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In-vitro Evaluation of Different Chemicals, Bioagents and Botanicals against *Xanthomonas citri* subsp *citri*

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Citrus canker caused by *Xanthomonas citri* subsp *citri* is one of the important disease in kagzi lime affecting the quality and market value of the fruits. The inhibitory activity of chemicals, bioagents and botanicals were evaluated against *Xanthomonas citri* subsp *citri* under in-vitro condition. Among the bactericides and fungicides tested 2-bromo- 2-nitropropane-1, 3-diol and copper hydroxide showed maximum inhibition of 16.39mm and 15.77mm respectively. However, the bioagents VK-6B and KK-3A recorded >90% inhibition among the thirteen bioagents evaluated. Similarly 21 different botanical were evaluated for their inhibition efficacy which revealed that Prosopis juliflora extract showed highest average inhibition of 10.83mm and 15.21mm in aqueous and alcoholic extracts respectively.

Keywords: Chemicals, Bioagents, Citrus canker, Botanical, *Xantomonas citri.*

Citrus canker caused by *Xanthomonas citri* subsp citri is a serious disease reducing the external quality of citrus fruits. It affects all types of citrus and severely infects on *Citrus aurantifolia* (lime). Canker occurs in all areas where lime grows in Karnataka state. Control of the disease requires integrated cultural practices and chemical sprays. Copper compound products are recommended for canker control. One major limitation of using chemical control agents is that phytopathogenic bacteria frequently develop a resistance to these compounds (Sigee,1993). In recent year much interest has been developed in the antimicrobial effects of medicinal plants and bacterial bioagents for plant disease control. Some plant extracts and bioagents were reported as effective inhibitors of phytopathogenic bacterial growth and was also suppressed by plant extracts and bioagents (Leksomboon et al,1998; Leksomboon et al,2000; Khodakaramian 2008; Garden et al,1978 and Grainge 1987). Plant extracts and bioagents are of interest as an alternative source of natural pesticide for controlling of plant pests.

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MATERIAL AND METHODS

The antibiotic compounds (2-Bromo – 2 nitropropane-1,3-diol, 2-Bromo – 2 nitropropane-1,3-diol (Immuno modulator), Kasugamycin, Plantamycin, Streptocycline and Validamycin) and fungicides (Carbendazim, Copper hydroxide, Copper oxychloride, Copper sulphate and Mancozeb) were evaluated in three different concentrations for their efficacy against *Xanthomonas citri* subsp *citri* by inhibition zone assay method.

The bacterium was multiplied by inoculating the culture into 250 ml of nutrient broth taken in 'Erleyenmayers' flask. The inoculated

flasks were incubated at 28±1°C for 48hr. The bacterial suspension was then seeded to the lukewarm nutrient agar medium. The seeded medium was poured into the sterilized petri plates and plates were allowed to solidify.

The chemicals and antibiotics were prepared at different concentrations as mentioned. The filter paper discs (Whatman No. 42) measuring 5mm in diameter were soaked in the respective chemical solution for 5-10 minutes and transferred onto the surface of the seeded medium in petriplates. The inoculated plates were kept in the refrigerator at 4 °C for 30 minutes to allow the diffusion of chemicals into the medium. Then plates were incubated at 28±1°C for 24 hr and observed for the production of inhibition zone around the filter paper discs. The results obtained were analysed statistically.

Similarly the bacterial antagonistic isolates maintained in the Department of Plant Pathology, College of Horticulture, Bagalkot *viz.*, were used to evaluate the efficacy of bioagents against *Xanthomonas citri* subsp *citri.*

The 13 isolates *viz.*, PM-1A, VK-6B, BK-6, KK-9A, VK-10C, BK-3, BK-5,BK-7, PM-2A, BK-8, BK-1L, KK-3 and CK-13A were tested using the dual culture technique, the 48 hr old pure culture of 13 bacterial bio-agents and *Xanthomonas citri* subsp *citri* were streaked in a single Plate containing NA medium with the help of inoculation loop. Two streaks of bio-agent were streaked first and the pathogen streaked at the middle of both streaks of bio agent. Later plates were kept for incubation at 30°C for 48 hours and finally the visual observation of inhibition of *Xanthomonas citri* subsp *citri* by bio-agent was recorded.

To identify the effective bioagents against *Xanthomonas citri* subsp *citri*, the scale developed by Ramesh 2015, which ranged from I to VI was used.

S. No.	Per cent inhibition of growth of *X. citri* subsp *citri* by bio-agent	Grade
1	>90	I
2	76-90	II
3	51-75	III
4	26-50	IV
5	1-25	V
6	0	VI

Aqueous extraction method

The plant extracts used in the study as mentioned in table 3 were collected and washed in the tap water and rinsed in the distilled water. The plant parts chopped in to small parts and taken fifty gram of sample and macerated in the surface sterilized pestle and mortar under aseptic condition by adding fifty ml of sterile water (1:1 w/v) and kept overnight at 4^0 C in a refrigerator for complete release of active component. After that the extract was filtered through two layered muslin cloth. The extracted solution was considered as stock solution of aqueous extract.

Alcoholic extraction method

Fifty gram of the of the respective plant parts was mixed with a fifty ml of ethyl alcohol and macerated in a pestle and mortar under aseptic condition. The sample was transferred to a beaker and kept overnight under refrigerated condition for evaporation of alcohol. The fifty ml of distilled water was added to make the 1:1 w/v. Finally the Alcohol extract was squeezed through double layered muslin cloth. The filtrate was collected used as stock solution of alcoholic extract.

The inhibition zone technique as followed in chemical evaluation were done to know the effect of botanicals on *Xanthomonas citri* subsp *citri* at different concentration of 1:1, 1:5 and 1:10. The Streptocycline 0.5g (500ppm) + Copper oxychloride 3g (0.3%) per liter of water treated as positive control and untreated paper discs considered as negative control.

RESULTS

The *in-vitro* evaluation of different antibiotics in inhibiting the growth of *Xanthomonas citri* subsp *citri* were assessed by paper disc method and results are presented in Table 1. Among the different antibiotics, 2-Bromo-2 nitropropane-1, 3-diol (14.67, 16.33 and 18.17mm) and Streptocycline (13.50, 15.00 and 16.33mm) showed highest inhibition at 300, 400 and 500ppm. Among the fungicides copper hydroxide (14.33, 15.67 and 17.33mm) and copper oxy chloride (11.00, 13.33 and 14.83mm) recorded highest inhibition at 1500, 2000 and 2500ppm respectively. Whereas plantamycin and copper sulphate recorded average values of 12.50 mm and 3.88mm respectively. However 2-Bromo – 2 nitropropane-1,3-diol

Table 1. Effect of antibacterial chemicals against *Xanthomonas citri* subsp *citri* under *in vitro* condition

Treatments	Antibacterial chemicals	Mean diameter zone of inhibition (mm)			Average
	Bactericides	300 ppm	400 ppm	500 ppm	
T_1	2-Bromo – 2 nitropropane-1,3-diol	14.67 (3.89)	16.33 (4.10)	18.17 (4.32)	16.39
T_2	2-Bromo – 2 nitropropane-1,3-diol (Immuno modulator)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_3	Kasugamycin	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_4	Plantamycin	10.50 (3.31)	12.67 (3.62)	14.33 (3.85)	12.50
T_5	Streptocycline	13.50 (3.74)	15.00 (3.93)	16.33 (4.10)	14.27
T_6	Validamycin	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_7	Control	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
	SEm±	0.04	0.04	0.05	
	CD (0.01)	0.09	0.14	0.18	
	Fungicides	**1500 ppm**	**2000 ppm**	**2500 ppm**	
T_1	Carbendazim	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_2	Copper hydroxide	14.33 (3.85)	15.67 (4.02)	17.33 (4.22)	15.77
T_3	Copper oxychloride	11.00 (3.38)	13.33 (3.71)	14.83 (3.91)	13.05
T_4	Copper sulphate	0.00 (0.70)	4.33 (2.19)	7.33 (2.79)	3.88
T_5	Mancozeb	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_6	Control	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
	SEm±	0.04	0.05	0.02	
	CD (0.01)	0.16	0.21	0.15	

Note: Figures in parentheses indicate Square root transformed values

(Immuno-modulator), kasugamycin, validamycin, carbendazim, mancozeb and untreated control recorded no inhibition zone in all the concentrations tested.

The results of the dual culture technique indicated that all 13 isolates inhibited growth of *Xanthomonas citri* subsp *citri*. A maximum inhibition of >90% was recorded by two isolates VK-6B (*Lysinibacillus xylanilyticus)* and KK-3A of bacterial bio-agents. Eight isolates *viz* PM-1A, KK-9A, VK-10C, BK-5, BK-3, CK-13A, PM-2A and BK-1L recorded 76-90% inhibition whereas three isolates BK-6, BK-8 and BK-7 recorded 26.-50% inhibition. The results are presented in Table 2.

The data on *in-vitro* evaluation of botanicals on the growth of *Xanthomonas citri* subsp. *citri* is presented in Table 3. In aqueous extraction method the results showed difference among the treatments. Among the botanicals tested prosopis with concentration of 1:1, 1:5 and 1:10 w/v was found to be most effective with the highest inhibition of 14.00, 10.00 and 8.50 mm

respectively followed by Kokum (13.50, 8.50 and 6.50) and Soapnut (11.00, 7.50 and 5.50 mm) respectively. There was no inhibition observed in remaining treatments. However positive check Streptocycline @ 500ppm + Copper oxy chloride @ 3000ppm showed maximum inhibition of 21.50 mm and found significantly different from all other botanicals tested.

The alcoholic extracts of 21 botanicals were evaluated against *Xanthomonas citri* subsp *citri* under *in-vitro* condition and results are presented in Table 4. The similar trends of results were obtained as in case of aqueous extraction. The results revealed that among all the botanicals, prosopis with concentration of 1:1, 1:5 and 1:10 w/v was found to be most effective with the maximum inhibition of 18.50, 15.50 and 11.63mm respectively. Prosopis treatment was followed by Kokum at 1:1, 1:5 and 1:10 w/v recorded inhibitory zone of 18.50, 13.00 and 10.50 mm respectively. However soapnut recorded inhibitory zone of 16.50, 12.50 and 8.50 mm at 1:1, 1:5 and 1:10 w/v concentrations respectively. There was no inhibition of bacterial growth in the remaining treatments at all the three concentrations. The standard check Streptocycline 500ppm + Copper oxychloride 3000ppm recorded maximum inhibition of 21.50 mm which found superior to all the botanicals tested. Whereas water control recorded no inhibition zone

Table 2. *In-vitro* evaluation of antagonistic bacterial bio-agents against *Xanthomonas citri* subsp *citri*

S. No.	Code of the bio agents	Grade (based on % inhibition bybio agent)
1	PM-1A	II
2	VK-6B (*Lysinibacillus xylanilyticus)*	I
3	BK-6	IV
4	KK-9A	II
5	VK-10C	II
6	KK-3A	I
7	BK-5	II
8	BK-3	II
9	CK-13A	II
10	BK-8	IV
11	PM-2A	II
12	BK-7	IV
13	BK-1L	II

DISCUSSION

A total of eleven chemicals were evaluated for their efficacy against *Xanthomonas citri* subsp *citri* by paper disc method and the mean inhibition diameter was recorded. Highest inhibition was noticed in 2-Bromo-2 nitropropane-1,3-diol (14.67, 16.33 and 18.17mm) and Streptocycline (13.50, 15.00 and 16.33mm) at 300, 400 and 500ppm respectively, followed by copper hydroxide (14.33, 15.67 and 17.33mm) and copper oxy chloride (11.00, 13.33 and 14.83) which recorded highest inhibition at 1500, 2000 and 2500ppm respectively.

Streptocycline is a protein inhibitor whereas streptomycin sulphate play role in interfering with formyl-methionine tRNA and binds to 30s RNA whereas tetracyclin hydrochloride interferes with amino-acyl tRNA. However 2-Bromo – 2 nitropropane-1,3-diol interacts with cysteine, cysteine methyl ester and glutathione in the presence of air and acts as catalyst for oxidation of thiol groups of disulfides (Julia 1988).

The present findings were supported by shahid *et al.* (2005) who evaluated the efficacy of different chemicals against *Xanthomonas campestris pv. citri* with varied concentration of 0.01, 0.1 and 1% to check the multiplication of bacteria by paper disc method and found that Agrimycin –100, Streptomycin sulphate, Vitavax and Dithane M–45 had maximum inhibiting capacity with 2.47, 2.28, 2.38 and 2.32 cm respectively.

Shahbaz *et al.* (2007) studied the efficacy of Agrimycin–100, Cupravit, Bavistin, Dithane M-45, Vitavax, Daconil, Antracol, Benlate and Nimrod at 1% concentration in *in-vitro* condition against *Xanthomonas campestris pv. citri*. Among which Agrimycin–100 was found to be the most effective chemical with 2.89cm of inhibition.

Similar studies were also reported by Rashid *et al.*, 2014 on *in-vitro* evaluation of six selected antibacterial chemicals *viz.* Cupravit 50 WP (Copper oxy chloride), Sulcox 50 WP (Copper oxy chloride), Champion 77 WP (Copper hydroxide), Indofil M-45 (Mancozeb), Dithane M-45 (Mancozeb) and Bavistin 50 WP (Carbendazim) where Indofil-M45 recorded maximum inhibition of *Xanthomonas axonopodis* pv. *citri*

Thirteen isolates were examined against *Xanthomonas citri* subsp *citri* for their efficacy in

Table 3. Effect of aqueous extracts of selected botanicals against *Xanthomonas citri* subsp *citri* under *in-vitro* condition

Treatments	Plant extracts	Mean diameter zone of inhibition (mm)			Average
		1:01	1:05	1:10	
T_1	*Adathoda vasica*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_2	*Calotropis gigantean*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_3	*Cinnamomum verum*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_4	*Cymbogon winteranius*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_5	*Eugenia caryophyllata*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_6	*Eugenia caryophyllata*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_7	*Eucalyptus citriodora*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_8	*Allium sativum*	12.00 (3.54)	0.00 (0.70)	0.00 (0.70)	4.00
T_9	Kalia bith	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{10}	*Garcinia indica*	13.50 (3.74)	8.50 (3.68)	6.50 (2.64)	9.50
T_{11}	*Lantena camera*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{12}	*Cymbopogon citrates*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{13}	*Salvadora persica*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{14}	*Azadiracta indica*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{15}	Nitro sulph	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{16}	Phosphorika	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{17}	*Pongamia pinnanta*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{18}	*Prosopis juliflora*	14.00 (3.81)	10.00 (4.00)	8.50 (3.00)	10.83
T_{19}	*Sapindus saponaria*	11.00 (3.39)	7.50 (3.61)	5.50 (2.44)	8.00
T_{20}	*Ocimum basilium*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{21}	Control	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{22}	Streptocycline + copper oxychloride	21.5 (4.69)			
	SEm±	0.05	0.05	0.04	
	CD (0.01)	0.15	0.12	0.11	

Note: Figures in parentheses indicate Square root transformed values 1:1, 1:5 and 1:10 = Concentrations (1 part of crude extract and 1, 5 and 10 part of water)

Table 4. Effect of alcohol extracts of selected botanicals against *Xanthomonas citri* subsp *citri* under *in-vitro* condition

Treatments	Plant extracts	Mean diameter zone of inhibition (mm)			Average
		1:01	1:05	1:10	
T_1	*Adathoda vasica*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_2	*Calotropis gigantean*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_3	*Cinnamomum verum*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_4	*Cymbogon winteranius*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_5	*Eugenia caryophyllata*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_6	*Eugenia caryophyllata*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_7	*Eucalyptus citriodora*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_8	*Allium sativum*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_9	Kalia bith	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{10}	*Garcinia indica*	18.50 (4.30)	13.00 (3.68)	10.50 (3.32)	14.00
T_{11}	*Lantena camera*	16.50 (3.61)	11.00 (3.39)	0.00 (0.70)	9.16
T_{12}	*Cymbopogon citrates*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{13}	*Salvadora persica*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{14}	*Azadiracta indica*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{15}	Nitro sulph	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{16}	Phosphorika	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{17}	*Pongamia pinnanta*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{18}	*Prosopis juliflora*	18.50 (4.36)	15.50 (4.00)	11.63 (3.48)	15.21
T_{19}	*Sapindus saponaria*	16.50 (4.13)	12.50 (3.61)	8.50 (3.00)	12.50
T_{20}	*Ocimum basilium*	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{21}	Control	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
T_{22}	Streptocycline + copper oxychloride	21.5 (4.69)			
	SEm±	0.04	0.06	0.04	
	CD (0.01)	0.11	0.20	0.11	

Note: Figures in parentheses indicate Square root transformed values 1:1, 1:5 and 1:10 = Concentrations (1 part of crude extract and 1, 5 and 10 part of water)

inhibiting the growth of the pathogen, in which two isolates VK-6B (*Lysinibacillus xylanilyticus*) and KK-3A showed maximum inhibition (>90%) and were scaled as grade I. Eight isolates *viz* (PM-1A, KK-9A, VK-10C, BK-5, BK-3, CK-13A, PM-2A and BK-1L) recorded 76-90% inhibition with II grade. The last three isolates BK-6, BK-8 and BK-7 recorded 26.-50% inhibition with IV grade.

The antagonistic activity of ten strains of *Pseudomonas fluorescens* and five strains of *Pseudomonas putida* were evaluated against *Xanthomonas axonopodis pv. citri*, under *in-vitro* condition. The strain *Pseudomonas fluorescens* - 19 had recorded a maximum inhibition with 6.40cm and there was no inhibition by the *Pseudomonas putida* strain 8, 13 and 18. The variation in the antagonistic activity of bacterial isolates might be due to the difference in the mode of action and mechanism (Khodakaramian *et al.*, 2008).

The study is supported by Mohammed *et al.* (2014) where a total of 22 potential bacterial antagonists isolated as epiphytes from the phylloplane of healthy citrus trees were screened for their *in vitro* efficacy against *Xcc*. These strains were identified as *Pseudomonas fluorescens* on the basis of biochemical and physiological tests and 16S rDNA. Out of these 22 potentially bacterial antagonists, five strains (KSA1, KSA9, KSA14, KSA17 and KSA20) showed high potential growth inhibition.

Similar kinds of investigations were also reported on pomegranate bacterial blight caused by *Xanthomonas axanopodis pv. punicae* by Yenjerappa (2009), where antagonists like *Bacillus subtilis* and *Pseudomonas fluorescens* were found significantly superior in inhibiting the growth of pathogen. Ramesh (2015) isolated 170 isolates from different pomegranate orchards and found 57 isolates suppressing the growth of pathogen out of which 8 isolates *viz*, PM-1A, VK-6B, BK-6, KK-9A, VK-10C, KK-3A, BK-5 and BK-3 showed the maximum inhibition.

Biological control can achieve the objective of disease suppression through a number of ways such as antibiosis, competition, mycoparasitism, cell wall degradation, induced resistance, plant growth promotion and rhizosphere colonization capability. The most effective bio-agent studied till date appears to antagonize pathogen using multiple mechanisms is *Pseudomonas*, which utilizes both antibiosis and induction of host resistance to suppress the disease causing microorganisms (Jan *et al.*, 2013)

Under biological control of plant diseases, various antagonistic organisms have been identified, which fight against the pathogens by different mechanisms *viz.*, competition, lysis, antibiosis, siderophore production and hyper parasitism (Vidyasekaran, 1999).

The antagonism of *Pseudomonas fluorescens* against some *Xanthomonas* spp. was reported by Unnamalai and Gnanamanickam (1984).

In aqueous extraction of botanicals, the results showed significant difference among the treatments. Among the botanicals tested *Prosopis juliflora* was found to be most effective with maximum average inhibition of 10.83 mm which was significantly different from all other treatments. This was followed by kokum and soapnut which recorded average inhibitory zone of 9.50 and 8.00 mm respectively. Even in the alcoholic extraction method the same trend has been observed with the average inhibition of 15.21, 14.00 and 12.50 mm by the prosopis, kokum and soapnut respectively.

Kokum rind possesses an important phenolic compound called as garcinol identified by Sutar *et al.* (2012), beside garcinol the other compounds like furfural and its derivatives, cyanidin- 3-glucose which are present as anthocyanin in the rind. This furfural and cyanidin -3-glucose is a potent antimicrobial. The extent to which these compounds get extracted in different solvents determine the degree of bactericidal action.

Sasitorn (2003) reported the use of 23 different herbal extracts, extracted by 95% ethyl alcohol at the concentration of 100,000, 50,000, 10,000, 5,000 and 1,000 ppm respectively and found that guava leaf extract could inhibit the growth of bacteria at 50,000 ppm, the myrobalan wood fruit extract at 10,000 ppm, the extract of beleric myrobalan fruit, nut gall fruit and pomegranate fruit peel could inhibit the growth of bacteria at all concentration.

Srinivasachary (1995) investigated that, *Ocimum* plant extract was more effective in inhibiting the growth of *Xanthomonas campestris* pv. *mori.* Similar studies were also reported on *in vitro* efficacy of different botanicals in inhibiting growth of *Xanthomonas axonopodis* pv. *punicae*

by Manjula (2002) who found that kolangi extract was superior followed by meswak, tulsi and patchouli. Yenjerappa (2009) reported that, garlic extract at 10 per cent concentration was significantly greater in efficacy than all other treatments followed by parthenium and lantana leaf extract and onion bulb.

The *in-vitro* efficacy of 15 different botanicals were tested against *Xanthomonas axonopodis* pv. *punicae* by Ramesh 2015 and revealed that garlic had recorded maximum average inhibition of 14.89 mm followed by kokum and prosopis with 13.20 and 11.44 mm respectively.

REFERENCES

1. Garden,L.,J.P. Chaumont, and R. Chartier. Antibacterial activity of 200 plant extracts against phytopathogenic bacteria. *Phytiatrie Phytopharmacie* (France) 1978; **27**: 285-294.
2. Grainge,M.D. and A.M. Alvarez. Antibacterial and antifungal activity of Artabotrys hexapetalus leaf extracts. *Intern Trop Plant Diseases.*,1987; **5**:173-179.
3. Jan, M. J., Nisar, A. D., Tariq, A. B., Arif, H. B. and Mudasir, A. B. Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *Int. J. Modern Pl. Anim. Sci.*, 2013; **1**(2): 39-57.
4. Khodakaramian, G. H., Heydari, A. and Balestra, G. M., Evaluation of pseudomonads bacterial isolates in biological control of citrus bacterial canker disease. *Int. J. Agril. Res.*, 2008; **3**(4):268-272.
5. Leksomboon, C., N. Thaveechai , W. Kositratana, and Y. Paisooksantivatana. Antiphytobacterial activity of medicinal plant extracts. *Kasetsart J. N. Sci.*, (Thai) **54** : 91-97.
6. Leksomboon, C., N. Thaveechai, and W. Kositratana. Effect of Thai medicinal plant extracts on growth of phytopathogenic bacteria, *In Proceeding of the 36th Kasetsart University Annual Conference, Plant Section,* 1998 February, *Kasetsart University, Bangkok.* 3-5.
7. Manjula, C. P. and Khan, A. N. A., Incidence of bacterial blight of pomegranate (*Punica granatum* L.) in Karnataka. Paper presented In: the *Ann. Meet. Symp. Plant Disease Scenario in Southern India,* Bangalore 2002, 19-21: 51-52.
8. Mohammad, A. A. S., Evaluation of saudi fluorescent pseudomonads isolates as a biocontrol agent against citrus canker disease caused by *Xanthomonas citri Subsp. citri. Egypt. Acad. J. Biolog. Sci.*, 2014; **6** (2): 1 –7.
9. Ramesh, I. Biological management of *Xanthomonas axonopodis* pv. *Punicae* (Hingorani and Singh) Vauterin *et al.,* causing bacterial blight of *punica granatum L.. M.Sc thesis, Univ. of Hortic. Sci., Bagalkot. Karnataka.,* 2015.
10. Rashid, M., Chowdhury. M. S. M. and Sultana. N. Prevalence of canker on seedlings of citrus (Citrus spp.) in selected areas of Bangladesh and its management, *The J. Plant Patho. Photon*., 2014; **114**: 177-187.
11. Sasitorn, V., Efficacy of thai herbal extract for growth inhibition of *Xanthomonas axonopodis* pv. *citri,* the bacterial canker of citrus. *Kasetsart J.* (*Nat. Sci.*) 2003; **37**:445 – 452
12. Shahbaz, T. S., Usman, G. M., Afzal, M., Abdul, R. and Amir, H. Incidence of citrus canker disease caused by *Xanthomonas campestris pv. citri* (hasse) dows on kinnow (*citrus reticulata*) and its chemotherapy. *Pak. J. Bot*., 2007; **39** (4): 1319-1327.
13. Shahid, M., Maher, S. T., Sahi, M., Usman, G. M., Inam, U. H., Imran, U. H., Yasir, I., Sarwar, M.S. and Tauqir, A. Evaluation of different toxicants against Xanthomonas campestris pv. citri (Hasse) Dows. *Int. J. Agri. Biol*., 2005; **7** (1):121-124.
14. Sigee,D.C. Bacterial Plant Pathology Cell and Molecular Aspects. *Cambridge University Press*, 1993; pp 325.
15. Srinivasachary. Etiology and ecology of the causal organism of bacterial blight of mulberry (*Morus alba* L.). *M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Bangalore, Karnataka India*. 1995; pp. 79.
16. Sutar, R. L., Mane, S. P. and Ghosh, J. S., Antimicrobial activity of extracts of dried kokum (*Garcinia indica* C). *Int. Food Res. J.*, 2012; **19** (3):1207-1210.
17. Unnamalai, N. and Gnanamanickam, S. S., *Pseudomonas fluorescens* is an antagonistic to *Xanthomonas citri* (Hasse) Dye. The incitant of citrus canker. *Curr. Sci.*, 1984; **53:** 703-704.
18. Vidyasekaran, P., Biotechnological approaches to suppress seed to plant transmission of seed borne pathogens. In : *Nat. Symp. Seed Sci. Tech., University of Mysore, manasagangotri, Mysore, India*, 5-7 August, 1999; pp. 41.
19. Yenjerappa, S. T. Epidemiology and management of bacterial blight of pomegranate caused *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh). *Ph. D. Thesis, Univ. Agric. Sci., Dharwad, Karnataka.*, 2009.