The Pharma Innovation Journal 2022; 11(1): 43-47

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(1): 43-47

www.thepharmajournal.com
Received: 02-10-2021
Accepted: 06-12-2021

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In vitro evaluation of differents bioagents, antibiotics and fungicides against bacterial blight of Clusterbean caused by *Xanthomonas axonopodis* pv. *cyamopsidis*

Anita Jat, PS Shekhawat, KK Saini, Tara Yadav and Rajpal Yadav

Abstract
Four bioagents, two antibiotics and two fungicides were evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis in vitro* condition to develop effective management strategies for bacterial blight of clusterbean. During *in vitro* studies, bioagents, antibiotics and fungicides were able to inhibit growth of the bacteria at all the concentrations tried. The maximum inhibition zone (30.50 mm) was shown by streptocycline followed by neomycin (24.50 mm), copper oxychloride (23.25 mm) and copper hydroxide (19.58 mm) at their highest concentration. Streptocycline @ 300 ppm and copper oxychloride @ 2000 ppm were found superior, as compared to others. None of the bioagent was found effective against the bacterial blight pathogen *X. axonopodis* pv. *cyamopsidis* except *P. fluorescens* where 10.70 mm mean diameter of inhibition zone was recorded.

Keywords: Clusterbean, bacterial blight, *in vitro*, *Xanthomonas*, bioagents and antibiotics

Introduction
Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] is an important annual legume crop of *kharif* season in arid and semi-arid regions of the Indian subcontinent. It is a self-pollinated, short duration legume crop generally cultivated under resource constrained conditions on marginal and sub marginal lands (Kumar, 2005) [7]. Clusterbean belongs to the tribe Indigoferae of the Leguminosae (Fabaceae) family with diploid chromosome number 2n=14. The crop is known for drought tolerance having deep root system (Kumar and Rodge, 2012) [6]. Clusterbean is a native to the Indian subcontinent. It is an erect, bushy, annual herbaceous legume up to 3 m height with trifoliate leaves up to 10 cm long and white flowers. The pods are straight, hairy, pale shiny green, up to 12 cm long and contain 5 to 12 hard seeds. The area under clusterbean production in India is 4.26 million ha with a production of 2.42 million tonnes and productivity of 567 kg/ha (Anonymous, 2020) [2]. Rajasthan is the biggest clusterbean producer state contributes about 80 per cent of the total clusterbean production in the country. In Rajasthan, area under the clusterbean crop is 35.30 lakh hectare with production of 14.04 lakh tonnes and productivity 398 kg/ha (Anon, 2020) [2]. Clusterbean is grown in all the districts of Rajasthan however, Alwar, Barmer, Bikaner, Churu, Hanumangarh, Jaipur, Jaisalmer, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Pali, Sri-Ganganagar and Sikar are major producer contributing about 80 per cent of the total clusterbean production in the state.
The disease has been resulted in enormous losses to clusterbean yield. Hence, it is the demand of the time to search for suitable and effective control measures against the disease. Therefore, the present investigation was planned to test the efficacy of different botanicals, bioagents, antibiotics and fungicides in management of bacterial blight disease of clusterbean caused by *Xanthomonas axonopodis* pv. *cyamopsidis.* The botanicals, bioagents, antibiotics and fungicides which were found best in these treatments were applied in integrated management schedule.

Material and Methods
Collection of diseased samples
Samples of naturally infected clusterbean plants were collected from Jaipur, Sikar and Bikaner districts during *kharif* 2018. The infected aerial parts of the diseased samples were carefully placed in polythene bags, properly tagged and brought to the laboratory. Samples were thoroughly washed with sterile distilled water. To confirm the presence of bacterium, ooze tests were performed regularly from different plant parts of clusterbean.

Isolation of the pathogen

Infected plant parts such as leaf, stem, pods and seeds showing typical symptoms of bacterial blight disease were taken for isolation of causal bacterium. For isolation, infected portions of plants were cut, surface sterilized with 0.1 per cent sodium hypochoride (NaOCl) solution for two minutes and rinsed thoroughly thrice with sterile distilled water. The diseased bits were then transferred individually into a few drops of sterile water on a sterilized glass slide under aseptic conditions. The diseased bits were given a cut with sharp sterilized blade. The bits were left for one minute to allow bacterial ooze to come out in water. A charged needle with ooze was streaked on nutrient agar Petri plates. Three Petri plates were streaked at a time, without recharging the needle loop.

Purification of the pathogen

This procedure was repeated several times using fresh sets of nutrient agar plates each time. The inoculated plates were incubated at 28±2 ºC for 48hrs in an inverted position. The suspected bacterial colonies were picked up with the help of sterilized inoculated loop and further streaked on the surface of nutrient agar medium. The inoculated plates were incubated at 28±2 ºC for 48 to 72hrs and obtained bright yellow colored bacterial colonies. The purified bacterial colonies were streaked on yeast extract glucose chalk agar slants and stored at 5 ºC in refrigerator and also in sterile distilled water tubes, by suspending 2-3 loops full of the bacterial culture for further studies.

Pathogenicity tests

Pathogenicity tests were conducted by inoculating one month old clusterbean plants raised in 25 cm earthen pots by spraying the bacterial suspension thrice at 24 hrs intervals. A fresh 72 hrs old bacterial culture, grown on nutrient agar media was always used for inoculations on the plants. The culture was harvested in 10 ml sterile water diluted to a concentration of $2.5x10^8$ cfu/ml and used immediately. The suspension was sprayed on plants with hand atomizer twice at 24 hrs interval. Suitable control was maintained using only distilled water in place of inoculum suspension. The forty inoculated plants were kept in cage house under high humid condition for 48 hrs then under natural cage house conditions. Uninoculated plants served as control. The plants were watered at frequent interval and regularly observed for the appearance of disease symptoms. Then pathogen was again reisolated from newly inoculated plants and compared with the original culture.

***In vitro* evaluation of bioagents, antibiotics and fungicides**

In vitro, evaluation of bioagents, antibiotics and fungicides were tested against bacterium at different four concentrations on bioassay medium (Waksman and Reilly, 1945) [16]. The experiemnts were conducted in CRD (Completely Randomized Design) with five replications. The observations were recorded on the basis of inhibition zone formed in plates by testing botanicals, bioagents, antibiotics and fungicides.

***In vitro* evaluation of bioagents**

Under *in vitro* conditions four bioagents *viz., Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their efficacy against the growth of *X. axonopodis* pv. *cyamopsidis* by inhibition zone assay method. One ml of bacterial suspension in sterile distilled water prepared by suspending 48 hrs old bacterial culture from yeast extract glucose chalk agar slants in 5 ml sterile water was incorporated in 250 ml of bioassay medium mixed thoroughly and 20 ml of medium was poured in each sterilized Petri plate. The seeded medium was poured into the sterilized plates and plates were allowed to solidify. A loopful culture of each of the antagonist was placed at one points in plates containing the seeded medium. The inoculated plates were then incubated at 28±2ºC for 72 hrs. Observations were recorded for the zone of inhibition produced by antagonists around the growth of the pathogen.

***In vitro* evaluation of antibiotics and fungicides**

Each chemical at four concentrations was evaluated for their efficacy against the growth of *X. axonopodis* pv. *cyamopsidis* by paper disc method (Waksman and Reilly, 1945) [16]. One ml of bacterial suspension in sterile distilled water prepared by suspending 48 hrs old bacterial culture from yeast extract glucose chalk agar slants in five ml sterile water was incorporated in 250 ml of bioassay medium mixed thoroughly and 20 ml of medium was poured in each sterilized Petri plate. The seeded medium was poured into the sterilized plates and plates were allowed to solidify. Different concentrations solution of antibiotics (150, 200, 250 and 300 ppm) and fungicides (500, 1000, 1500 and 2000 ppm) were prepared for each chemical. The filter paper discs (Whatman No.42) measuring 10 mm in diameter were soaked in the respective chemical solution for 10 min and transferred onto the surface of the seeded medium in Petri plates (3 discs/ plate). The inoculated plates were kept in the refrigerator at 5 ºC for 4 hrs to allow the diffuse of chemicals into the medium. Then, plates were incubated at 28±2ºC for 72 hrs and observed for the production of inhibition zones around the filter paper discs.

Table 1: List of fungicides and antibiotics

Common name	Trade name	Chemical name	Company
Streptocycline	Labistryn	N-methyl-L-glycosamine, streptose and streptidine	Hindustan antibiotics ltd., Pimpri (Pune)
Neomycin	Neosporin	B-bulfate, bacitracin zink powder and polymyxin	Glaxosmith kline pharmaceuticals limited (India), Bangalore
Copper oxychloride	Blitox-50% WP	Copper oxychloride -50	National pesticide and chemicals, Amravati (Maharastra)
Copper hydroxide	Kocide	Copper hydroxide	DuPont (India) ltd., Mumbai

Result and Discussion

***In vitro* evaluation of bioagents:** The four bioagents *viz., Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their efficacy against the growth of *X. axonopodis* pv. *cyamopsidis* by inhibition zone assay method. The data depicted in table 2 and fig.1 revealed that none of the bioagent was found effective against the bacterial blight pathogen *Xanthomonas axonopodis* pv. *cyamopsidis* except *P. fluorescens* which showed 10.70 mm mean diameter of inhibition zone.

Table 2: *In vitro* evaluation of bioagents against *Xanthomonas axonopodis* pv. *cyamopsidis* caused bacterial blight of clusterbean

S. No.	Treatments	Mean diameter of inhibition zone (mm)
1.	*Trichoderma viride*	5.12
2.	*Trichoderma harzianum*	4.20
3.	*Pseudomonas fluorescens*	10.70
4.	*Bacillus subtilis*	6.70
5.	Control	00.00
	S.Em±	0.67
	CD at 5%	2.08

All data are mean of five replications

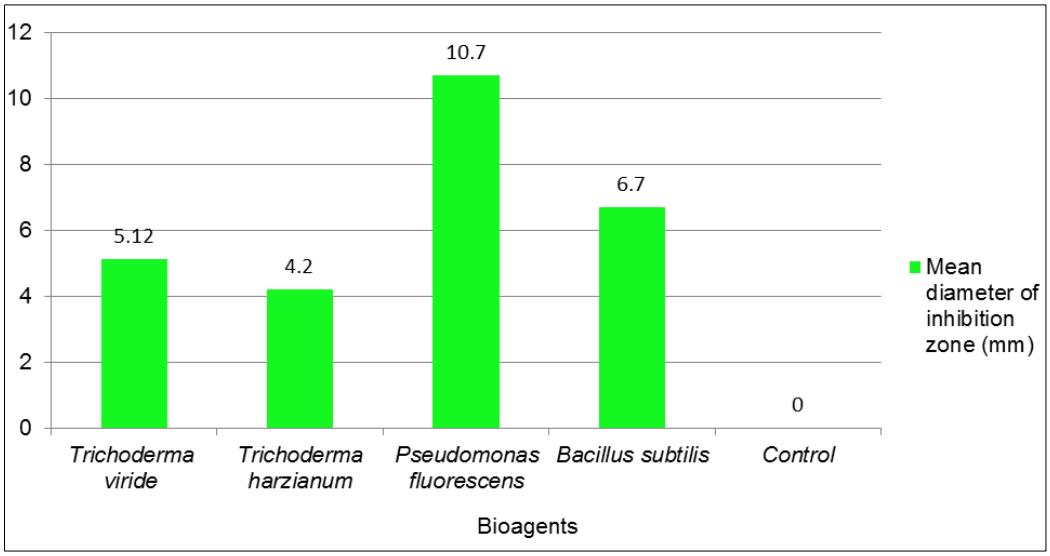

Fig 1: *In vitro* evaluation of bioagents against *Xanthomonas axonopodis* pv. *cyamopsidis* caused bacterial blight of clusterbean

However, similar attempts had been also made by several workers on clusterbean as well as other crops. Sain and Gour (2009) [14], Raju *et al.* (2012) [13], Shankar *et al.* 2015 [15], Yenjerappa *et al.* (2011) [17], Kumar and Kumar (2017) [8], Patil *et al.* (2017b) [11], Kumar and Jahangirdar (2017) [11], Apet *et al.* (2018b) [18] recorded *P. fluorescens* as effective in mangement of oily spot of pome granate and Bhure *et al.* (2019) [4] who found *P. fluorescens* as the best bioagent in management of bacterial blight disease of clusterbean.

In vitro evaluation of antibiotics and fungicides

Table 3: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis* pv. *cyamopsidis* caused bacterial blight of clusterbean

S. No.	Treatments	Concentration (ppm)	Mean diameter of inhibition zone (mm)
1.	Streptocycline	150	21.66
		200	24.50
		250	28.66
		300	30.50
2.	Neomycin	150	17.50
		200	19.51
		250	22.48
		300	24.50
3.	Copper oxychloride	500	15.20
		1000	17.45
		1500	20.80
		2000	23.25
4.	Copper hydroxide	500	9.50
		1000	13.46
		1500	16.66
		2000	19.58
5.	Control	00.00	0.00
S.Em±		-	0.43
CD at 5%		-	1.24

All data are mean of five replications

The efficacy of two antibiotics and two copper-based fungicides were tested against the bacteria by paper disc diffusion technique at 150, 200, 250 and 300 ppm and 500, 1000, 1500 and 2000 ppm concentrations. The data presented in table 3 and fig.2 revealed that both antibiotics and fungicides were significantly inhibiting the growth of *X.*

axonopodis pv. *cyamopsidis.* The maximum inhibition zone (30.50 mm) was shown by streptocycline at 300 ppm concentration followed by neomycin (24.50 mm), copper oxychloride (23.25 mm) and copper hydroxide (19.58 mm) against *X. axonopodis* pv. *cyamopsidis* at their highest concentration (Plate 1). In a similar study, Raju *et al.* (2012) [13], Patil *et al.* (2017a) [10], Amit *et al*. (2017) [1], Jadhav *et al.* (2018) [5] and Prasad *et al*. (2018) [12] said that the streptocycline (250 ppm) showed best result in management of bacterial blight in rice followed by copper oxychloride (2.0%).

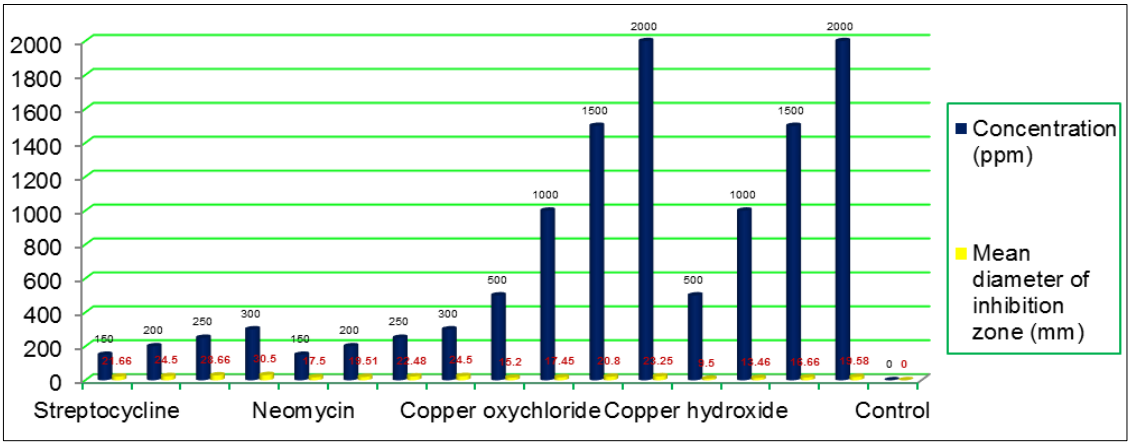

Fig 2: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis* pv. *Cyamopsidis* caused bacterial blight of clusterbean

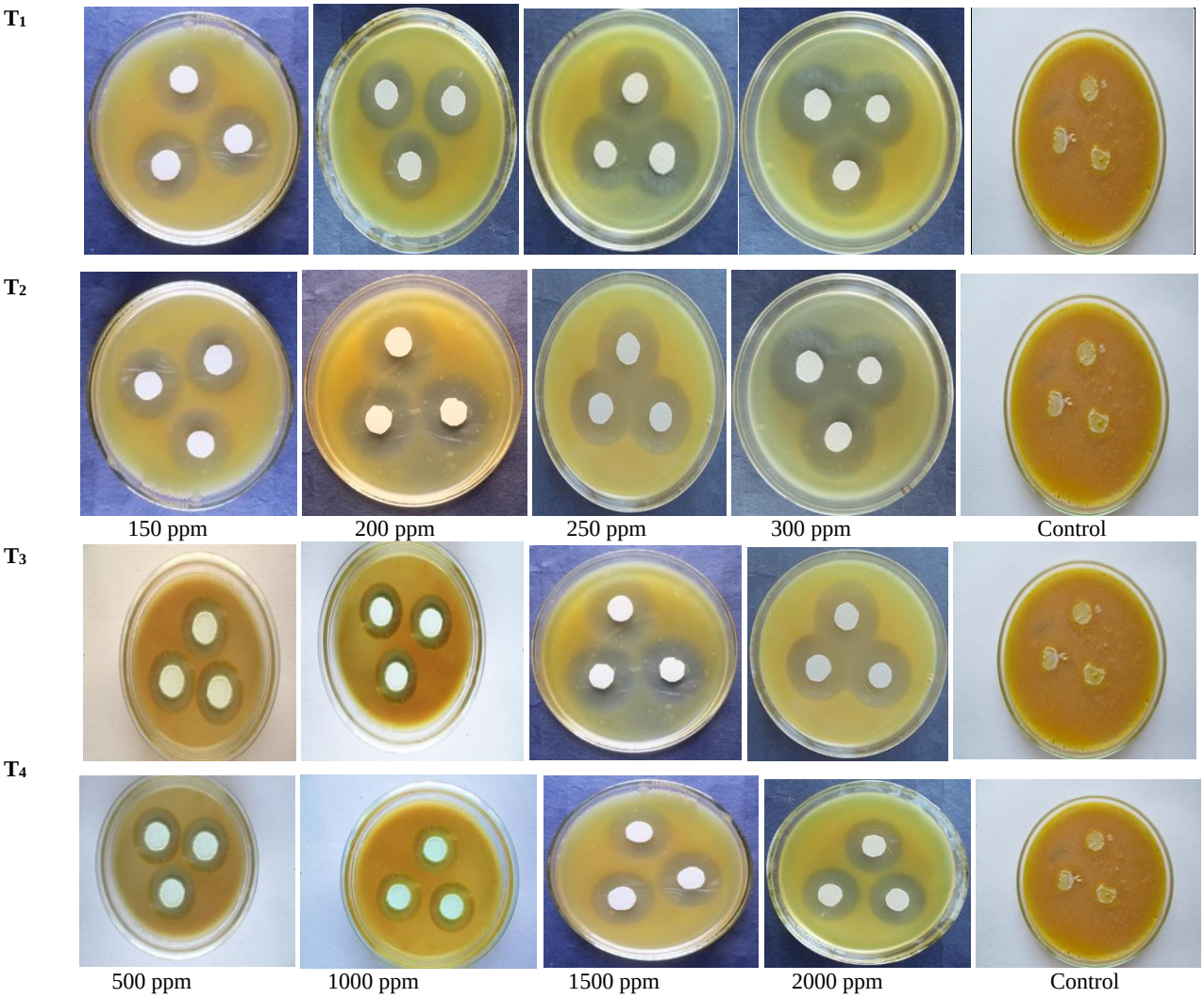

Plate 1: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis* pv. *Cyamopsidis* caused bacterial blight of clusterbean

Conclusion

During *in vitro* studies, bioagents, antibiotics and fungicides were able to inhibit growth of the clusterbean bacterial blight bacterium at all the concentrations tried. *P. fluorescens*, streptocycline @ 300 ppm and copper oxychloride @ 2000 ppm were found superior, as compared to others bioagents, antibiotics and fungicides.

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