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EVALUATION OF BIOAGENTS AGAINST *XANTHOMONAS AXONOPODIS* PV. *CYAMOPSIDIS*

PRATEEK KUMAR[1,*], DALVINDER PAL SINGH[2], RAKESH PUNIA[1], ARVIND[3] AND PRAGATI YADAV[4]

[1] Department of Plant Pathology, [2]Department of Genetics and Plant Breeding (Forage Section), [3]Department of Entomology, [4]Department of Agronomy
CCS Haryana Agricultural University, Hisar-125 004 (Haryana), India
(e-mail : prateekberwalhau@gmail.com)

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SUMMARY

Cluster bean (*Cyamopsis tetragonoloba* L. Taub.) (2n=14) is an indigenous, self-pollinated, drought tolerant legume crop. This crop has recently gained the status of industrial crop due to the high galactomannan content in the endosperm of its seed. This crop is vulnerable to bacterial leaf blight as one of the most destructive diseases of cluster bean caused by *Xanthomonas axonopodis* pv. *cyamopsidis* and confines cluster bean productivity in all growing regions. Biological control is an ecological-friendly approach to suppress the growth of phytopathogens. In this study, four bio-agents *viz. Bacillus subtilis, Providencia* sp., *Trichoderma viride* and *Trichoderma harzianum* were evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis in vitro* condition. *Providencia* sp. showed maximum antibacterial activity with 14.12 per cent zone inhibition followed by *Bacillus subtilis* (12.13%). The fungal antagonists *viz. Trichoderma viride* (7.11%) and *Trichoderma harzianum* (5.95%), were found least effective against the tested pathogen. *Providencia* sp.@ 25 per cent w/v reduce disease incidence of 50 per cent under screen house conditions followed by *Bacillus subtilis* (35.71%). Among the treatments, the lowest disease reduction and highest per cent disease incidence was observed in treatment with *Trichoderma harzianum* (7.14). Therefore, bioagents can be used as a sustainable tool for management of plant diseases towards sustainable agriculture.

Key words: *Biocontrol, Providencia, Bacillus subtilis, Trichoderma viride, Xanthomonas axonopodis* pv. *cyamopsidis,* Bacterial blight

Cluster bean [*Cyamopsis tetragonoloba* (L.)] is an annual legume crop that is primarily grown in arid and semi-arid regions under resource-constrained conditions. A plant with deep roots in the Leguminosae (Fabaceae) family, cluster beans are renowned for their ability to withstand extreme temperatures and drought. India, Pakistan, the United States, Italy, Morocco, Germany, and Spain are the main nations that cultivate cluster bean. Approximately 80% of the cluster beans produced worldwide are produced in India. In India, cluster bean is mostly grown for gum in the desert states of Rajasthan, Haryana, Gujarat, and Punjab; in other locations, however, they are grown for vegetables. Cattle feed/fodder, green manure, and vegetables are just a few uses for cluster beans. Its soft green pods are an inexpensive and high-quality source of nutrients. Moreover, high-protein cattle feed made from cluster bean meal and seed is utilized (Ghorpade *et al.,* 2018). Known by another name, “guaran,” cluster bean gum is a naturally occurring hydrocolloid found in the endosperm of seeds. It has become the most significant naturally occurring, safe, non-toxic agrochemical. After the seed is dehusked, ground endosperm is the main source of gum production. Cluster beans are a new industrial crop with significant potential for earning foreign cash due to their variety of the following clusterbean gum derivatives are significant from a commercial standpoint: guar gum acryl amide, oxydized guar gum, acetate guar gum, sulphated guar gum, hydroxy and carboxy guar gum, and carboxyl methyl hydroxyl propyl guar gum. Numerous sectors, including textile, paper, cosmetics, mining, petroleum, pharmaceuticals, food processing, oil drilling, and explosives, use its derivatives. Due to its favorable climate and rain-fed cultivation in the northwest Indian states of Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh, and Madhya Pradesh, India is one of the world’s top producers of cluster bean, contributing approximately 82% of the crop’s total production. 3.14 million hectares of cluster beans were grown in India in 2019–20, yielding 1.52 million tonnes of production and 484 kg/ha of productivity. The state that produces the most cluster beans is Rajasthan, followed by Haryana. Haryana's contribution to India's clusterbean output fluctuates from 18% to 30% depending on the year. The districts of Bhiwani, Dadri, Gurgaon, Mahendragarh, Rewari, and Hisar in Haryana are those that produce cluster bean (Agarwal *et al.*, 2018). Farmers in Andhra Pradesh have begun cultivating this crop for seeds on more than 1000 hectares after realizing there were more opportunities with it in past years in Rajasthan and Haryana. Anthracnose (*Colletotrichum capsici* f.sp. *cyamopsidis*), bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*), alternaria leaf spot (*Alternaria cyamopsidis*), dry root rot/leaf blight (*Fusarium solani* and *Rhizoctonia solani*), and wilt (*Fusarium caeruleum*) are significant diseases of arid legume cluster bean. The most damaging disease to cluster beans among all of these is bacterial leaf blight, which is caused by *Xanthomonas axonopodis* pv. *Cyamopsidis* (Giri *et al*., 2008) Bacterial leaf blight of clusterbean (*Cyamopsis tetragonoloba* (L.) Taub) caused by *Xanthomonas axonopodis* pv. *cyamopsidis* is one of the important diseases of clusterbean crop from economic point of view as it confines the crop productivity in all growing regions. This disease affects all the above ground plant parts and cause yield losses to the extent of 60 per cent. The devastating nature of the disease makes its management more difficult and huge importance whereas lack of resistant germplasm in cluster bean encouraged us to study of effectiveness of various botanicals and bio-agents against this phytopathogenic bacteria and evaluation of resistance in the available genetic material of cluster bean for eco-friendly and economical management of the disease.

Most of the rhizospheric antagonistic microorganism viz. *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma viride* and *Trichoderma harzianum* increases plant resistance by improving the plant growth and responses of the host due to their better root colonization which plays important role in disease suppression. These antagonists can directly suppress plant pathogens by producing antibiotics enzymes like chitinases, glucanases, proteases, and siderophores and indirectly through mechanisms for better competitive ability for nutrients and space. These have been reported to reduce the disease incidence significantly under controlled as well as under natural field conditions (Kumhar *et al.,*2018).

Researchers have used various traditional disease management strategies as control measures so far but not much has been done in the evaluating the antagonistic nature of bioagents against bacterial leaf blight in guar. Therefore, objectives of the research is to find out efficacy of bio-rational components against *X. axonopodis* pv. *cyamopsidis.*

MATERIALS AND METHODS

In-vitro evaluation of bio-agents

The antagonistic effect of bio-agents viz. *Bacillus subtilis, Providencia* sp.*, Trichoderma harzianum* and *Trichoderma viride* were evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis* under in-vitro conditions using dual culture technique as per standard lab protocol *In vitro* evaluation of botanicals components against *Xanthomonas axonopodis* pv. *cyamopsidis.*

Procedure

A suspension of *Xanthomonas axonopodis* pv. *cyamopsidis* was multiplied in nutrient broth (20 ml). The sterilized Petri plates were filled with 15–20 ml of nutrient agar (NA) media, which was then let to solidify. 100 µl of bacterial suspension (1.0 × 108 cfu ml^{-1}) were spread onto the surface of nutrient agar plate using sterile cotton swabs. The medium-containing Petri plates were inoculated with a loop full culture of the antagonistic organism in the centre. In the case of fungi that function as antagonists, 5 mm diameter mycelial discs from a culture that was actively growing were positioned in the middle of the plates. The plates were then incubated for 72 hours at 28°C. The findings of the observations were calculated and statistically evaluated for the construction of an inhibitory zone surrounding the antagonistic microorganisms around the growth of the pathogen.

The percent growth inhibition in each treatment was calculated according the formula given by 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

Design: CRD

Replications: 4

Evaluation of efficacy of selected bio-agents against *Xanthomonas axonopodis* pv. *cyamopsidis* under screen house conditions

Cluster bean crop was raised in the earthen pots containing sandy-loam soil mixed with well-decomposed FYM in the screen house of Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. Susceptible variety (Pusa Navbahar) of cluster bean was sown in pots on 30th July, 2021 at uniform depth and distance to get optimum plant stand in pot. Rouging was done to remove weeds. Plants were irrigated using tap water as and when required. The pots containing 25 to 30 days old plants were artificially inoculated with *Xanthomonas axonopodis* pv. *cyamopsidis* by spraying the bacterial suspension of 1×10^8 cfu ml^{-1}. The pots were covered with the polythene bags for next 48 h to maintain humidity. After 6 to 8 days of inoculation, when characteristic blight symptoms were shown on plants, bio-agents found effective in inhibiting bacterial blight under *in vitro* condition were sprayed over the crop plants.

The suspension of various concentrations was prepared in sterilized water just before inoculation. Total number of replications were three.

Observation on per cent disease incidence was recorded. Disease incidence was determined as number of plants affected per pot and expressed in percentage.

Disease incidence was calculated by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

RESULTS AND DISCUSSION

In vitro* evaluation of bio-agents against *Xanthomonas axonopodis* pv. *cyamopsidis

The antagonistic microorganisms *viz. Bacillus subtilis, Providencia* sp., *Trichoderma viride* and *Trichoderma harzianum* were evaluated *against Xanthomonas axonopodis pv. cyamopsidis* in vitro condition by dual culture technique as explained in the material and methods. The maximum per cent zone inhibition of 14.12 per cent was recorded from *Providencia* sp. which was statistically at par with *Bacillus subtilis* (12.13%). However, the fungal antagonists viz. *Trichoderma viride* and *Trichoderma harzianum* were found least effective in inhibiting the growth of the test pathogen and resulted in 7.11 and 5.95 per cent growth inhibition as compared to control.

This shows that both the bacterial antagonists are more effective in controlling *Xanthomonas* as compared to fungal antagonists. *Trichoderma harzianum* shows minimum inhibition percent zone as compared to other antagonists. The results are presented in Table 1, Fig. 1 and Plate 1.

TABLE 1
In vitro evaluation of bio-agents against *Xanthomonas axonopodis* pv. *cyamopsidis*

Treatments	Per cent zone inhibition (%)
Bacillus subtilis	12.13 (20.39)
Trichoderma viride	7.11 (15.45)
Trichoderma harzianum	5.95 (14.09)
Providencia sp.	14.12 (22.05)
Control	0.00 (0.57)
S. Em±	0.314
C. D. (P=0.05)	0.979
C. V. (%)	3.493

*Mean of four replications.
Figures given in parenthesis represent angular transformed value.

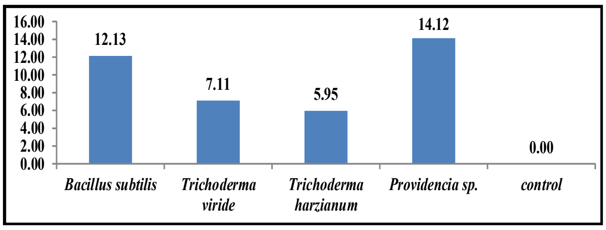

Fig. 1. *In vitro* evaluation of bioagents against *Xanthomonas axonopodis* pv. *cyamopsidis*.

The antagonistic microorganisms viz. *Bacillus subtilis, Providencia* sp., *Trichoderma viride* and *Trichoderma harzianum* were evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis* in vitro condition by dual culture technique as explained in the material and methods.

The maximum per cent zone inhibition of 14.12 per cent was recorded from *Providencia* sp. which was statistically at par with *Bacillus subtilis* (12.13%).

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Results of present investigation are in agreement with the findings of Lodha (2001), Gena *et al.* (2008), Sain and Gour (2010), Rana *et al.* (2011), Bharti *et al.* (2020) and Jat *et al.* (2022). Lodha (2001) reported that seed treatment with B. subtilis significantly

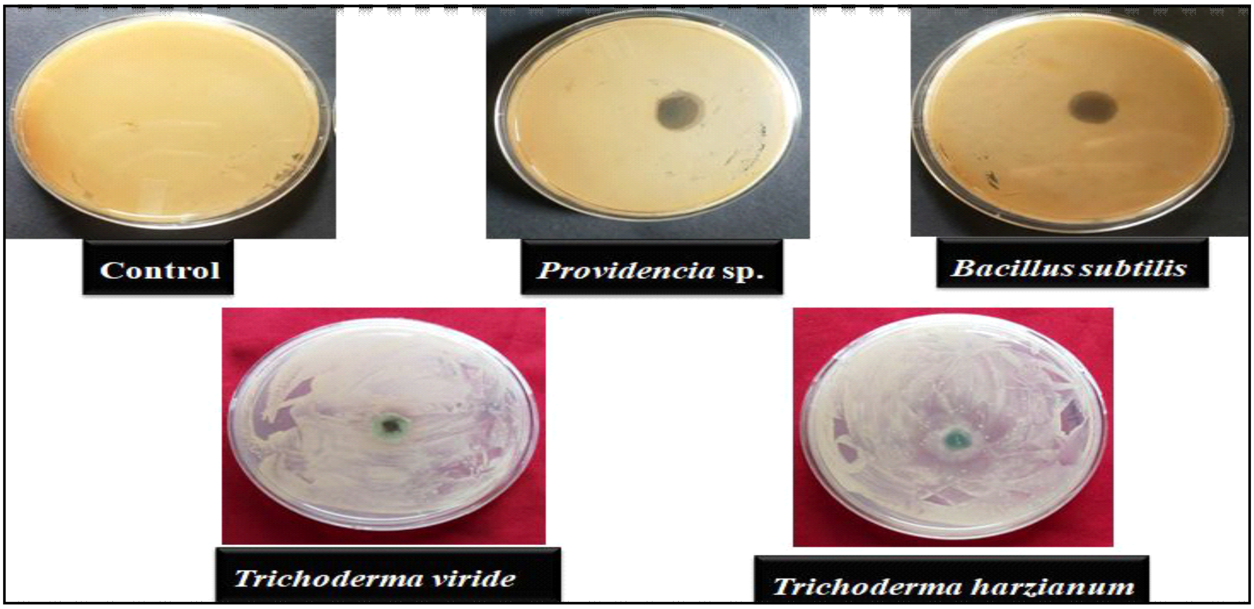

Plate 1. Zone inhibition by inhibition by bioagents against *Xanthomonas axonopodis* pv. *cyamopsidis*.

reduced the bacterial blight disease of cluster bean. Dutta and Dureja (2004) reported that the most effective phylloplane bacterium strain of *Bacillus*, Plb-3 produced four metabolites and Ba-II was the most inhibitory against X. *axonopodis* in vitro. Babu *et al.* (2007) revealed that antibacterial activity of botanicals was due to the presence of phenolic and acidic fractions.

Gena *et al.* (2008) 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. Plant growth promoting rhizobacteria possess the growth promoting functions, which not only play important role as antibacterial property, but also enhances seed germination up to 98 per cent and plant tolerance against bacterial blight of cluster bean (*Cyamopsis* (L.) Taub.) caused by *Xanthomonas axonopodis* pv. *cyamopsidis*. They reported that PGPR-1, PGPR-4 (*Bacillus* subtilis), PGPR-7 (*Pseudomonas fluorescens*) and PGPR-12 reported maximum in vitro growth inhibition and increased plant vigour (Sain and Gour, 2009).

Sain and Gour (2010) reported that *Pseudomonas* sp. and *Bacillus* sp. found highly efficient against X. *campestris* pv. *malvacearum*. *Bacillus subtilis* was found effective in suppressing the bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* by 12.71 per cent (Ambadkar *et al.,* 2015).

Bharti *et al.* (2020) also found that among the eight bio-agents studied the *Pseudomonas fluorescens* was found most effective for inhibiting the growth of *Xanthomonas axonopodis* pv. *cyamopsidis* followed by *Bacillus* subtilis. Similarly, Jat *et al.* (2022) reported that none of the bioagent among *Trichoderma* viride, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* was found effective against the bacterial blight pathogen *X. axonopodis* pv. *cyamopsidis* except P. *fluorescens* which showed 10.70 mm mean diameter of inhibition zone. Not much investigation have been carried regarding antagonistic effect of *Providencia* sp. against X. *axonopodis* pv. *cyamopsidis*. However it had been found that *Providencia* sp. like other PGPRs helped in the growth promotion of their host and survived by using the nutrients generated by plant roots as metabolites for their growth and support plant growth through a variety of mechanisms such as, the generation of hormones that stimulate growth and the control of plant diseases. Rana *et al.* (2011) concluded that PGPR strains enhanced the seedling length significantly but *Providencia* (AW5) showed a two-fold increase in percentage germination as compared to untreated controls. Siderophores and HCN synthesis was intricately linked to antifungal action and evaluated under in vitro conditions for their antagonistic capacity against a virulent isolate of *Fusarium oxysporum*. Results showed that among all ten isolates (MB108, MB109, MB104, MB044, MB068, MB012, MB050, MB051, MB106 (*Providencia* sp.) and MB112)

TABLE 2

Tests of efficacy of potential bioagents against Xanthomonas axonopodis pv. cyamopsidis under screen house conditions

S. No.	Treatments	Disease incidence (%)	Disease reduction (%)
1.	Bacillus subtilis	60.00 (50.75)	35.71
2.	Providencia sp.	46.67 (43.06)	50.00
3.	Trichoderma harzianum	86.67 (72.27)	7.14
4.	Control	93.33 (81.14)	0.00
	C. D. (P=0.05)	16.31	-
	S. Em±	5.23	-
	C. V. (%)	17.67	-

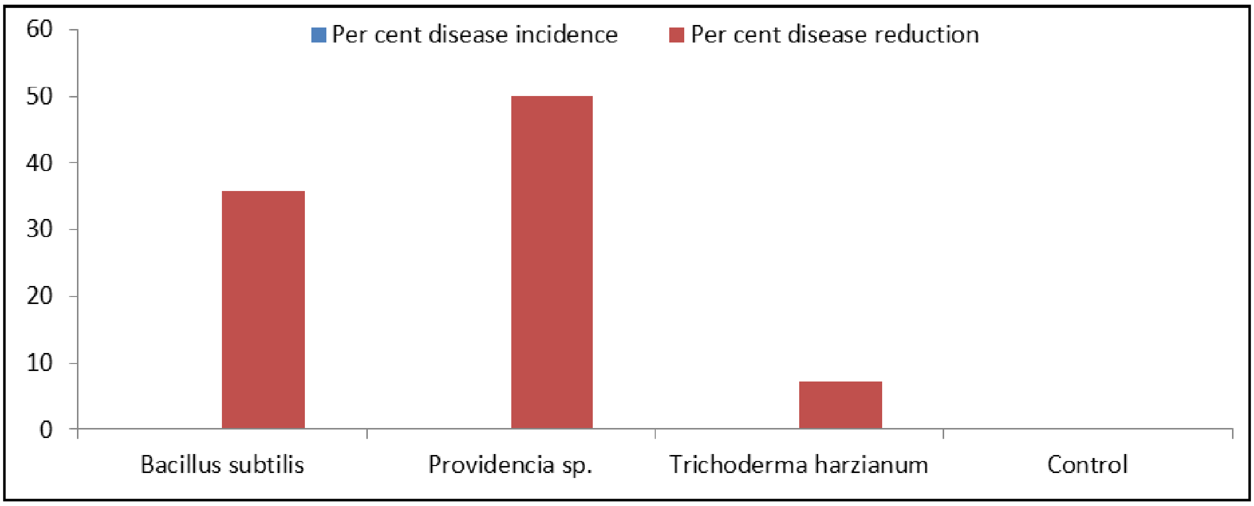

Fig. 2. Efficacy of potential bioagents concentration against *Xanthomonas axonopodis* pv. *cyamopsidis* under screen house conditions.

exhibited higher inhibition capacity than control. (Toloza-Moreno, 2020).

This shows that both the bacterial antagonists are more effective in controlling *Xanthomonas* as compared to fungal antagonists. *Trichoderma harzianum* shows minimum inhibition percent zone as compared to other antagonists. The results are presented in Table 1, Fig 1 and Plate 1.

Tests of efficacy of selected bioagents against *Xanthomonas axonopodis* pv. *cyamopsidis* under screen house conditions

The results presented in the Table 2 and Fig 2 indicated under screen house conditions, minimum per cent disease incidence (PDI) of 46.67 per cent was observed in treatment of *Providencia* sp. followed by *Bacillus subtilis* with PDI of 60.00 per cent as compared to control where the PDI was maximum (93.33 per cent). *Trichoderma harzianum* reduced the disease by 86.67 per cent.

Sain and Gour (2009) also evaluated that isolates of PGPR viz. PGPR-12 (P. fluorescens), PGPR-4(B. subtilis), PGPR-5 (B. subtilis) and PGPR-7 (P. fluorescens) significantly reduced the disease index as compared to other strains against X. axonopodis pv. cyamopsidis under field condition. Kanwar et al. (2016) also reported that foliar spray of neem oil (2%) combined with neem cake application @2 q ha-1 and Trichoderma @2 kg ha-1 applied with 100 kg FYM resulted in minimum per cent disease index (PDI) of 13.2 under natural conditions.

CONCLUSION

Providencia sp. showed maximum antibacterial activity with 14.12 per cent zone inhibition of *X. axonopodis* pv. *cyamopsidis* under in vitro conditions followed by *Bacillus subtilis* with 12.13 per cent zone inhibition. *Trichoderma viride* (7.11%) and *Trichoderma harzianum* (5.95%), were found least effective as in inhibiting the growth of the test pathogen. Under screen house conditions results indicated that minimum per cent disease incidence

(PDI) of 46.67 per cent was observed in treatment of *Providencia* sp. followed by treatments of *Bacillus subtilis* with PDI of 60.00 as compared to control where the PDI was maximum (93.33%).

The outcome of the study firmly conclude that the use of various bioagents formulations can perform very well for the management of blight disease in cluster bean.

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