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Assessment of different chemical compounds and bioagents against Bacterial blight pathogen of cotton (*Xanthomonas axonopodis* pv. *malvacearum*) under *in vitro* conditions

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Abstract
Bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum* is one of the most destructive diseases of cotton (*Gossypium* spp.), causing severe yield losses upto 38.78%. Present investigations on the disease (*Xam)* were carried out to fulfill the objectives defined, at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani. Among the various chemicals and bioagent tested under *in vitro* condition against *Xanthomonas axonopodis* pv. *malvacearum*, streptocycline 100 ppm + copper oxychloride 3000 ppm was significantly superior with maximum inhibition zone of 26.67 mm followed by streptocycline 100 ppm + carbendazim 1500 ppm (26.00 mm), streptocycline 100 ppm + copper hydroxide 3000 ppm (24.30 mm) and minimum inhibition zone was found in *Pseudomonas fluorescens* 2 X 10^6 cfu/ml (11.00 mm).

Keywords: *Gossypium,* minimum, inhibition

Introduction
Cotton (*Gossypium spp.*) is the most extensively cultivated commercial crop and is a major fibre crop of global importance and has high commercial value. Cotton locally known as “White Gold” is also a king of cash crop. Cotton is grown commercially in the temperate and tropical regions of more than 70 countries. Cotton mainly grown for fibre needs of the human. Cotton almost accounts 65% of fibre production in India. Edible oil is extract from cotton seed and de-oiled cakes are used as a cattle feed, which is a good source of high quality protein for animals. Cotton cake after extraction of oil is used as good organic manure which contain near about 6% nitrogen, 3% phosphorus and 2% potash. Cotton crop in India is known to suffer from number of diseases. Important diseases of cotton are given in the following.

Fungal diseases
Wilt: *Fusarium oxysporum f.sp vasinfectum*
***Verticillium* wilt:** *Verticillium dahilae*
***Alternaria* blight:** *Alternaria macrospora*
Grey mildew: *Ramularia areola*
Anthracnose: *Colletotrichum gossypii*
***Ascochyta* blight:** *Ascochyta gossypii*

Bacterial diseases
Bacterial blight: *Xanthomonas axonopodis* pv. *malvacearum*
Crown gall: *Agrobacterium tumefaciens*

Viral diseases
Cotton leaf curl virus (CLCuV)
Of this bacterial blight is one of the most important diseases of cotton and was first reported from Albama (USA) by Atkinson in 1891. The disease was first reported in India from Rajapalayam, Tamil Nadu in 1918.
The disease causes severe losses because it reduces photosynthetic activity by destroying the chlorophyll content in leaves and stem. Bacterial blight of cotton has been found to cause losses in seed cotton yields to the extent of 50% (Simpson, 1956).

Bacterial blight cause yield loss of 38.78%. Losses are less when only leaves are infected but when stem lesions are formed, the losses may be high as up to 90%.

Materials and Methods

Isolation of the pathogen

The diseased specimens of bacterial blight of cotton were collected from the experimental plots of Cotton Research Scheme, V.N.M.K.V., Parbhani were subjected to isolations on selective synthetic media. The leaves and bolls were washed and pieces of infected tissue were cut aseptically from the edge of typical spots with a little portion of healthy tissue. These tissues were surface disinfected in Petri plates containing 95% alcohol for few seconds and immediately placed on blotting paper for the removal of excess alcohol. After this it was again disinfected in mercuric chloride ($HgCl_2$) solution for 30 sec and subsequently washed in sterile distilled water for 3-4 times to remove the traces of mercuric chloride. The disinfected pieces were transferred by means of sterile forceps to a drop of sterile water on a sterile glass slide and teased by means of sharp sterile razor blade.

The water drop from slide was taken with a bacterial inoculation loop and streaked on a petriplate containing King's 'B' media, NA media and YGCA media under aseptic condition. These plates were incubated at 27±1 °C in BOD incubator for 72 hours. After completion of incubation period, the plates were observed for development of the colonies of *Xanthomonas axonopodis* pv. *malvacearum*.

Pathogenicity test

The virulence and pathogenic nature of the isolated *Xanthomonas axonopodis* pv. *malvacearum* bacteria was proved by spraying the bacterial suspension on the cotton plant. Five seeds of the Desi variety PA 255 and Bt cotton variety Ajeet 199 BG-II were sown in each pot filled with soil and FYM in 2:1 proportion and immediately watered with sterilized distilled water. Two weeks after germination, only four seedlings were maintained in each pot. When the plants were 4-6 weeks old, a bacterial suspension (10^8 cfu/ml) was prepared as an inoculums for pathogenicity test. The underside of the leaf surface was sprayed with water and dusted with carborendum powder. Further, these leaves were smeared with bacterial suspension by means of sterile cotton swab. Simultaneously, one of the pot were kept untreated control.

In vitro evaluation of fungicides and bioagents Fungicides

Antibiotics each at three different concentrations were evaluated for their sensitivity against the growth of *Xanthomonas axonopodis* pv. *malvacearum* by inhibition zone assay method. The bacterium was mutiplied by inoculating the culture in YGCA broth. The bacterial suspension was then seeded to the YGCA medium. *In vitro* experiment detail is mainly 3 replication and 12 treatments.

Treatment Details

Tr. No	Treatments	Trade name	Concentration
T_1	Streptomycin sulphate	Streptocycline	50,75, 100 ppm
T_2	Streptomycin + Tetracycline	Plantomycin	50,75, 100 ppm
T_3	2-bromo-2-nitropropane-1,3-diol	Bactrinashak	50,75, 100 ppm
T_4	Copper hydroxide 77 WP	Kocide	2000,2500,3000 ppm
T_5	Copper oxychloride 50WP	Blitox	2000,2500, 3000 ppm
T_6	*Pseudomonas fluorescens*	-	$2x10^6$ cfu/ml
T_7	Carbendazim (Methyl -2- benzimidazole carbamate)	Bavistin	750,1000,1500 ppm
T_8	Bordeaux mixture	-	5000,10000 ppm
T_9	Streptomycin sulphate + Copper hydroxide 77 WP	-	50 + 2000, 75 + 2500, 100 +3000 ppm
T_{10}	Streptomycin sulphate + Copper oxychloride 50WP	-	50 + 2000, 75 + 2500, 100 +3000 ppm
T_{11}	Streptomycin sulphate + Carbendazim	-	50 + 750, 75 + 1000, 100 +1500 ppm
T_{12}	Control		

The antibiotic solutions were prepared at different concentration. The filter paper discs measuring 5 mm diameter were soaked in respective antibiotic solutions and it were transferred to the medium of plates.

The inoculated plates were kept in the refrigerator at 5 ^{0}C to allow the diffusion of chemical in to the medium. The plates were then incubated at 27 ^{0}C and observations for the production of inhibition were observed.

The bio efficacy of these antibiotics and fungicides were evaluated at different concentrations mention in the treatment details.

Observations regarding the inhibition zone by antiobiotics and bioagent were recorded at 48 – 72 hours after inoculation. The inhibition zone was calculated by the formula given by Vincent (1927).

$$\text{Percent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition of growth

C = Growth (mm) of test bacteria in untreated control plates

T = Growth (mm) of test bacteria in treated plates

Results and Discussions

In vitro evaluation of different chemicals and bioagent against *Xanthomonas axonopodis* pv. *Malvacearum*

The results on the efficacy of various chemicals and bioagent inhibiting the growth of *Xanthomonas axonopodis* pv. *malvacearum* under *in vitro* condition are presented in Table 1, PLATE I, Fig. 1.

At 'A' concentration presented in Table 1, PLATE I, Fig. 1., bacterial inhibition zone was ranged from 10.60 mm (bactrinashak) to 19.6 mm (streptocycline + copper oxychloride). However it was significantly highest with streptocycline 50 ppm plus copper oxychloride 2000 ppm (19.6 mm), but was at par with streptocycline 50 ppm plus carbendazim 750 ppm with inhibition zone of 18.67 mm,

followed by streptocycline 50 ppm plus copper hydroxide 2000 ppm (18.00 mm) and was found at par with carbendazim 750 ppm with inhibition zone of 17.7 mm. This was followed by bordeaux mixture 2500 ppm (17.00 mm), streptocycline 50 ppm (15.7 mm), copper hydroxide 2000 ppm (15.00 mm), copper oxychloride 2000 ppm (12.00 mm), plantomycin 50 ppm (11.30 mm), *Pseudomonas fluorescens* 2 X 10^6 cfu/ml (11.00 mm) and the minimum mean inhibition zone was in bactrinashak 50 ppm (10.6 mm), later four antibiotics found at par in succession. The maximum% inhibition found in streptocycline 50 ppm plus copper oxychloride 2000 ppm (21.85%) and the minimum% inhibition was found in bactrinashak 50 ppm (11.85%).

At 'B' concentration presented in Table 1, PLATE I, Fig. 1. bacterial inhibition zone was ranged from 11.00 mm (*Pseudomonas fluorescens*) to 25.60 mm (streptocycline + copper oxychloride). However it was significantly highest with streptocycline 75 ppm plus copper oxychloride 2500 ppm (25.60 mm), followed by streptocycline 75 ppm plus carbendazim 1000 ppm (22.30 mm), streptocycline 75 ppm plus copper hydroxide 2500 ppm (22.00 mm), bordeaux mixture 5000 ppm (21.00 mm), later three chemicals found at par in succession. This was followed by, carbendazim 1000 ppm with inhibition zone of 20.30 mm, streptocycline 75 ppm (19.00 mm), copper hydroxide 2500 ppm (18.30 mm), later three chemicals found at par in succession. This was followed by, copper oxychloride 2500 ppm (14.00 mm), plantomycin 75 ppm (13.67 mm), bactrinashak 75 ppm (12.33 mm) and the minimum mean inhibition zone was in *Pseudomonas fluorescens* 2 X 10^6 cfu/ml (11.00 mm), later four treatments found at par in succession. The maximum% inhibition found in streptocycline 75 ppm plus copper oxychloride 2500 ppm (28.52%) and the minimum% inhibition was found in *Pseudomonas fluorescens* 2 X 10^6 cfu/ml (12.44%).

At 'C' concentration presented in Table 1, PLATE I, Fig. 1., bacterial inhibition zone was ranged from 11.00 mm (*Pseudomonas fluorescens*) to 26.67 mm (streptocycline + copper oxychloride). However it was significantly highest with streptocycline 100 ppm plus copper oxychloride 3000 ppm (26.67 mm), followed by streptocycline 100 ppm plus carbendazim 1500 ppm (26.00 mm), streptocycline 100 ppm plus copper hydroxide 3000 ppm (24.30 mm), bordeaux mixture 10000 ppm (24.00 mm), carbendazim 1500 ppm with inhibition zone of 21.30 mm, streptocycline 100 ppm (20.67 mm), copper oxychloride 3000 ppm (20.00 mm), later three chemicals found at par in succession. This was followed by plantomycin 100 ppm (18.7 mm), copper hydroxide 3000 ppm (18.33 mm), bactrinashak 75 ppm (15.00 mm) and the minimum mean inhibition zone was in *Pseudomonas fluorescens* 2 X 10^6 cfu/ml (11.00 mm). The maximum% inhibition found in streptocycline 100 ppm plus copper oxychloride 3000 ppm (29.63%) and the minimum% inhibition was found in *Pseudomonas fluorescens* 2 X 10^6 cfu/ml (12.44%)

Results obtained were in accordance with Shah *et al.* (1991) [5], Raut *et al.* (2010) [4], Jagtap *et al.* (2012) [3].

Table 1: *In vitro* efficacy of chemicals and biocontrol agent against *Xanthomonas axonopodis* pv. *Malvacearum*

Tr. No	Treatments	Conc. (ppm)	Mean inhibition zone (mm)			% inhibition (%)		
			A	B	C	A	B	C
T_1	Streptocycline	50 (A),75 (B), 100 (C)	15.7 (24.64)	19.0 (27.34)	20.67 (28.52)	17.41	21.11	22.96
T_2	Plantomycin	50 (A),75 (B), 100 (C)	11.3 (21.08)	13.67 (22.87)	18.7 (26.99)	12.96	15.19	20.74
T_3	Bactrinashak	50 (A),75 (B), 100 (C)	10.6 (20.10)	12.33 (21.67)	15.0 (23.99)	11.85	13.7	16.67
T_4	Copper hydroxide	2000 (A), 2500 (B), 3000 (C)	15.0 (24.09)	18.3 (26.73)	18.33 (28.87)	16.67	20.37	23.33
T_5	Copper oxychloride	2000 (A), 2500 (B), 3000 (C)	12.0 (21.40)	14.0 (23.05)	20.0 (28.08)	13.33	15.55	22.22
T_6	*Pseudomonas fluorescens*	2 X 10^6 cfu/ml	11.0 (20.41)	11.67 (21.08)	13.0 (22.32)	12.44	12.44	12.44
T_7	Carbendazim	750 (A), 1000 (B), 1500 (C)	17.7 (26.29)	20.3 (28.33)	21.3 (29.12)	19.63	22.59	23.7
T_8	Bordeaux mixture	2500 (A), 5000 (B), 10000 (C)	17.0 (25.75)	21.0 (28.87)	24.0 (31.05)	18.89	23.33	26.67
T_9	Streptocycline + Copper hydroxide	50+2000 (A), 75+2500 (B), 100+3000 (C)	18.0 (26.52)	22.0 (29.59)	24.3 (31.31)	20.0	24.44	27.04
T_{10}	Streptocycline + Copper oxychloride	50+2000 (A), 75+2500 (B), 100+3000 (C)	19.6 (27.84)	25.6 (32.26)	26.67 (32.84))	21.85	28.52	29.63
T_{11}	Streptocycline + Carbendazim	50+750 (A), 75+1000 (B), 100+1500 (C)	18.67 (27.07)	22.3 (29.86)	26.0 (32.50)	20.74	24.81	28.89
T_{12}	Control (Untreated)	-	0	0	0	0	0	0
	SE ±		0.73	1.19	1.47			
	CD at 5%		2.17	3.51	4.31			

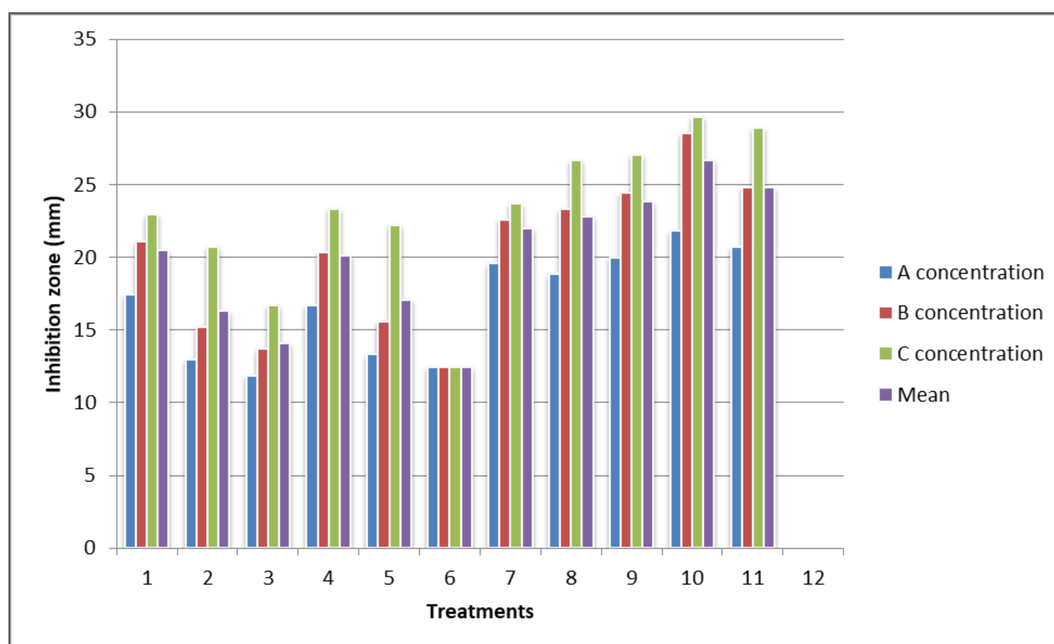

Fig 1: *In vitro* efficacy of chemicals and biocontrol agent against *Xanthomonas axonopodis* pv. *Malvacearum*

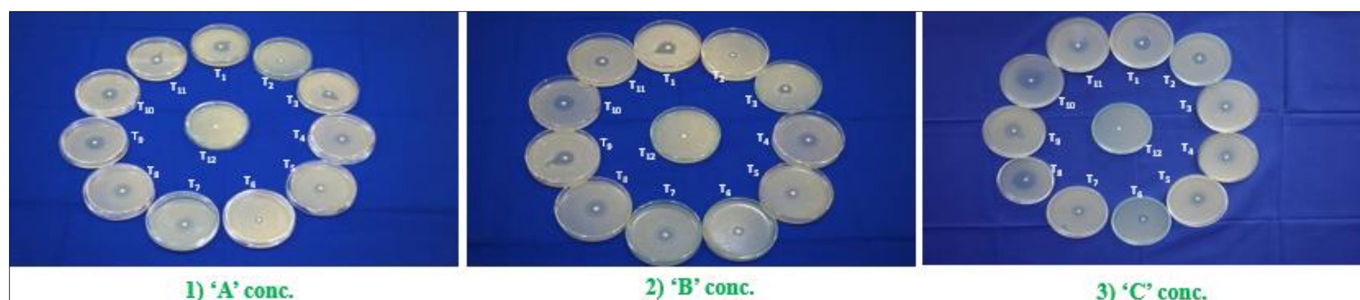

Fig 2: *In vitro* efficacy of chemicals and bioagent at different concentrations (* see the concentration on Table no. 1) against Xam

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