International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(6): 1237-1239

Received: 04-09-2020
Accepted: 10-10-2020

SM Chapke
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

DS Bharti
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

PL Sontakke
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

MG Patil
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

DN Dhutraj
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Corresponding Author:
SM Chapke
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

In vitro efficacy antibiotics against Bacterial leaf spot of chilli caused by *Xanthomonas axonopodis* pv. *vesicatoria*

SM Chapke, DS Bharti, PL Sontakke, MG Patil and DN Dhutraj

DOI: https://doi.org/10.22271/chemi.2020.v8.i6r.10930

Abstract
The *in vitro* study was conducted to evaluate efficacy of antibiotics against *Xanthomonas axonopodis* pv. *vesicatoria* causing bacterial leaf spot of chilli. Three antibiotics *viz*., Streptocycline, Aureofungin, Kasugamycine and fungicide Copper oxychloride, (each @250 and 500) were evaluated *in vitro* against *Xanthomonas axonopodis* pv. *vesicatoria* causing bacterial leaf spot of chilli. All the treatments significantly inhibited bacterial growth of *Xanthomonas axonopodis* pv. *vesicatoria* over control.

Keywords: *Xanthomonas axonopodis*, antibiotics, streptocycline, copper oxychloride

Introduction
Chilli (*Capsicum annum* L.) also known as red pepper is the member of family Solanaceae. Several sources concordantly put the origin of chilli in Bolivia or Brazil. Chilli originally from South America are referred as chillies, chilli, hot pepper, bell pepper, red pepper, pod pepper, cayenne pepper, paprika, pimento and *capsicum* in different parts of the world. Two species of chilli are under cultivation, the *Capsicum annum* L. is small in size, more pungent types, whereas, the *Capsicum frutescence* L. is somewhat larger, mild to moderately pungent types and referred as 'Dhobli Mirchi' and is used mostly as green vegetable.

India is the largest producer, consumer and exporter of chillies in the world. The important states growing chilli are Andhra Pradesh, Maharashtra, Orissa, West Bengal, Karnataka, Rajasthan and Tamil Nadu. As per the latest statistics, India produced 2955 thousand million tonnes of green chillies from an area of 292 thousand hectares in the year 2017-18 and produced 1304.38 thousand million tonnes of green chillies from an area of 794.12 thousand hectares in the year 2017-18. (Anonymous 2018) [2]. Andhra Pradesh is the leading both in area and production contributing 25% area and 46% of production (Anonymous, 2018) [2]. In Maharashtra State, the chilli is grown on area of 30.99 thousand hectares with annual production of 359.77 thousand tonnes and productivity of 2.08 (MT/ha) in years 2016-17. (Horticulture statistics at glance, 2017).

Among diseases, bacterial leaf spot of chilli, caused by *Xanthomonas axonopodis* pv. *vesicatoria* is one of the most important and was first observed in 1914 in South Africa. Bacterial leaf spots on the fruits have been shown to account for up to 52 per cent causes weight loss in infected fruits (Jones *et al*., 1986) [7].

The disease is considered to be a major constraint to chilli production all over the world (Blancard, 1997) [3]. It attacks every part of the chilli plant. Infection on leaves causes defoliation, resulting in reduced marketable fruit weight for both staked and unstaked tomatoes (Dougherty, 1978; Pohronezny and Volin, 1983) [5, 9], and increase exposure of fruits to sun scald. But the main economic effect of the disease is the reduction in fruit weight and quality. Bacterial spots on the fruits have been shown to account for up to 52 per cent weight loss in infected fruits (Jones *et al*., 1986) [7].

There are large number of chemicals/antibiotics available in the market as bactericides and their efficacy and stability needs to be verified in *in vitro* studies so as to incorporate the effective ones in the management packages. The effectiveness of bio-control depends on the choice of efficient species or isolates. The bio-control agents may act on the pathogen through antibiosis, competition for nutrients, parasitism of pathogen, disease suppression due to

prevention of colonization of the pathogen and induction of resistance in plants. Under artificial inoculation conditions, application of bioagents *viz., Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus subtilis*, *Trichoderma harzianum*, *Trichoderma viride* and *Streptomyces griseoviridis* isolates were antagonistic to tomato bacterial spot disease. Application of bioagents as soil or seedling treatments were more effective than their application as foliar treatment. By considering this disease, efficient bioagents need to be explored to fit into the disease management. (Narayanasamy, 2011) [8].

Material and Methods

In vitro evaluation of antibiotics

Antibiotics each at two different concentrations were evaluated for their sensitivity against the growth of *Xanthomonas axonopodis* pv. *vesicatoria* by inhibition zone assay method. The bacterium were mutiplied by inoculating the culture in NA media. The bacterial suspension were then seeded to the NA medium. The antibiotic solutions were prepared at different concentration. The filter paper discs measuring 5mm 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˚C to allow the diffusion of chemical in to the medium. The plates were then incubated at 27 ^{0}C and observation 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 antibiotics were recorded at 48 – 72 hours after inoculation. The inhibition zone were calculated by the formula given by Vincent (1927) [11].

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

Where

I = Per cent inhibition of growth

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

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

Results and Discussion

In vitro efficacy of antibiotics

An investigation was carried out to evaluate commercially available chemicals to find out their or efficacy against the growth of *Xanthomonas axonopodis* pv. *vesicatoria* under in *in vitro* condition. Evaluation of antibiotics was done by paper disc method. The results on the efficiency of various antibiotics and antibiotics mixed with fungicide in inhibiting the growth of bacterium expressed as inhibition zone (mm) are presented in (Table 1, Fig 1 and Plate 1).

Among different treatments, streptocycline + copper oxy chloride had showed highest inhibition (30.66 mm) and significantly superior over all treatment followed by streptocycline at 500 (28.11 mm) and streptocycline at 250 (24.77 mm). All other chemicals *viz.*, aureofungin + copper oxy chloride, aureofungin moderately effective but were significantly different from each other, and Kasagumycin were less effective and were significantly different with each other.

Interaction effect among the chemicals and concentration indicated that, Streptocycline (250 ppm) + COC (0.25% ppm) and Streptocycline at 500 ppm were highly effective with an inhibition zone of (30.66mm) and (28.11mm) respectively followed by Streptocycline 250 ppm (24.77mm). The moderately effective treatments were Aureofungin at 500 ppm + Copper hydroxide at 0.25% ppm (24.00 mm), at 500 ppm (20.66 mm), Aureofungin at 250 ppm (18.88 mm). The less effective treatment were Kasagumycin at (250 ppm + COC 0.25% ppm) with an inhibition zone (17.77 mm) and Kasagumycin at 500 ppm (15.66 mm). Kasugamycin at 250 ppm (13.88 mm) were significantly different with each other. The results of present investigation also revealed that antibiotics were effective in controlling bacterial leaf of chilli such as Streptocycline + Copper oxy chloride and Streptocycline used in the present investigation inhibited the growth of the pathogen with better inhibition zone.

Among the various antibiotics tested at 250 and 500 ppm concentrations (alone and their combination) for their efficacy against inhibiting the growth of *Xanthomonas axonopodis* pv. *vesicatoria*. The maximum inhibition zone was observed in Streptocycline + COC at (250 and 0.25%) Streptocycline at 250 and 500 ppm which found to be significantly superior over all other antibiotics tested.

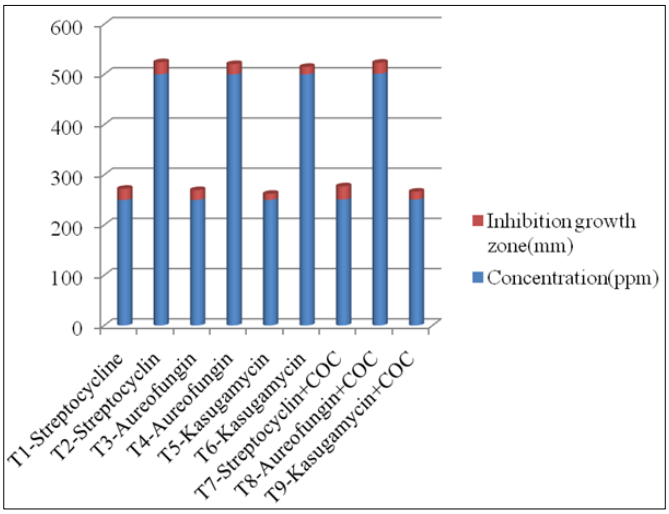

Fig 1: Effect of different antibiotics against *Xanthomonas axonopodis* pv. *vesicatoria* at different concentration in *in vitro*

Table 1: *In vitro* efficacy of antibiotics against *Xanthomonas axonopodis* pv. *vesicatoria* causing bacterial leaf spot of chilli

Tr. no.	Treatments	Conc. (ppm)	Zone of inhibition (mm)	Inhibition per cent over control
T_1	Streptocycline	250	22.00 (27.97)	24.77 (29.84)
T_2	Streptocycline	500	24.00 (29.33)	28.11 (32.01)
T_3	Aureofungin	250	19.33 (20.08)	18.88 (25.75)
T_4	Aureofungin	500	20.33 (20.80)	20.66 (27.03)
T_5	Kasugamycin	250	12.00 (20.27)	13.88 (23.66)
T_6	Kasugamycin	500	14.67 (22.52)	15.66 (23.31)
T_7	Streptomycin +Copper oxycholoride	250+0.25%	26.33 (30.87)	30.66 (33.62)
T_8	Aureofungin+Copper oxycholoride	500 +0.25%	21.67 (27.74)	24.00 (29.66)
T_9	kasugamycin+Copper oxycholoride	250+0.25%	15.67 (23.32)	(17.77) (24.93)

T_{10}	Control		90.00 (00.00)	0.00
	S.E. +		0.55	
	C.D. at 1%		1.63	

* Arcsine values

Plate 1: *In vitro* effect of antibiotics against *Xanthomonas axonopodis* pv. *vesicatoria* at different concentration

These results of the present study are in line with the findings of many earlier workers who studied antibiotics action against many phytopathogenic *Xanthomonas axonopodis* spp. *viz*., Chirame *et al*., (1993) [4] evaluated the *in vitro* antibiotics of streptocycline, paushamycin and aureofungin against *X. campestris* pv *citri*. Of the 3 antibiotics tested, the biggest zone of inhibition was produced by streptocycline (500 p.p.m.) and the smallest zone of inhibition was produced by aureofungin (10 p.p.m.).

Ingole *et al*., (2004) [6] studied *in vitro* effects of antibiotics *viz.* streptocycline, streptomycin sulfate and paushamycin (each at 0.2, 0.25 and 0.3%) against *X. axonopodis* pv. *glycines*. The antibiotics were tested in combination with copper oxychloride at different concentrations (25, 50 and 100 ppm for streptocyclin and streptomycin sulfate, and 50, 100 and 150 ppm for paushamycin). Among the concentrations, streptomycin sulfate and copper oxychloride at 0.25%+100 ppm, streptocycline and copper oxychloride at 0.3%+50 ppm and 0.25%+100 ppm, and paushamycin combined with copper oxychloride at 0.3%+50 ppm and 0.25%+150 ppm were found the most effective.

Dhutraj (2011) studied the efficacy of antibiotics *viz*., Streptocyc1in, Aureofungin 100, Plantamycine and Bactasan evaluated and recorded significant inhibition of *Xanthomonas axonopodis* pv. *vesicatora*, over untreated control. However, antibiotic Streptocycline at all test concentrations recorded significantly highest inhibition. The second best antibiotic found was Aureofungin.

Raju *et al*., (2012) [10] evaluated the different bactericides to inhibit the pathogen *Xanthomonas axonopodis* pv. *punicae*. Among the different chemicals, streptocycline + COC with an inhibition zone of 3.3 cm exhibited superior efficacy followed by streptocycline (2.80 cm) and COC (2.65 cm).

Ambadkar *et al.*, (2015) [1] studied the efficacy of different antibiotics for management of bacterial blight disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae. In vitro* study revealed that antibiotic streptocycline showed maximum inhibition zone at 250 and 500 ppm concentrations against *Xanthomonas axonopodis* pv. *punicae*, followed by Tetracycline and Bacterinol respectively.

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