International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(6): 1233-1236

Received: 10-08-2020
Accepted: 15-09-2020

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In vitro efficacy antibiotics against Bacterial blight of clusterbean caused by *Xanthomonas axonopodis* Pv. *cyamopsidis*

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DOI: https://doi.org/10.22271/chemi.2020.v8.i6r.10929

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

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

Introduction
Cluster bean [*Cyamopsis tetragonoloba* L. Taub.] (2n=14) is a drought tolerant leguminous vegetable belonging to *Leguminoceae* (*Fabaceae*) family and temperature tolerance commonly known as 'guar'. It is also known by other names such as khutti, dararretic, guari etc. In India guar is known as poor man's crop and has been grown for years in arid and semi arid conditions in southern Asia as a kharif crop. Primarily, guar is a drought hardy, deep rooted, summer annual legume, grown mainly in the dry habitat. This crop has a great industrial importance in recent years, mainly due to the presence of gum (galactomannan) in its endosperm, which constitutes about 30-32 per cent of the whole seed. Clusterbean seed is used as a concentrate for animals and for extraction of gum (Choudhary *et al.*, 2014) [7]. Clusterbean gum and its derivatives are used in various industries like textile, paper, cosmetics, mining, petroleum, pharmaceutical, food processing, oil drilling, explosives, well drilling (Rathore *et al.*, 2010) [20].

Clusterbean is commercially grown in India, Pakistan and U.S.A. and to a limited extent in Australia, Brazil and South Africa. Overall, India produces around 80% of global cluster bean production. It is cultivated on more than 4 m ha in India, Rajasthan alone accounts for around 80% of the area and production. Owing to its demand in the international market, it has been introduced in the non-traditional growing areas like Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra and Chhattisgarh. Further, its cultivation is also being taken up under irrigated conditions during summer (Bhatt, 2017) [4]. In India 2016-17 clusterbean area, production and productivity is 42.6 lakh hecter, 24.2 thousand ton and 567 kg/ha respectively. In Maharashtra area, production and productivity is 21.6 lakh ha, 0.0063 thousand ton and 360 kg/ha respectively. The leaf blight pathogen *Xanthomonas axonopodis* pv. *cyamopsidis* causes drastic reduction in plant stand and yield as high as 58% in cultivar. It is seed borne and can survive in seeds for up to one year. The disease appears both as leaf spot and blight simultaneously. The spots initially are intraveinal, round, water soaked or oily in appearance and well defined on the dorsal surface of leaf. The bacterial blight of guar is characterized by the appearance of 'V' shaped lesions, which rapidly spread to the entire lamina giving it a totally blighted appearance.

Material and Methods
In vitro evaluation of Bioagents
Antibiotics each at two different concentrations were evaluated for their sensitivity against the growth of *Xanthomonas axonopodis* pv. *cyamopsidis* by inhibition zone assay method.

The bacterium were multiplied 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 ˚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).

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

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

Experimental details

Design : CRD

Replications : Three

Treatments : Ten

Table 1: Treatment details

Treatments	Chemical name	Concentration
T_1	Streptocycline	250ppm.
T_2	Streptocycline	500ppm
T_3	Aureofungin	250ppm
T_4	Aureofungin	500ppm
T_5	Kasugamycin	250ppm
T_6	Kasugamycin	500ppm
T_7	Streptocycline + Copper oxychloride	250ppm+0.25%
T_8	Aureofungin + Copper oxychloride	500ppm+0.25%
T_9	Kasugamycin + Copper oxychloride	250ppm+0.25%
T_{10}	Control (Untreated)	

***In vitro* efficacy of Bioagents**

The results indicated that Streptocycline + Copper oxychloride had showed significant superiority over other treatments with highest inhibition (22.20 mm) at 250 ppm + 0.25 per cent followed by Streptocycline at 500ppm(21.67 mm) and Streptocycline 250 ppm (16.50 mm). However, all other chemicals *viz*., Aureofungin 500 ppm + Copper oxychloride 0.25%, Aureofungin 500 ppm, Aureofungin 250 ppm, Kasugamycin 250 ppm + Copper oxychloride 0.25%, Kasugamycin 500 ppm were on par with each other, whereas, Kasugamycin was least effective.

The interaction effect among the chemicals and concentrations indicated that Streptocycline (250 ppm) + Copper oxychloride (0.25%) and Streptocycline (500 ppm) alone were found significantly superior over other treatments with an inhibition zone of 22.20 mm and 21.67 mm, respectively. Thus, all the antibiotic tested were found antibiotic against *Xanthomonas axonopodi* pv. *cyamopsidis* and significantly inhibited its bacterial growth, over untreated control. *In vitro* evaluation of antibiotics provides the preliminary information about the efficacy of particular chemical in a shortest period of time and therefore it serves as a basis for further field assay. Thirumalachar *et al.* (1956) reported the bioefficacy of streptomycin sulphate in inhibiting *Xanthomonas axonopodis* pv. *cyamopsidis* under *in vitro* conditions.

Among the different chemicals evaluated in the present investigation, Streptocycline + Copper oxychoride exhibited superior efficacy in inhibiting *Xanthomonas axonopodis* pv. *cyamopsidis* with an inhibition zone of 22.20 mm followed by Streptocycline 500 ppm (21.67 mm) alone. However both the treatments were on par with each other. All other chemicals *viz*., Aureofungin 500 ppm + COC 0.25%, Aureofungin 500 ppm, Aureofungin 250 ppm, Kasugamycin 250 ppm + COC 0.25% were found to be moderately effective but were significantly different from each other. Kasugamycin 500 ppm and Kasugamycin 250 ppm were less effective. In respect of concentration and combination of antibiotics and chemical, efficacy varied significantly.

Table 2: *In vitro* efficacy of Bioagents against *Xanthomonas axonopodis* Pv. *Cyamopsidis*

Tr. No.	Treatments	Concentration	Mean inhibition zone(mm)
T_1	Streptocycline	250	16.50 (23.20)*
T_2	Streptocycline	500	21.67 (27.87)
T_3	Aureofungin	250	9.87 (18.32)
T_4	Aureofungin	500	11.43 (19.93)
T_5	Kasugamycin	250	5.56 (14.75)
T_6	Kasugamycin	500	5.93 (14.80)
T_7	Streptocycline+ Copper oxychloride	250+0.25%	22.20 (28.75)
T_8	Aureofungin + Copper oxychloride	500+0.25%	13.70 (21.67)
T_9	Kasugamycin + Copper oxychloride	250+0.25%	7.76 (16.36)
T_{10}	Control		0.00 (0.00)
SE(m)+			0.13
CD@1%			0.46

*Figures in Parenthesis are arcsine transformed values

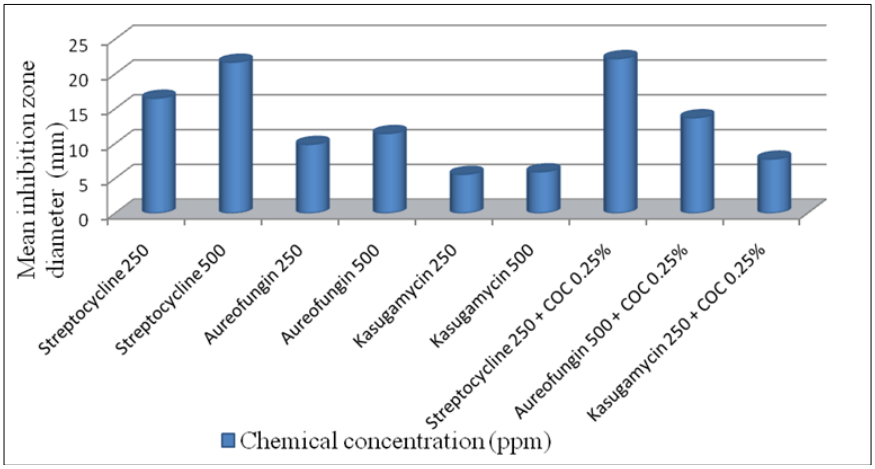

Fig 1: *In vitro* efficacy of Bioagents against *Xanthomonas axonopodis* pv. *cyamopsidis*

Fig 2: *In vitro* efficacy of different Bioagent on mycelial growth of *Xanthomonas axonopodis* pv. *cyamopsidis*

Conclusion and discussion

Considering the magnitude of the disease and its resultant losses, the investigation was undertaken to study the disease and pathogen thoroughly to bring out an appropriate management aspects to mitigate the problem effectively. *In vitro* evaluation of antibiotics revealed that Streptocycline + Copper oxychloride (250ppm + 0.25%) with an inhibition zone of 22.20 mm exhibited superior efficacy against the growth of *X. axonopodis* pv. *cyamopsidis* followed by Streptocycline 500ppm (21.67 mm) alone and Streptocycline 250ppm (16.50mm). Streptocycline (250ppm) in combination with Copper oxychloride (0.25%) was found to be the most efficient in controlling the pathogen under *in vitro* condition. Our results similar with earlier workers who studied antibiotics action against many phytopathogenic *Xanthomonas axonopodis* spp. *viz*., Lambani *et al.* (2017) [11] studied efficacy of different antibiotics and chemicals was assessed *in vitro* by paper disc method the combination tested the maximum inhibition (3.41 cm) was recorded in streptocycline at 750 ppm + copper oxychloride (0.3%) followed by same combination with reduced concentration, streptocycline at 500 ppm + copper oxychloride (0.2%) was 3.09 cm.

Dhutraj (2011) [8] studied the efficacy of antibiotics *viz*., Streptocycline, Aureofungin 100, Plantamycine and Bactasan evaluated and recorded significant inhibition of *Xanthomonas axonopodis* pv. *vesicatora*, over untreated control. However, antibiotic Streptocyclin at all test concentrations recorded significantly highest inhibition. The second best antibiotic found was Aureofungin.

Antre *et al.* (2016) [3] studied efficacy of total 18 the chemicals and bioagents against *Xanthomonas axonopodis* pv. *punicae* by using disc diffusion method. Maximum zone of inhibition were recorded in chemical treatments. Copper oxychloride (0.3%) + Streptomycin sulphate (500 ppm) was found significantly superior in inhibiting the growth of bacteria with 21.33mm zone of inhibition.

Ambadkar *et al.*, (2015) [2] 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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