In vitro evaluation of bio-control agents against leaf spot disease caused by *Cercospora* sp.

Abstract

Okra (*Abelmoschus esculentus*) is an important vegetable crop that cultivated in tropical and subtropical regions of the world. It is mostly grown for its delicate fruit, stem, flower and bud. Like other crop, okra is also attacked by many diseases. Among all diseases, leaf spot is major disease which causes heavy loss to the crop. During isolation and identification it was identified as *Cercospora* and varied in their cultural and morphological character. Pathogenecity test was also proved by using Koch's postulates. During course of study, 4 bio control agents were evaluated under *in vitro* condition for their effectiveness. Among all bio control agents *Bacillus subtilis* showed maximum inhibition at different time intervals of 68.14 %, 76.00 %, 81.17 % at 72 hrs, 120hrs, 168 hrs respectively followed by *Trichoderma harzianum* with 66.02 %, 72.27% and 77.25 % respectively at 72hrs, 120 hrs, 168 hrs. Similarly other bio control agents –*Trichoderma asperellum* and *Pseudomonas fluorescens* were also found effective with inhibition of 62.60 %, 70.74%, 76.24% and 60.20%, 69.45%, 75.05% respectively at 72hrs, 120hrs, and 168hrs. The result from present investigation revealed that *Bacillus subtilis* was found most effective in the suppression of pathogen growth. In future investigations, this bio-control agent might be further assessed for the effective treatment of okra leaf spot disease.

Keywords: *Cercospora, Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis.*

Introduction

The most well-known and widely used species of the Mallow (Malvaceae) family, okra (*Abelmoschus esculentus*) is an important vegetable crop cultivated in tropical and subtropical regions of the world Oyelade *et al*.,(2003); Andras *et al*.,(2005);Naveed *et al*.,(2009);Saifullah & Rabbani, (2009). This crop can be grown in both huge commercial farms and garden .It serves a crucial part in the nutrition of human as excellent source of protein, carbohydrates, vitamins, calcium, potassium, enzymes, and minerals and having antioxidants, anti-infilamantory and, antimicrobial properties. According to FAOSTAT 2020 report, total world production of okra about 10.8 million tonnes. India is the leading producer of okra with 6,466 million metric tons contributing about 60% to the world production. In India, Gujarat ranks first with 1,019.42 ('000 metric tonnes), followed by west Bengal and Bihar (National Horticulture Board 2021-22). In Bihar, in term of production Araria is leading producer of okra with 10600 ('000 metric tonnes) followed by Arwal and Aurangabad (Statistical Handbook, Department of Agriculture, GOB, 2017-2018). Numerous diseases, including bacterial, viral, and fungal ones, have been documented in India and attributed for the low yield of okra (Sastry and Singh, 1974). Among various okra diseases such as Alternaria (Atia and Tohamy , 2004), leaf blight, Yellow vein mosaic, Fusarial wilt, Powdery mildew, okra leaf curl virus (Atiri and Fayoyin, 1989) damping off, the leaf spot caused by *Cercospora* is becoming more significant these days.

Material and Methods

Collection of disease sample

The leaf spot affected samples of okra plants were collected for the pathogen's isolation and further research. Sterilized paper bags were used for collection of sample and brought to the lab for further detailed study and maintained at a temperature of 5°C in the refrigerator until the isolation of the causative organisms was completed.

Isolation of pathogen

Okra leaves showing symptoms of leaf spot was collected and used for isolation of pathogen. In running tap water, leaves were thoroughly cleaned and then using a sterile razor blade, small bits of the affected part and the surrounding healthy tissues were cut away. Those bits were sterilized with 0.1% mercuric choloride for 30 seconds and then washed three times with sterile distilled water. The cleaned leaf bits were aspectically plated in 9 cm diameter petri dishes containing potato dextrose agar medium (4 pieces per plate) after being blotted between sterile Whatman No.1 filter paper. For the growth of the pathogen, these inoculated petri plates were incubated at 28± 2°C in a Biological Oxygen Demand (BOD) incubator. On PDA plates, fungus started to grow after a few days, turning white to greyish. To grow a pure culture of the isolates, the mycelia growing from the tissues were transferred into new PDA medium that contained 1.0 mg/ ml streptomycin sulphate added to it. The fungus was identified based on its cultural and morphological characters (under microscope).Then, on PDA slants, the pure cultures that had been obtained was kept. The BOD incubator was set to 28± 2°C to incubate the slants. For the entire duration of study of the cultures were maintained on PDA and revived each month. After ten days of inoculation, the colony colour and diameter of each colony was measured.

***In vitro* evaluation of bio-control agents**

By using a dual culture method, bio-control agents were evaluated against pathogen. For this test, a five mm disc of an actively growing *Cercospora* culture was placed on one side of a petri plate, and a five mm disc of test bio-control agent was placed on the opposite side of the plate. Four replication of each treatment was made, and suitable control was also kept with inoculation of *Cercospora* culture only. For five days, the plates were incubated at 28±2 °C to calculate the per cent inhibition of *Cercospora* growth by each test bio- agents, the colony diameter of *Cercospora* in dual culture was measured. The Vincent's (1947) formula given by calculated the percentage of mycelial growth inhibition over control.

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

Where,

I = Per cent inhibition of mycelial growth over control

C = Mean maximum colony diameter in control (mm)

T = Mean maximum colony diameter in treatment (mm)

Table -1 List of bio- control agents evaluated against the *Cercospora* sp.

S. No.	Bio-control agents
1	*Trichoderma harzianum*

2	*Trichoderma asperellum*
3	*Pseudomonas fluorescens*
4	*Bacillus subtilis*

Result and discussion

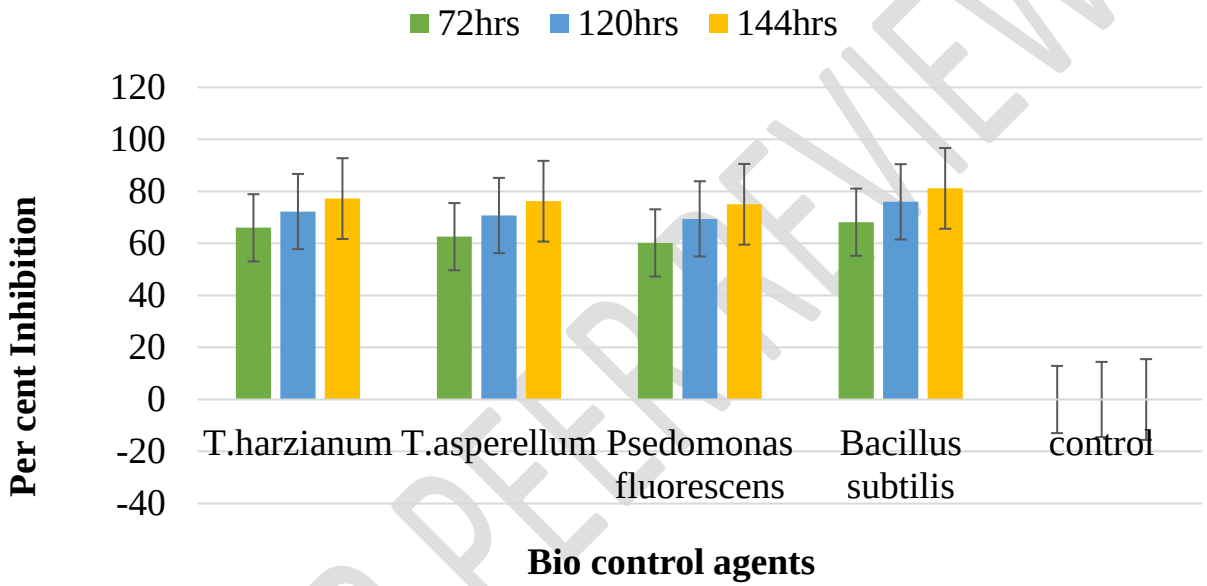
It is evident from the result that all the test bio control agents were significantly effective in suppressing the target pathogen. It is also clear that *Bacillus subtilis* was the most effective in suppression of the pathogen growth which was restricted upto 11.14 mm after 72 hrs, 13.20 mm at 120 hrs and 16.00 mm at 168 hrs thus showing 68.14 % inhibition, 76.0% inhibition and 81.17 % inhibition over control after 72 hrs, 120 hrs and 168 hrs of inoculation respectively. The next most effective bio-control agent was *Trichoderma harzianum* which confined the pathogen growth upto 11.89 mm at 72 hrs, 15.25 mm at 120 hrs and 19.33 mm at 168 hrs thus inhibiting the pathogen growth by 66.02 %, 72.27% and 77.25 % respectively at 72 hrs, 120 hrs and 168 hrs. The other bio-control agents –*Trichoderma asperellum* and *Pseudomonas fluorescens* were also found to be promising in effectively inhibiting the pathogen growth by 62.60%, 70.74%, 76.24% and 60.20%, 69.45%, 75.05% at 72 hrs, 120hrs and, 168hrs respectively. Earlier also effectiveness of various bio-control agents has been reported by different workers. Hamden *et al*., (2023) reported that among bacterial and fungal bio-control agents, bacterial bio -control agent like *Bacillus subtilis* showed maximum inhibition, whereas among fungal bio-control agent, *Trichoderma* sp showed maximum inhibition of the pathogen -*Cercospora* causing leaf spot in sugarbeet. Various strains of the *Bacillus* species have been recognized for their ability to generate lipopeptides Zuber *et al*., (1993). In other studies also biological agents such as Bacillus subtilis and Trichoderma sp., exhibited strong potential in suppressing the growth of fungal pathogens Derbalah *et al*., (2013); Esh *et al*., (2011); El-Kazzaz *et al*., (2002). Sen *et al*., (2023) reported that among 7 *Tricoderma* isolates which significantly reduced the growth of *Alternaria alternata* and *Curvularia lunata.*

Table: 2.Effect of different bio-control agents for their growth at different time intervals against *Cercospora* sp

S.No	Biocontrol agent	72 hrs		120 hrs		168 hrs	
		G (mm)	PI (%)	G (mm)	PI (%)	G (mm)	PI (%)

1	*Trichoderma harzianum*	11.89	66.02	15.25	72.27	19.33	77.25
2	*Trichoderma asperellum*	13.09	62.60	16.09	70.74	20.19	76.24
3	*Pseudomonas fluorescens*	13.93	60.20	16.80	69.45	21.20	75.05
4	*Bacillus subtilis*	11.14	68.17	13.20	76.00	16.00	81.17
5	Control	35.00	-	55.00	-	85	-
C.D. (at 5 %)		0.63		1.09		1.28	
SE (±m)		0.33		0.39		0.43	
C.V. (%)		2.84		1.35		0.67	

Fig 1 : Graph showing the effect of different bio-control agents for their growth at different time intervals against *Cercospora sp*



72hrs **120 hrs** **168 hrs**

Trichoderma harzianum

Trichoderma asperellum

Pseudomonas fluorescens

Bacillus subtilis

Control

Plate 1 : Evaluation of potential bio-control for their effect against *Cercospora* sp.

References

Andras, C.D., Simandi, B.,Orsi, F., Lambrou, C., Tatla, D.M., Panayiotou, C., Domokos, J., & Doleschall. F. (2005). Supercritical carbon dioxide extraction of Okra (Hibiscus esculenstu L.)seeds. *J. Sci. Food Agric*., 85: 1415-1419.

Atia, M. M. M., & Tohamy, M. R. A. (2004). First record of Alternaria leaf spot disease on okra in Egypt. *Egyptian Journal of Phytopathology*, 32(1/2), 141- 142.

Atiri, G. I., & Fayoyin, G. A. (1989). Horizontal resistance to okra leaf curl virus in okra germplasm. *Annals of applied biology*, 114, 152-153.

Derbalah, A. S., El-Moghazy, S. M., & Godah, M. I. (2013). Alternative Control Methods of Sugar-beet Leaf Spot Disease Caused by the Fungus Cercospora beticola (Sacc). *Egyptian Journal of Biological Pest Control*, 23(2)

Esh, A. M. H., El-Kholi, M. A., and Taghian, S. (2011). Antagonistic activities of Bacillus amyloliquefaciens from phyllosphere of sugar beet against Cercospora beticola sacc. *Journal of Plant Protection and Pathology*, 2(1): 99- 116.

El-Kazzaz, M. K., Badr, M. M., El-Zahaby, H. M., and Gouda, M. I. (2002). Biological control of seedling damping-off and root rot of sugar beet plants. Pant Protection Science-Prague, 38: 645-647.

FAOSTAT (2018): http://faostat.fao.org/default.aspx

https://agricoop.gov.in/en/ActsDepartment of agriculture and farmer welfare 2020.

https://agriexchange.apeda.gov.in/India%20Production/India_Productions.aspx?cat=Vegetables&hscode=1079; national horticulture board 2021.

M, Saifullah., & Rabbani, MG. (2009). Evaluation and characterization of okra (Abelmoschus esculentus L. Moench.) genotypes. SAARC J. Agric. 7: 92-99.

Naveed, A., Khan, A.A., & Khan, I.A. (2009). Generation means analysis of water stress tolerance in okra (Abelmoschus esculentus L.). *Pak. J. Bot.*, 41: 195- 205. 58

Sen , A., Rai,D.,& jaiswal,A.(2023). Isolation and in vitro screening of native Tricoderma isolates against economically important pathogens of Japanese Mint (Mentha arvensis).*Emer Life sci Res* 9(2): 19- 26.

Oyelade, O.J., Ade-Omowaye, B.I.O., and Adeomi, V.F. (2003). Influence of variety on protein, fat contents and some physical characteristics of okra seeds. *J. Food Eng*., 57: 111-114. 67. Z.

Zuber, P., Nakano, M. M., & Marahiel, M. A. (1993). Peptide antibiotics, p. 897–916. In A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), Bacillus subtilis and other gram-positive bacteria. American Society for Microbiology, Washington, D.C.