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Efficacy of fungicides botanicals bioagents against *Xanthomonas axonopodis* pv. *citri*

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Abstract
Citrus canker disease of acid lime caused by *Xanthomonas axonopodis* pv. citri is an important disease in many parts of MH region. *In vitro* experiment were conducted to study efficacy of fungicides botanicals bioagents against *Xanthomonas axonopodis* pv. *citri.* The growth characteristics of all the isolates were studied on nutrient agar medium. All seven isolates were found Gram –ve, short rod and positive for biochemical tests *viz.* KOH, catalase test, starch hydrolysis, Indole production, acid and gas production and gelatein liquefacation. The efficacy of bioagents, botanicals and chemicals was studied by paper disc method. The Copper oxychloride (0.2%) + streptomycin sulphate (200 ppm) was found significantly effective in inhibiting growth of bacteria.

Keywords: fungicides, botanicals, *Xanthomonas axonopodis* pv

Introduction
Citrus is an important value as fruit crop. Present day citrus is delectable, juicy, and seedless is of great nutritional significance as well (Khan *et al*., 1992) [1, 5]. Additionally, it possesses enormous therapeutic qualities (Chaudhry *et al*., 1992) [1]. Citrus is a member of Rutaceae family and grown in varying densities in countries with tropical or subtropical climates.
Citrus plant is attacked by a number of diseases like citrus canker, gummosis, citrus decline, citrus tristeza virus, greening, etc. Citrus canker is caused by "*Xanthomonas campestris* pv. *citri"* that is probably the worst enemy to citrus plants (Sahi *et al*., 2007). Citrus canker disease is of regular occurrence on several citrus cultivars in varying degrees of incidence depending on the climatic conditions. The bacterium, *Xanthomonas* causes different symptoms ranging from pustules to necrotic lesions consisting of erumpent corky tissue surrounded by water soaked tissues and yellow halo on leaves, stems and fruits (Zekri *et al*., 2005; Graham *et al*., 2004; Das, 2003) [7, 2]. *Xanthomonas axonopodis* pv. *citri* is a rod shaped, gram negative bacterium with single polar flagellum.

Material and Methods
Collection, isolation and maintenance of bacterial strains.
The citrus canker diseased sample were collected from different district of Maharashtra state viz. Akola, Warora, Nagpur, Parbhani, Sangli, Rahuri, Pune (Table 1). A total of seven isolates of pathogen were obtained from infected leaves, twigs of acid lime showing typical symptoms of citrus canker. These isolates were isolated by tissue isolation method. A bacterial suspension of each specimen was then cultured on NA medium. Following incubation, colonies similar to *Xanthomonas* were maintained on NA medium at room temperature by adopting subsequent subculturing at periodical, regular intervals. Three days old cultures were used for further studies.

Table 1: List of isolates of *Xanthomonas axonopodis* pv. *citri*

Sr. No	Location	District	Code no.
1	AICRP on fruits Dr. P.D.K.V. Akola	Akola	Xac1
2	Warora	Chandrapur	Xac2
3	Nagpur	Nagpur	Xac3
4	Parbhani	Parbhani	Xac4
5	Sangli	Sangli	Xac5
6	M.P.K.V.Rahuri	Ahmednagar	Xac6
7	C.O.A.Pune 05	Pune	Xac7

Morphological studies

The confirmations of the *Xanthomonas axonopodis* pv.*citri* isolates were performed with the following studies.Pure culture of selected isolates were streaked on Nutrient agar medium separately for colony development. The individual colonies were examined for colony colour and shape

Biochemical test

All the isolates of *Xanthomonas axonopodis* pv. *citri.* were compared on the basis of their biochemical tests viz., starch hydrolysis, Gelatin liquefaction, Indol Production, KOH test, Gram's reaction, Acid and gas production, catalase test.

***In vitro* efficacy of different Botanicals, Bioagents, Chemicals against *Xanthomonas axonopodis* pv.*citri* by Paper disc method**

Sensitivity of the Akola isolates (Xac1) was tested by modified paper disc inhibition assey method.Derived concentration of the botanicals, bioagents & chemicals were freshly prepared in sterile distilled water. Similarly the neem extract solution at diifferent concentration i.e 1%, 2.5%, 5% and bioagents solution i.e *Pseudomonas fluorescence* ($1x10^8$cell) and *Bacillus subtilis* ($1x10^8$cell) culture prepared. The filter paper disc (Whatman No. 42) measuring 5 mm in diameter were soaked in the respective solution for 5 minutes and transferred onto the surface of the seeded medium in petriplates. The plates were incubated at 25-27 ^{0}C for 72 hours and observed for the production of inhibition zone around the filter paper discs. The results obtained were analysed statistically. paper disc soaked in sterile distilled water served as control.

Results and Discussion.

Morphology and Biochemical Characteristic of *Xanthomonas axonopodis* pv. *citri*

All the isolates were studied with respect to their colony colour, shape and Grams staining reaction. The results are presented in Table 4 revealed that bacterial cells appeared short rod and Gram negative. Isolates Xac1, Xac2, Xac3 produced yellow colonies however Xac4, Xac5, Xac 6, Xac7 showed pale yellow colour colonies on NA medium. The biochemical tests for their identification, some of the tests were performed for comparing the characteristics depicted in Burgey's manual of Systematic Bacteriology. Seven isolates with respect of KOH, catalase, starch hydrolysis, Indole production, acid and gas production, Gelatein liquefacation were studied. The results are presented in table 2.

Table 2: Morphological and Biochemical characteristic *Xanthomonas axonopodis* pv. *citri* isolates

Sr. No.	Isolates	Xac1	Xac2	Xac3	Xac4	Xac5	Xac6	Xac7
1	Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
2	Colony colour	yellow	yellow	yellow	Pale yellow	Pale yellow	Pale yellow	pale yellow
3	Gram reaction	-ve	-ve	-ve	-ve	ve	-ve	-ve
4	Starch hydrolysis	+++	+++	+++	+	+	+	+
5	Catalase	+++	+++	+++	+++	+++	+++	+++
6	Indole production	+++	+++	+++	+++	+++	+++	+++
7	KOH	+++	++	+++	+++	++	++	++
8	Gelatein liquefication	+++	+++	+	++	++	+	++
9	Acid and gas production	++	++	++	++	++	++	++

- negativevarying degree of reaction
+ positive
: + poor
: ++ moderate
: +++ strong

Manjula (2002) [6] reported that, seven isolates of the pomegranate bacterium were small rods, appeared singly, Gram negative. Gottwald *et al*. (2002) [4] who reported *Xanthomonas axonopodis* is a rod shaped, Gram negative bacterium. Das (2003) [2] reported that the bacteria is rod shaped measuring 1.5-2.0 x 0.5-0.75µm, Gram negative.Biochemical tests viz., KOH, catalase, starch hydrolysis, gelatein liquefaction, acid and gas production, Indole production confirms the bacterial pathogen *Xanthomonas axonopodis* pv.*citri.* The cultured showed variation among the isolates of *Xanthomonas axonopodis* pv.*citri.* Similar variation among the isolates has been earlier noted by Raut (1990) [8] studied 15 isolates of *Xanthomonas axonopodis* pv. *mangiferae indicae* for different physiological and biochemical properties viz. H_2S production, action on carbohydrates, gelatin test, KOH test etc. Das (2003) [2] reported that the bacterial cells of *Xanthomonas citri* are positive for hydrolysis of starch, liquefaction of gelatin, catalase. Das (2005) [3, 7] studied different isolates of *Xanthomonas axonopodis* pv. *citri* for different physiological and biochemical properties viz. H2S production, gelatin liquefaction, KOH test etc solubility test, catalase test, acid production from D-xylose, glucose

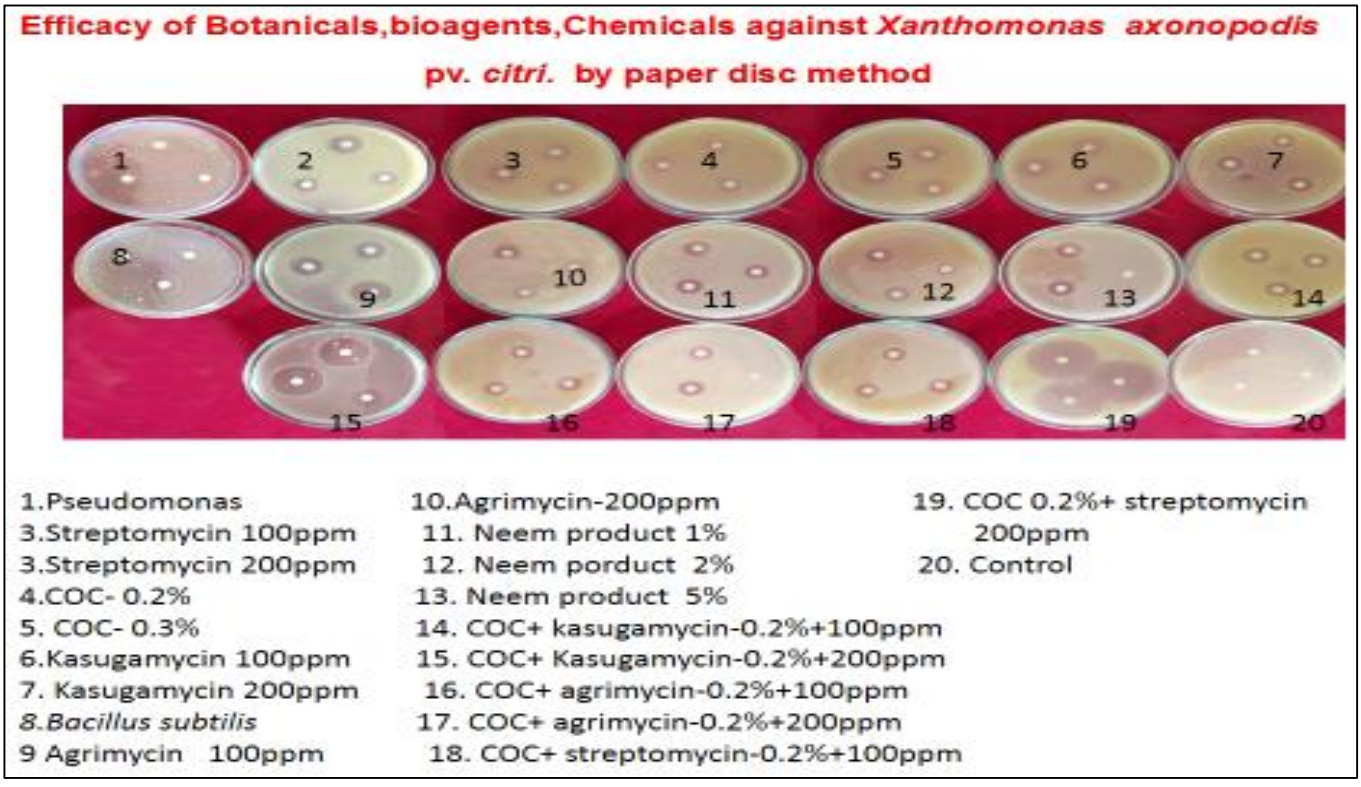
Efficacy of different chemicals, botanicals and bioagents against *Xanthomonas axonopodis* pv. *citri* by Paper disc method:

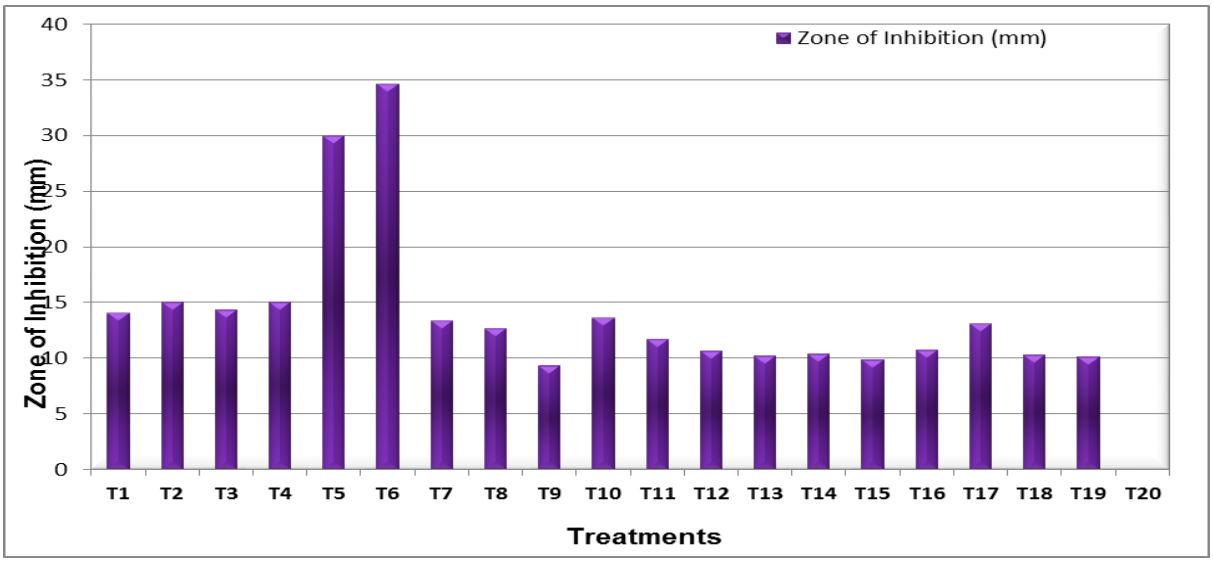
In order to asses the efficacy of chemicals, botanicals and bioagents against Akola isolates of *Xanthomonas axonopodis* pv. *citri* (Xac1) an experiment was conducted and the evaluation was made by paper disc method. The data presented in Table 3, revealed the significant differences among the different treatment.

Table 3: Efficacy of different chemicals, botanicals and bioagents against *Xanthomonas axonopodis* pv. *citri* by paper disc method

Sr. no.	Treatment	Conc.	Zone of inhibition (mm)
T_1	Streptomycin sulphate	100 ppm	14.10*(22.02)
T_2	Streptomycin sulphate	200 ppm	15.06*(22.83)
T_3	Copper oxychloride	0.2%	14.33*(22.04)
T_4	Copper oxychloride	0.3%	15.06*(22.83)
T_5	COC + Streptomycin sulphate	0.2%+100 ppm	30.00*(33.21)
T_6	COC + Streptomycin sulphate	0.2%+200 ppm	34.66*(36.05)
T_7	Kasugamycin	100 ppm	13.33*(21.39)
T_8	Kasugamycin	200 ppm	12.66*(20.84)
T_9	Agrimycin	100 ppm	9.33*(17.76)
T_{10}	Agrimycin	200 ppm	13.66*(21.67)
T_{11}	COC + Agrimycin	0.2%+100 ppm	11.66*(19.94)
T_{12}	COC + Agrimycin	0.2%+200 ppm	10.66*(19.05)
T_{13}	COC+ Kasugamycin	0.2%+100 ppm	10.23*(18.63)
T_{14}	COC+Kasugamycin	0.2%+200 ppm	10.36*(18.76)
T_{15}	Neem extract	1%	9.86*(18.30)
T_{16}	Neem extract	2.5%	10.73*(19.11)
T_{17}	Neem extract	5%	13.13*(21.24)
T_{18}	*Pseudomonas fluorescens*	$1x10^8$ cfu /ml	10.33*(18.73)
T_{19}	*Bacillus subtilis*	$1x10^8$ cfu /ml	10.13*(18.56)
T_{20}	Control		00.00* (0.368)
	'F' test		Sig.
	SE (m) $\pm$		0.008
	CD (P) = 0.01		0.023

The treatment number T_6 i.e. combination of copper oxychloride (0.2%) + streptomycin sulphate (200 ppm) were significantly superior in inhibiting the growth of bacteria (34.66mm). which in at par with T_5 i.e. COC (0.2%) + streptomycin sulphate (100 ppm) 30.00mm. Among the bioagents *Pseudomonas fluorescence* showed maximum zone of inhibition (10.33mm) followed by *Bacillus subtilis* (10.13mm).In botanical neem extract (5%) showed (13.13mm) zone of inhibition followed by neem extract 2.5% (10.73mm) and 1%(9.86mm). Sharma *et al*. (1981), who reported that, the combination of streptocycline and copper oxychloride was most effective in inhibiting the growth of *Xanthomonas vesicatoria* as assessed *in vitro* by paper disc method. Das (2005) [3, 7] reported that COC (0.3%) + streptomycin (100 ppm) inhibiting the maximum growth of *Xanthomonas axonopodis* pv. *citri.*





Efficacy of chemicals, botanicals and bioagents against *Xanthomonas axonopodis* pv. *citri* by paper disc method

References

1. Chaudhry NA, Khan AR. Hameedullah. Introduction of acclimatized exotic citrus. Citrus fruit varieties at Horticultural Research Station, Sahiwal. Proc. 1st Int. Sem. Citriculture in Pakistan, University of Agriculture Faisalabad–Pakistan, 1992, 15.
2. Das AK. Citrus canker; A review. J Appl. Hort. 2003; 5(1):52-60.
3. Das S. Variability among the isolates of *Xanthomonas axonopodis* pv. *citri*. M.Sc. Thesis (Unpub.) Dr. P.D.K.V. Akola. 2005, 23- 29.
4. Gottwald TR, Graham JH, Schubert TS. Citrus canker the Pathogen and its impact. Online. Plant helth progress. 2002, 812-01RV
5. Khan MM, Khan MA, Inam-ul Haq M, Ahmad R. Aziz I. Incidence of citrus canker caused by *X. campestris* pv. *citri* orchard in Faisalabad District. In: Proceed. 1st Inter. sem. citriculture in Pakistan. Dec. 2-5. University of Agriculture Faisalabad. 1992; 311-314
6. Manjula CP. Studies on bacterial blight of pomegranate (*Punica granatum* L.) caused by *Xanthomonas axonopodis* pv. *punicae.* M.Sc. (Agri.) Thesis, Univ. Agric. Sci., Bangalore, Karnataka (India), 2002.
7. Zekri M, Chamberlain H, Timmer P, Roberts P, Muchove R. Field identification of citrus canker symptoms and decontamination procedures Uni. Florida. IFAS extension, 2005.
8. Raut BT. Studied on leaf spot of mango caused by *Xanthomonas campestreis* pv. *mangiferae indicae* Ph.D. Thesis (unpub) U.A.S Dharwad. 1990, 104
9. Sharma RR, Thind BS, Singh N. *In vitro* and *in vivo* evaluation of chemicals against *Xanthomonas vesicatoria*, the causal agent of bacterial leaf spot of chillies. Indian J Mycol. Pl. Pathol. 1981; 11(2):178-181.