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## *In vitro* efficacy bioagents against bacterial blight of clusterbean caused by *Xanthomonas axonopodis* pv. *cyamopsidis*

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**Abstract**

The different bioagents were evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis* causing bacterial blight of clusterbean under *in vitro* condition. Among all the eight bioagent viz., *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Bacillus subtilis*, *Trichoderma konigii*, *Azospirillum sp.*, *Acetobacter sp.* and *Pseudomonas fluorescences* were evaluated *in vitro* against *Xanthomonas axonopodis* pv. *cyamopsidis*. All the treatments significantly inhibited bacterial growth of *Xanthomonas axonopodis* pv. *cyamopsidis* over untreated control. Among eight bioagent *Pseudomonas fluorescences*, *Bacillus subtilis* and *Acetobacter sp.* significantly inhibited the bacterial growth each at 81.26%, 78.95% and 76.72% respectively.

**Keywords:** *Xanthomonas axonopodis*, *Pseudomonas fluorescences*, *Bacillus subtilis*

**Introduction**

Cluster bean [*Cyamopsistetragonoloba* 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 mans crop and has been grown for years in arid and semi arid conditions in southern Asia as a kharif crop (Kumar, 2005) [13]. 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) [6].

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) [15]. 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) [3]. In India 2016-17 clusterbean area, production and productivity is 42.6 lakh hecter, 24.2 thousand ton and 567 kg/ha respectively (Anonymous, 2017) [1]. In Maharashtra area, production and productivity is 21.6 lakh ha, 0.0063 thousand ton and 360 kg/ha respectively (Anonymous, 2017) [1]. 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 (Sain and Gour, 2009) [16]. 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**

A total of eight bioagents (as detailed under treatments) were evaluated *in vitro* against *Xanthomonas axonopodis*, by inhibition zone assay method. A suspension of *Xanthomonas* the sterilized petriplates and allowed to solidify.

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A loopful *axonopodis* pv. *Cyamopsidis* multiplied in nutrient broth (20 ml) was mixed with nutrient agar medium (1000 ml) contained in flask. Fifteen to twenty ml of seeded medium was poured into culture of the antagonistic organism was placed in the centre of petriplates containing the seeded medium. In case of fungal antagonists, mycelial discs of 5 mm (dia.) size taken from actively growing culture were placed in the centre of the plates. The inoculated plates were then incubated at 28°C for 72 hours. The experimental details were as given below:

### Experimental details

Design: CRD

Replications: Three

Treatments: Nine

### Treatment details

Tr. No.	Treatments	Tr. No.	Treatments
T <sub>1</sub>	<i>Trichoderma viride</i>	T <sub>6</sub>	<i>Azospirillum</i> sp.
T <sub>2</sub>	<i>Trichoderma harzianum</i>	T <sub>7</sub>	<i>Acetobacter</i> sp.
T <sub>3</sub>	<i>Trichoderma hamatum</i>	T <sub>8</sub>	<i>Pseudomonas fluorescens</i>
T <sub>4</sub>	<i>Bacillus subtilis</i>	T <sub>9</sub>	Control (untreated)
T <sub>5</sub>	<i>Trichoderma konigii</i>		

Observations were recorded for the production of inhibition zone around the antagonistic microorganisms around the growth of the pathogen and the results obtained were calculated and analyzed statistically.

Per cent inhibition of the test pathogen by the bioagents, over untreated control was calculated by applying following formula (Vincent, 1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Per cent inhibition

C = Diameter of pathogen colony in control

T = Diameter of pathogen colony in treatment

### In vitro efficacy of bioagents

Results (Table 5) revealed that all the bioagents evaluated exhibited fungistatic/antifungal activity against *Xanthomonas axonopodis* pv. *cyamopsidis* and inhibited its growth, over untreated control (Plate-V).

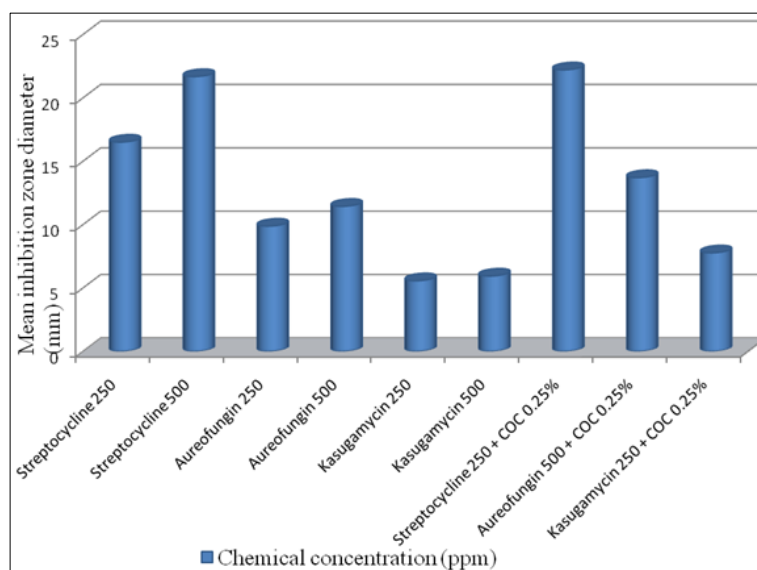
However, *T. viride* was found most effective with least mycelial growth (16.86 mm) and highest mycelial inhibition (81.26%), followed by *T. harzianum* (18.94 mm and 78.95%), *T. hamatum* (20.97 mm and 76.72%), *Aspergillus niger* (36.17 mm and 59.81%), *T. konigii* (39.16 mm and 56.48%), *Pseudomonas fluorescens* (47.50 mm and 47.42%) and *Bacillus subtilis* (50.17 mm and 44.25%).

The hyphae of *Trichoderma* wrap around the pathogen fungi and produce antibiotics and extracellular enzymes, which lyses the cell wall of these pathogens that damage them. The invading fungus eventually collapse and disintegrates.

**Table 1:** In vitro efficacy of bioagents against *Xanthomonas axonopodis* pv. *Cyamopsidis*

Tr. No.	Treatments	Mean Inhibition Zone (mm)*
T <sub>1</sub>	<i>Trichoderma viride</i>	1.80 (3.42)*
T <sub>2</sub>	<i>T. harzianum</i>	2.60 (8.90)
T <sub>3</sub>	<i>T. hamatum</i>	3.39 (10.45)
T <sub>4</sub>	<i>Bacillus subtilis</i>	11.50 (2.40)
T <sub>5</sub>	<i>T. konigii</i>	2.40 (8.87)
T <sub>6</sub>	<i>Azospirillum</i> sp.	5.76 (13.71)
T <sub>7</sub>	<i>Acetobacter</i> sp.	8.90 (16.35)
T <sub>8</sub>	<i>Pseudomonas fluorescens</i>	12.64 (20.97)
T <sub>9</sub>	Control	0 (0.00)
	S. E. ±	0.04
	C.D. (P=0.01)	0.19

\*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. Among the eight bioagents studied the *Pseudomonas fluorescens* was found most effective for inhibiting the growth of *Xanthomonas axonopodis* pv. *cyamopsidis* followed by *Bacillus subtilis*. These findings are similar to the results reported by Jindal *et al.* (1989) who tested the efficacy of *Bacillus spp.* to inhibit the growth of *Xanthomonas axonopodis* pv. *vignicola* and found that *Bacillus subtilis* was effective in controlling bacterial blight of cowpea.

Massomo *et al.* (2004)<sup>[14]</sup> also reported the inhibitory action of *Bacillus subtilis* against the bacterium *Xanthomonas axonopodis* pv. *vignicola* causing bacterial blight of cowpea which is similar to the observations recorded in the present study. Apet *et al.* (2018)<sup>[2]</sup> studied efficacy of different bioagent viz., *T. hamatum*, *B. subtilis*, *P. fluorescence*, *T. virens*, *T. Harzianum* and *T. viride* against *Xanthomonas axonopodis* pv. *punicae*. Maximum inhibition was observed due to the *Pseudomonas fluorescens* (12.82 mm), while minimum with *T. virens* (2.38mm).

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