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RESEARCH PAPER

In-vitro evaluation of chemicals, antibiotics, selected nutrients and bio-agents against bacterial leaf spot of betel vine

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Abstract: An *in vitro* experiment was conducted on evaluation of chemicals, antibiotics, selected nutrients and bio-agents against bacterial leaf spot of betel vine caused by *Xanthomonas axonopodis* pv. *beticola* in department of Plant Pathology, College of Agriculture , University of Agricultural Sciences, Dharwad. *In vitro* evaluation of bactericides, bio-agents and selected nutrients against the pathogen served as a guideline for the field level management of bacterial leaf spot disease which was seriously affecting the betel vine production. Results revealed that, among those, K-cyclne at 750 ppm concentration with the inhibition zone of 25.33 mm, Bordeaux mixture at 3000 ppm concentration (12.00 mm), K-cycline at 500 ppm concentration with COC at 2000 ppm concentration (25.33 mm), $CuSO_4$ at 0.5 per cent concentration (5.60 mm) and *Pseudomonas fluorescens* (11.00 mm) were found superior over rest of antibiotics, chemicals, combination of antibiotics and chemicals, selected nutrients and bio-agents against *Xanthomonas axonopodis* pv. *beticola,* respectively.

Key words: Bacterial leaf spot, Betel vine, Antibiotic, Bordeaux mixture

Introduction

Betel vine (*Piper bitle* L.) is an important horticultural evergreen perennial climber having social, medicinal, religious and commercial values, mainly grown for its heart shaped deep green leaves. It belongs to the family Piperaceae which include black pepper and kava. The most probable place of origin of betel vine is central and eastern Malaysia (Chattopadhyay and Maity, 1967). The betel is mainly grown in tropical and subtropical region, with a shade and high moisture condition with relative humidity (40-80 %) and temperature ranging from 15-40ºC. The important varieties which are cultivated in Karnataka are kari yele, mysore yele and ambadi yele. In Karnataka, betel vine is grown in an area of 6988 ha with a production of 1, 31,1795 lakh leaves with a productivity of 18.86 lakh leaves per ha (Anon., 2012).

In all betel vine growing areas of India and other countries its growth and yield performance are being threatened by varied fungal and bacterial diseases. Among all the diseases, bacterial leaf spot of betel vine is one of the important disease which is becoming more severe in recent years. Bacterial leaf spot disease is caused by *Xanthomonas axonopodis* pv. *betlicola.* Characteristic symptoms of the disease are moist oily patches on underneath of leaves as start up symptoms, gradually they enlarge and turn into brown or black in color. Under high severity, these patches can spread to the stem resulting in shedding of leaves and nodes, consequently the plant will die. The disease can easily spread into surrounding vines. Bacterial leaf spot of betel vine widely spreads though rain splash and wind and causes significant yield loss. There is a need to develop integrated disease management (IDM) practice for mitigating the plant diseases. Hence, commercially available antibiotics, other antibacterial chemicals, bio-agents and botanicals were evaluated against the disease under *in vitro.*

Material and methods

The present investigation was carried out in the year of 2015-16, to evaluate the different antibiotics, chemicals and bio-agents to find out their effectiveness against *X. a. pv. betlicola* under *in vitro* condition. Commercially available antibiotics like Streptocycline, K-cycline and Bromopol (Immunomodulator) were used. Chemicals like Copper oxychloride and Copper hydroxide were used. The bio-agents like *Trichoderma viridae, Bacillus subtilis, Pseudomonas fluorescens* and some selected nutrients were tested against *Xanthomonas axonopodis* pv. *betlicola* by *in vitro* by inhibition zone assay and their concentrations are furnished below.

(A) Chemicals and antibiotics

Trade name	Antibiotics and chemicals name	Concentration (ppm)
Bordeaux mixture		2000, 2500, 3000
Blitox	Copper oxychloride	2000, 2500, 3000
Kocide	Copper hydroxide	2000, 2500, 3000
Streptocycline	Streptomycine sulphate (90% + tetracycline hydroxide 10%)	300, 500, 700
K-cycline	Streptomycine sulphate (90% + tetracycline hydroxide 10%)	300, 500, 700
Bromopol	2-Bromo-2-Nitro propane -1-3-diol	300, 500, 700
Streptocycline + copper oxychloride		(300 + 2000) (500 + 2000) (700 + 2000)
Streptocycline + copper		(300 + 2000)

Contd...

hydroxide	(500 + 2000)
	(700 + 2000)
K-cycline + copper	(300 + 2000)
oxychloride	(500 + 2000)
	(700 + 2000)
K-cycline + copper	(300 + 2000)
hydroxide	(500 + 2000)
	(700 + 2000)
Bromopol + copper	(300 + 2000)
oxychloride	(500 + 2000)
	(700 + 2000)
Bromopol + copper	(300 + 2000)
hydroxide	(500 + 2000)
	(700 + 2000)
Untreated control	-

(B) Selected nutrients

Selected nutrients	Source	Concentrations (%)
Zinc	$ZnSO_4$	0.1, 0.25, 0.5
Copper	$CuSO_4$	0.1, 0.25, 0.5
Manganese	$MnSO_4$	0.1, 0.25, 0.5
Calcium	$CaSO_4$	0.1, 0.25, 0.5
Boron	Sodium tetra borate	0.1, 0.25, 0.5
Magnesium	$MgSO_4$	0.1, 0.25, 0.5
Iron	$FeSO_4$	0.1, 0.25, 0.5

(C) Bio-agents

Bio-agents	Sources
Bacillus subtillis	IOF, UAS, Dharwad
Pseudomonas fluorescens	IOF, UAS Dharwad
Trichoderma viride	IOF, UAS Dharwad

A loopfull of 72 h old culture of *Xanthomonas axonopodis pv. betlicola (Xab)* was multiplied on NGA (15ml) in Petri plates was mixed with 5 ml distilled sterilized water solution of 300 µl was spread to NGA. Sterilized filter paper discs (Whatman No. 1) measuring 5 mm diameter were soaked in above mentioned concentrations of antibiotics, chemicals and bio-agents for 10 min and placed on the surface of the NGA medium which was seeded with *Xanthomonas axonopodis* pv. *betlicola.* The sterilized filter paper discs were dipped in sterilized water served as control. The inoculated plates were incubated at 27±1°C for 78 h.

Observations were recorded by measuring the diameter of the inhibition zone around the filter paper disc for each concentration and analysed statistically. The experiment was repeated twice. Each concentration was repeated thrice and discs were dipped in sterile water served as control.

Results and discussion

Results indicated that, among the antibiotics evaluated K-cycline had showed significantly superior over other treatments with highest inhibition of 21.23 mm at 700 ppm followed by K-cycline (18.83 mm) at 500 ppm and Streptocycline at 700 ppm (17.83 mm). Whereas the Bromophol showed least effective at 700 ppm (2.00 mm) and 500 ppm (1.0 mm) and at 300 ppm concentration Bromophol failed to produce any inhibition zone (Table 1). The effect of K-cycline and Streptocycline were on par with each other at all the concentrations. Interaction effect between the antibiotics and concentrations indicated that K-cycline at 700 ppm and 500 ppm were found significantly superior over other treatments with an inhibition zone of 21.33 and 18.83 mm, respectively. Among the antibacterial chemicals evaluated Bordeaux mixture had showed significantly superior over other treatments with highest inhibition of 12.00 mm at 3000 ppm followed by 11.00 mm at 2500 ppm and 8.33 mm at 2000 ppm. Whereas the effect of Copper oxychloride found least effective at 3000 ppm (7.33 mm), 2500 ppm (6.33 mm) and at 300 ppm (4.50 mm). Whereas Copper hydroxide was failed to inhibit *X. a*. pv. *betlicola* (Table 1). Interaction effect among the chemicals and concentrations indicated that Bordeaux mixture at all the concentrations was

Table 1. Evaluation of antibiotics and antibacterial chemicals on the growth of *Xanthomonas axonopodis* pv. *betlicola* under *in vitro*

Treatments			Mean inhibition zone (mm)	
Antibiotics	300 ppm	500 ppm	700 ppm	Mean
Streptocycline	16.00(4.12)*	16.83(4.22)	17.83(4.33)	16.88(4.22)
K-cycline	17.33(4.28)	18.83(4.45)	21.33(4.72)	19.16(4.49)
Bromopol	0.00(1.00)	1.00(1.41)	2.00(1.73)	1.00(1.41)
Untreated control	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
Source	S.Em±		C.D. at 1 %	
Treatment (T)	0.009		0.027	
Concentration (C)	0.008		0.024	
Interaction (T×C)	0.016		0.050	
Chemicals	2000 ppm	2500 ppm	3000 ppm	
Copper oxychloride	4.5(2.34)*	6.33(2.70)	7.33(2.88)	6.05(2.65)
Copper hydroxide	0.0(1.00)	0.0(1.00)	0.0(1.00)	0(1.0)
Bordeaux mixture	8.33(3.05)	11.00(3.46)	12.00(3.60)	10.44(3.38)
Untreated control	0(1.0)	0(1.0)	0(1.0)	0(1.0)
Source	S.Em±		C.D. at 1 %	
Treatment (T)	0.03		0.09	
Concentration (C)	0.02		0.07	
Interaction (T×C)	0.05		0.15	

* $\sqrt{x+1}$ transformed values

found significantly superior over other treatments. The results were in agreement with Kumar and Jahagirdar (2017), they reported that, 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 34.1 mm.

Among the different antibiotics and chemicals evaluated in combination against the growth of the pathogen, K-cycline (700 ppm) + COC (3000 ppm) was found significantly superior over other treatments with inhibition zone of 25.33 mm followed by K-cycline (500 ppm) + COC (3000 ppm) with inhibition zone of 23.83 mm followed by Streptocycline (700 ppm) + COC (3000 ppm) with inhibition zone of 22.33 mm. Efficacy between the concentrations was significant in all the chemicals at all the concentrations. Interaction effect between the combinations and concentrations was found significant in all the treatments at all the concentrations (Table 2). Similar results were reported by (Singh, 1996) Streptocycline 250 ppm was the most effective in combination with Bordeaux mixture and Copper oxychloride followed by Streptocycline and Deoxycycline 250 ppm alone. Among the bactericides, K-cycline was found effective against all isolates of *Xap*, which was significantly superior to rest of the bactericides and was followed by Streptocycline and Plantamuine. Least inhibition was found in Bronip and COC (Basama *et al.,* 2013) .

Among the seven nutrients tested, $CuSO_4$ has recorded highest an inhibitory zone of 5.60 mm which is significantly superior to other micro and secondary nutrients tested followed by $ZnSO_4$ (2.33 mm) and FeSO4 (2.08 mm). $MnSO_4$, $CaSO_4$, boron and $MgSO_4$ were failed to inhibit the pathogen. $CuSO_4$ has recorded maximum inhibition zone of 4.16 mm at 0.1 per cent, 5.50 mm at 0.25 per cent and 7.50 mm at 0.5 per cent concentrations respectively (Table 3). Basamma *et al.* (2013) reported that among the nine nutrients tested, $CuSO_4$ has recorded highest mean inhibitory zone of 8.9 mm which is significantly superior to other micro and secondary nutrients tested followed by micron special (boron, $MgSO_4$ and $ZnSO_4$) (4.1 mm) at all concentrations tested. $MnSO_4$, $CaSO_4$, boron, sulphur, $FeSO_4$ were failed to inhibit the pathogen *X. a.* pv. punicae. $CuSO_4$ has recorded inhibition zone of 6.0 mm at 0.05 per cent, 7.5 mm at 0.1 per cent, 9.5 mm at 0.2 per cent and 12.5 mm at 0.5 per cent concentrations respectively.

Among the three bio-agents evaluated, *Trichoderma viridae* was found ineffective against the pathogen and *Pseudomonas fluorescens* was found significantly effective with inhibition zone 11.00 mm followed by *Bacillus subtilis* with inhibition zone of 7.33 mm (Table 4). Among the three bio-agents,

Table 2. Evaluation of antibiotics and antibacterial chemicals in combination on the growth of *Xanthomonas axonopodis* pv. *betlicola* under *in vitro*

Treatments	Mean inhibition zone (mm)			
Combination	300 ppm + 2000 ppm	500 ppm + 2000 ppm	700 ppm + 2000 ppm	Mean
Streptocycline + copper oxychloride	19.16 (4.49)*	20.83(4.67)	22.33(4.83)	20.77(4.66)
K-cycline + copper oxychloride	21.00(4.69)	23.83(4.98)	25.33(5.13)	24.5(5.04)
Bromopol + copper oxychloride	4.50(2.34)	7.83(2.97)	9.66(3.26)	7.33(2.88)
Streptocycline + copper hydroxide	15.50(4.06)	7.33(4.28)	18.00(4.35)	16.94(4.23)
K-cycline + copper hydroxide	17.00(4.26)	19.00(4.47)	21.00(4.69)	19.00(4.47)
Bromopol + copper hydoxide	0.00(1.00)	1.00(1.41)	2.00(1.73)	1.00(1.41)
Untreated control	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.0)
Source	S.Em. ±		C.D. at 1 %	
Treatment (T)	0.04		0.12	
Concentration	0.02		0.08	
(C)Interaction (T×C)	0.07		0.21	

* transformed values

Table 3. Evaluation of micro and secondary nutrients on the growth of *Xanthomonas axonopodis* pv. betlicola under *in vitro*

Nutrients	Mean inhibition zone (mm)			
	0.1 %	0.25 %	0.5 %	Mean
$ZnSO_4$	0.00(1.00)*	2.00(1.73)	5.00 (2.45)	2.33(1.72)
$CaSO_4$	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
$MgSO_4$	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
$FeSO_4$	0.00(1.00)	2.10(1.76)	4.10(2.27)	2.08(1.67)
$MnSO_4$	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
$CuSO_4$	4.16(2.27)	5.50(2.48)	7.50(2.91)	5.72(2.55)
Boron	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
Untreated control	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
Source	S.Em. ±		C.D. at 1 %	
Nutrients (N)	0.06		0.12	
Concentration (C)	0.04		0.08	
Interaction (B×C)	0.10		0.22	

* transformed values

Pseudomonas fluorescence was found significantly effective followed by *Bacillus subtilis. Pseudomonas fluorescens* was found moderately effective in inhibiting the pathogen *Xanthomonas axonopodis* pv. *punicae* by producing inhibition zone of 10.8 mm, whereas, *Pseudomonas aeruginosa, Bacillus subtilis* and *Bacillus polymyxa* were not effective in inhibiting the pathogen (Jalaraddi, 2006). Borah *et al.* (2008) reported the effectiveness of *Bacillus subtilis* and *Pseudomonas fluorescens* in inhibiting the growth of *Xanthomonas axonopodis* pv. *betlicola* causing bacterial leaf spot of betel vine. *In vitro* screening of four bio-agents, *viz., Trichoderma harzianum, Aspergillus terreus, Bacillus subtilis* and *Pseudoonas fluorescens w*as done against *Colletotrichum capsici, Xanthomonas axonopodis* pv. *betlicola* and complex of *C. capsici* + *Xanthomonas axonopodis* pv. *betlicola. B. subtilis* significantly inhibited the growth of *C. capsici* (79.5%) and *Xanthomonas axonopodis* pv. *betlicola* (68.1%) and their complex (76.4%) (Deka *et al.,* 2008).

Conclusion

K-cycline was found effective against *Xanthomonas axonopodis* pv. *betlicola,* which was significantly superior to rest of the bactericides. Among the chemicals, Bordeaux mixture was found effective against *Xanthomonas axonopodis* pv. *betlicola*, which was significantly superior to rest of the chemicals. Among the antibiotics and chemicals in combination, K-cycline in combination with COC was found effective against *Xanthomonas axonopodis* pv. *betlicola.* Among bio-agents, *Pseudomonas fluorescens* was found very effective in inhibiting the growth of the pathogen. Among plant extracts, meswak extract was proved as very effective in inhibiting the maximum growth of the pathogen followed by garlic extract. Among nine nutrients, $CuSO_4$ proven has proven has best.

Table 4. Evaluation of bio-agents on the growth of *Xanthomonas axonopodis* pv. *betlicola* under *in vitro*

Treatments	Mean inhibition zone (mm)
Bacillus subtillis	7.33(2.91)*
Pseudomonas fluorescens	11.00(3.46)
Trichoderma viride	0.00(1.00)
Untreated control	0.00(1.00)
S.Em. ±	0.08
C.D. at 1 %	0.40

* $\sqrt{x+1}$ transformed values

References

Anonymous, 2012, *Horticultural Statistics of Karnataka state,* Department of Horticlture, Karnataka

Basamma, J., Benagi, V. I., Nargund. V. B. and Nirmalnath, J., 2013, *In vitro* prospects of *botanicals,* bio-agents and micronutrients in the inhibition of *Xanthomonas axonopodis* pv. *punicae* causing bacterial blight of pomegranate. *J. Pure Appl. Microbiol.*, 8 (1): p. 38.

Borah, P. K., Deka, U. K., Dutta, P. K. and Robin G., 2008, Management of leaf spot disease complex of betel vine by bio-agents and plant extracts. *Indian Phytopathol.,* 61 (3): 337-342.

Chattapdayay, S. P. and Maity, S., 1967, *Diseases of Betel Vine and Species,* ICAR, New Delhi. pp. 23-25.

Deka, U. K., Dutta, P. K., Robin, G. and Borah, P. K., 2008, Management of leaf spot disease complex of betel vine by bio-agents and plant extracts. *Indian Phytopathol.,* 61 (3): 337-342.

Jalaraddi, J. N., 2006, Biological control of Bacterial blight of pomegranate caused by *X. a.* pv. *punicae M. Sc. (Agri.) Thesis,* Univ. Agric. Sci., Bangalore, Karnataka (India).

Kumar, L. and Jahagirdar, S., 2017, *In vitro* evaluation of antibiotics, chemicals and bio-agents against *Xanthomonas axonopodis* pv. *glycines. Pl. Arch.*, 17 (1) : 742-744.

Singh, B. P. and Chand, J, N., 1971, Studies on diseases of betel (*Piper betle*) in Jabalpur. A. bacterial leaf spot caused by *Xanthomonas betlicola. Sci. Cult.*, 37: 209-210.

Singh, D., 1996, Chemical control of bacterial leaf spot of betelvine *(Piper betle* L.). *Rec. Hort.,* 3(1): 139-142.