41 cm. Among the seven bio-agents evaluated, *P. fluorescens* recorded highest inhibitory zone (2.63 cm) against *X. axonopodis* pv. *glycines* which were followed by *Bacillus amyloliquifaciens* (0.93 cm). PPFM-20 (0.89 cm) and PPFM-71 (0.88 cm) which are on par with each other.

Keywords: *In vitro*, Bactrinashak, Bordeaux mixture, *P. fluorescens*, *X. axonopodis* pv. *glycines*, PPFM strains.

Soybean (*Glycine max* (L.) Merrill) is a protein rich oilseed crop. Though, soybean is a legume crop but it is widely used as oilseed due to its poor cooking ability on account of inherent presence of trypsin inhibitor that limits its usage as pulse crop. It can supply the much needed protein to human diets, because it contains more than 40 per cent protein of superior quality and all the essential amino acids particularly glycine, tryptophan and lysine, similar to cow's milk and animal proteins. Soybean also contains about 20 per cent oil with an important fatty acid, lecithin, Vitamin- A and D. In India, annual yield losses due to various diseases are estimated as 12 per cent of total production. Over hundred pathogens are known to affect soybean, of which 66 fungi, six bacteria and eight viruses have reported to be associated with soybean seeds (Sinclair, 1978).

In India, it occupies an area of 10.02 million ha with a production of 11.64 million tonnes and productivity of 1062 kg per ha (Anon., 2015). Madhya Pradesh is often called as 'Soybean state' or 'Fort of soybean' due to its contribution to the extent of 70 per cent of area and 64 per cent of production. In Karnataka, it is grown on an area of 0.29 million ha with production of 0.24 million tones and productivity of 828 kg per ha (Anon., 2015).

MATERIALS AND METHODS

Antibiotics and chemicals

The bacterium was multiplied by inoculating a heavy suspension (1×10^5 cfu/ ml) into nutrient agar contained in 500 ml Erlenmeyer flask, so as to get the bacterium lawn on the medium. The inoculated flasks were incubated at $27 \pm 1^\circ\text{C}$ for 48 hr. The bacterial culture was seeded with nutrient agar. The seeded medium was poured into sterilized petriplates and allowed to solidify.

The previously sterilized filter paper discs (Whatman No. 1) were soaked separately in

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different bacterial solutions at specified concentrations for 10 minute and transferred onto the surface of the seeded medium at the center. Then to allowed the diffusion of chemical into the medium the plates were incubated at $27 \pm 1^\circ\text{C}$ for 72 hr. After incubation period was over, the plates were observed for the production of inhibition zone around the filter paper discs. The bacterium grown on nutrient agar without any chemicals served as control. The results obtained were analyzed statistically.

Bioagents

Trichoderma viride, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus amyloliquifaciens*, and Pink

Table 1. *In vitro* evaluation bioagents against *Xanthomonas axonopodis* pv. *glycines*

Bioagents	Inhibition zone (cm)
<i>Bacillus amyloliquifaciens</i>	0.93 (1.28)
<i>Bacillus subtilis</i>	0.82 (1.25)
<i>Bacillus subtilis</i> (Serenade 1)	0.53 (1.23)
<i>Bacillus subtilis</i> (Serenade 2)	0.68 (1.29)
Pink pigmented facultative methylotrophes (PPFM -20)	0.89 (1.37)
Pink pigmented facultative methylotrophes (PPFM -71)	0.88 (1.37)
<i>Pseudomonas fluorescens</i>	2.63 (1.90)
<i>Trichoderma harzianum</i>	0.00 (1.00)
<i>Trichoderma viride</i>	0.00 (1.00)*
S.Em±	0.02
CD at 1%	0.12

* $\sqrt{x+1}$ values

pigmented facultative methylotrophs (PPFM 20 and PPFM 71 strains) obtained from Institute of Organic Farming (IOF) and Department of Microbiology, UAS, Dharwad respectively were tested for their antagonistic activity against *Xanthomonas axonopodis* pv. *glycines* as evidenced by inhibition zone assay method. 300?l (5×10^5 cfu/ml) of this solution was seeded to nutrient agar and five mm diameter sterilized filter paper disc (Whatman No. 1) was soaked for ten minutes in the suspension of all bioagents and PPFM (a loopful of culture was mixed in 1 ml of sterile water). Sterilised filter paper discs soaked in sterile water served as control. Such discs were placed on the surface of the nutrient agar medium seeded with *X. a.* pv. *glycines*. Five replications were maintained. The inoculated plates were incubated at $28 \pm 1^\circ\text{C}$ for 72 h. The effect of antagonistic bacteria in inhibiting the growth of *X. a.* pv. *glycines* was detected by observing the zone of inhibition around the filter paper disc in each antagonistic bacteria in centimeter and the observation on zone of inhibition was recorded and analysed statistically.

RESULTS AND DISCUSSION

Antibiotics

The results on *in vitro* evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines* are presented in Table 9 and Plate 15. The results indicated that among the antibiotics screened at 250, 500 and 750 ppm. The 250 ppm concentration the maximum inhibition zone (2.50 cm) was recorded in Streptomycine sulphate which differed significantly from rest of the treatments

Table 2. *In vitro* evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines*

Antibiotics	Inhibition zone (cm)			
	Concentrations (ppm)			
	250	500	750	Mean
Agrimycine- 100	1.90 (1.70)	1.94 (1.71)	1.96 (1.72)	1.93 (1.71)
Bactrinashak	1.81 (1.67)	1.85 (1.68)	1.87 (1.69)	1.84 (1.68)
Streptomycine sulphate	2.50 (1.97) *	2.61 (1.97)	2.69 (1.99)	2.60 (1.83)
Source	S.Em±	CD at 1%		
Chemicals (A)	0.37	1.22		
Concentration (C)	0.12	0.84		
Interactions (A×C)	0.24	0.94		

* $\sqrt{x+1}$ values

followed by 1.90 and 1.81 cm in Agrimycine-100 and Bactrinashak. At 500 ppm concentration the inhibition zone increased in all the treatments. At 750 ppm, the maximum inhibition (2.69 cm) was recorded in Streptomycine sulphate which was on par with Streptomycine sulphate at 500 ppm (2.61 cm), followed by 1.96 cm and 1.87 cm in Agrimycine-100 and Bactrinashak. Among the different concentration tested, inhibition zone was on at 500 and 750 ppm irrespective of antibiotics used. The mean of all the concentration of antibiotics, revealed that the maximum inhibition zone was observed in Streptomycine sulphate (2.60 cm) followed by 1.93 cm and 1.84 cm in Agrimycine 100 and Bactrinashak.

The results on inhibition zone of different chemicals screened against *X. a.* pv. *glycines* are presented in Table 10 and Plate 16. The results revealed that the among the chemicals Boreaux mixture at 1 per cent recorded maximum inhibition zone (1.08 cm) followed by copper oxychloride at 0.3 per cent (0.94 cm). Copper oxychloride at 0.2

per cent (0.79 cm) and copper hydroxide at 0.3 per cent (0.76 cm) were moderate in inhibiting the growth of the pathogen. However, among the chemicals, combination tested the maximum inhibition (3.41 cm) was recorded in streptocycline at 750 ppm + copper oxychloride (0.3%) followed by same combination with reduced concentration, streptocycline at 500 ppm + copper oxychloride (0.2%) was 3.09 cm.

The results on *in vitro* evaluation of bioagents against *Xanthomonas axonopodis* pv. *glycines* are presented in Table 8, Plate 14. The maximum inhibition zone (2.63 cm) was recorded in *Pseudomonas fluorescens* which was followed by 0.93 cm and 0.89 cm in *Bacillus amyloliquifaciens* and Pink pigmented facultative methylotrophes (PPFM 20) strain. The other bioagents such as (Pink pigmented facultative methylotrophes) PPFM 71 and *Bacillus subtilis* recorded a inhibition zone of 0.88 cm and 0.82 cm respectively. The commercial formulation of *Bacillus subtilis*, (Serenade) at 1000 ppm 2000 ppm was least effective which recorded 0.53 cm and 0.68cm. There was no

Table 3. *In vitro* evaluation of chemicals against *Xanthomonas axonopodis* pv. *glycines*

Chemicals	Concentration (%)	Inhibition zone (cm)	Mean (cm)
Bordeaux mixture	0.5	0.88	0.98 (1.42)
		(1.37) *	
		1.08	
Copper hydroxide	0.2	(1.40)	0.71 (1.33)
		0.68	
		(1.29)	
Copper oxychloride	0.3	0.76	3.29 (2.07)
		(1.36)	
		0.79	
Streptocycline + Copper oxychloride	0.2	(1.32)	3.29 (2.07)
		0.94	
		(1.39)	
Streptocycline + Copper oxychloride	500 ppm+ 0.2	3.09	3.29 (2.07)
		(2.02)	
		3.41	
Source	SEm	(2.12)	CD at 1%
		0.03	
		0.06	
Chemicals (B)	0.03	0.10	
Concentration (C)	0.02	0.06	
Interactions (B×C)	0.04	0.13	

* $\sqrt{x+1}$ values

inhibition zone observed in case of *Trichoderma viride* and *T. harzianum*.

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