Environment and Ecology 40 (1) : 11—14, January—March 2022
ISSN 0970-0420

In vitro Evaluation of Various Bioagents Against Spore Germination Inhibition of *Erysiphe cichora cearum* DC. in Bhendi

Ashwini R, Amaresh Y. S., Yenjerappa S.T., Sunil Kulkarni, S. G. Hanchinal, Shekhargouda Patil

Received 24 October 2021, Accepted 10 December 2021, Published on 8 January 2022

ABSTRACT

Bhendi powdery mildew caused by *Erysiphe cichoracearum* DC. affects all the stages of the plant growth by causing premature defoliation and resulting 17.0 to 86.6% yield loss in bhendi. Looking into significance of disease various bioagents were evaluated under *in vitro* conditions for their efficacy against *E. cichoracearum* by spore germination technique. Results revealed that, irrespective of concentrations of the bio agents tested, the treatment involving *P. fluorescence* (Pf-1) recorded maximum mean per cent inhibition of spore germination (64.20%) followed *P. fluorescence* (Pf-2) (62.21 %). Whereas, least mean per cent spore germination inhibition (44.13 %) was recorded in *A. quisqualis* (AQ/06) followed by *B. subtilis* (BS-9) (46.01 %) and B. subtilis (BS-5) (48.59 %).

Ashwini R*, Amaresh, Y. S., Yenjerappa, S.T., Sunil Kulkarni, S. G. Hanchinal, Shekhargouda Patil
Department of Plant Pathology, College of Agriculture, Raichur University of Agricultural Sciences, Raichur 584–104, Karnataka, India
Email : ashupatil158915@gmail.com
*Corresponding author

Keywords *Erysiphe cichoracearum*, bio agents, *P. fluorescence, A. quisqualis, B. subtilis.*

INTRODUCTION

Bhendi (*Abelmoschuses culentus* (L.) Moench) is globally important annual vegetable belongs to a family malvaceae, it is most broadly distributed vegetable all over the world. Many factors responsible for yield loss of the crop, one of them are the diseases which are the major constraints for low yield of bhendi (Sastry and Singh 1974). A number of fungal, bacterial and viral diseases have been reported in India. Among the fungal diseases affecting bhendi crop, powdery mildew caused by *Erysiphe cichoracearum* DC is the most important disease causing considerable yield losses. Continuous use of systemic or same fungicides in the management of disease leads to the development of resistance in the pathogen and also causes deleterious effect on existing ecosystem. Hence, screening of bio-agents viz., *Trichoderma* sp. *Pseudomonas fluorescens*, *Bacillus subtilis* and *Ampelomyces quisqualis* for their antagonistic activity against the pathogen under *in vitro* conditions for the management of the disease is essentially required.

MATERIALS AND METHODS

The effect of cultural filtrate of *Trichoderma asperellum* Samuels, Lieckf. Nirenberg, *Trichoderma*

Table 1. *In vitro* efficacy of various bio agents against *Erysiphe cichoracearum* by spore germination technique. *Mean of three replications, **Angular transformed value.

Sl. No.	Bio agents	Isolate number	Per cent inhibition of conidial germination at different concentrations 0.1%	0.25 %	0.5 %	Mean
1	*Trichoderma asperellum*	Tr-14	49.68*(44.82)**	56.26 (48.60)	63.43 (52.79)	56.45 (48.71)
2	*Trichoderma asperellum*	Tr-10	48.54 (44.16)	55.43 (48.12)	61.26 (51.51)	55.07 (47.91)
3	*Bacillus subtilis*	Bs-5	41.36 (40.02)	47.52 (43.58)	56.91 (48.97)	48.59 (44.19)
4	*Bacillus subtilis*	Bs-9	42.05 (40.43)	44.36 (41.76)	51.64 (45.94)	46.01 (42.71)
5	*Pseudomonas fluorescence*	Pf-1	54.55 (47.61)	65.43 (53.99)	72.64 (58.46)	64.20 (53.25)
6	*Pseudomonas fluorescence*	Pf-2	52.56 (46.47)	63.64 (52.92)	70.43 (57.06)	62.21 (52.07)
7	*Trichoderma harzianum*	Th-1	45.32 (42.31)	51.49 (45.85)	53.64 (47.09)	50.15 (45.09)
8	*Trichoderma harzianum*	Th-2	44.56 (41.88)	49.26 (44.58)	52.43 (46.39)	48.75 (44.28)
9	*Ampelomyces quisqualis*	AQ/06	40.52 (39.53)	43.64 (41.35)	48.25 (44.00)	44.13 (41.63)
	Mean		46.57 (43.03)	53.00 (46.72)	58.95 (50.16)	–
			SEm (±)	CD at 1 %		
	Bioagents (B)		0.15	0.57		
	Concentration (C)		0.07	0.26		
	B×C		0.27	1.02		

harzianum Rifai, *Bacillus subtilis* (Ehrenberg) Cohn. and *Pseudomonas fluorescens* Migula isolates and one commercially available bio agent i.e., *Ampelomyces quisqualis* Ces., efficacy was studied against *E. cichoracearumat* 0.1, 0.25 and 0.5% concentrations under *in vitro* conditions by spore germination technique. Nutrient broth and King's-B broth were prepared and inoculated with respective antagonist (s). Cultural filtrates of the respective antagonist (s) grown in broth for 7-8 days were filtered using what man paper no. 41 in laminar air flow and recovered cultural filtrates. The various bioagents used in the study were procured from Biocontrol Laboratory, Department of Plant Pathology, UAS, Raichur.

One drop of the respective antagonist culture filtrate suspension was placed separately on a slide and one drop of spore suspension of *E. cichoracearum* was placed exactly on this respective drop so that required concentration was obtained in each of the treatment. Three replications were maintained for each of the treatment. These cavity slides were incubated at room temperature (25 ± 1^{0}C) for 24 h. The observation on spore germination was recorded at 24 h after incubation under microscope at 40X magnification. Cavity slides with only sterile water and spores of pathogen served as a control. Observation on number of spores germinated was recorded.

Per cent conidial germination was calculated by the following formula.

$$\text{Per cent germination} = \frac{A}{B} \times 100$$

Where,

A = Number of conidia germinated

B = Number of conidia observed

Per cent inhibition over the control was calculated by using the formula given by Vincent (1947).

$$\text{Percent inhibition of spore germination (I)} = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of spore germination.

C = Germination of conidia in control.

T= Germination of conidia in treatment.

RESULTS AND DISCUSSION

In order to explore possible antagonistic potential,

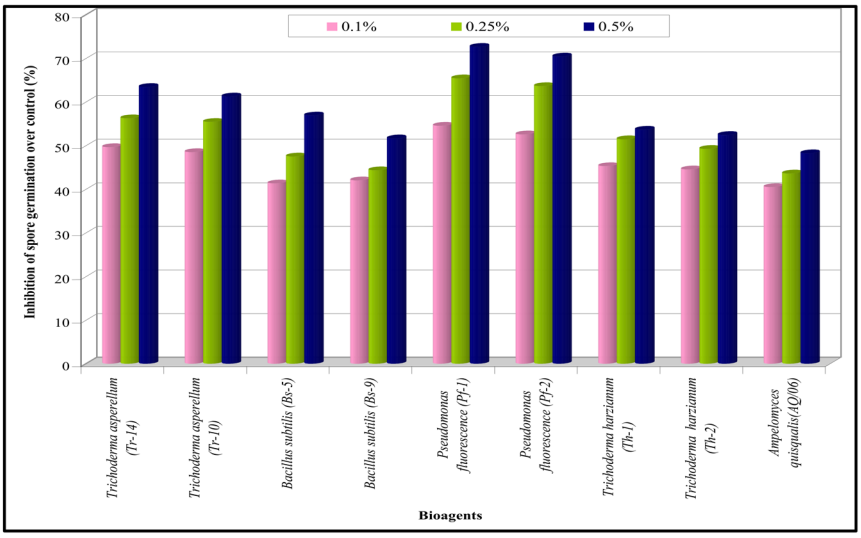

Fig. 1. *In vitro* efficacy of various bioagents against *Erysiphe cichoracearum* by spore germination technique

different strains of *Trichoderma* spp. viz., *Trichoderma asperellum* (Tr-14), *T. asperellum* (Tr-10), *Trichoderma harzianum* (Th-1), *T. harzianum* (Th-2) along with the strains of *Bacillus subtilis* (BS-5) and *B. subtilis* (BS-9) *Pseudomonas fluorescence* (Pf-1)and *P. fluorescence* (Pf-2) and one commercially available product i.e., *Ampelomyces quisqualis* (AQ/06) were tested for their efficacy against *E. cichoracearum* at three different concentrations (0.1, 0.25, 0.5 %) through spore germination technique under *in vitro* conditions as explained in "Material and Methods". The results pertaining presented in Table 1.

The results clearly indicated that, various bioagents had significant effect on inhibition of conidial germination of *E. cichoracearum.* Among which *P. fluorescence* (Pf-1) was significantly superior with maximum inhibition of spore germination (72.64 %) followed by *P. fluorescence* (Pf-2) (70.43 %) (Fig. 1). The next best treatments were *T. asperellum* (Tr-14), *T. asperellum* (Tr-10), *T. harzianum* (Th-1), *T. harzianum* (Th-2) with spore germination inhibition of 63.43, 61.26, 53.64, 52.43% at 0.5% concentration respectively, earlier reports of Harman *et al*. (2004) and Kumar *et al.* (2012) suggested that, *Trichoderma* spp. produce various sensing enzymes and number of synergistic cell-wall degrading enzymes and other substances that exhibit a wide antimicrobial spectrum. However, combination of several modes of action is responsible for bio control (Elad 2000). Least inhibition (48.25 %) was recorded in *A. quisqualis* (AQ/06) followed by *B. subtilis* (BS-5) (56. 91%), *B. subtilis* (BS-9) (51.64 %) at 0.5% concentration. However, all the bio agents showed same trend of spore germination inhibition at lower concentrations (0.1 and 0.25 %) also. It was noticed that in all the bio agents evaluated, maximum inhibition was recorded in higher concentration compared to lower concentration. However, irrespective of concentrations of the bio agents tested, the treatment involving *P. fluorescence* (Pf-1) recorded maximum mean per cent inhibition of spore germination (64.20%) followed *P. fluorescence* (Pf-2) (62.21 %) because the anti-fungal metabolite 2,4-diacetyl phloroglucinol produced by *P. fluorescence* strains play major role in antifungal activity (Ganeshan and Kumar 2005). Whereas, least mean per cent spore germination inhibition (44.13 %) was recorded in *A. quisqualis* (AQ/06) followed by *B. subtilis* (BS-9) (46.01 %) and *B. subtilis* (BS-5) (48.59 %).

Biological control through the use of antagonistic micro-organisms is a potential, non-chemical means of managing plant disease by reducing inoculum levels of pathogen. Such management would help in preventing the pollution and also the health hazards. Antagonistic potential of different bio control agents has been reported by several earlier workers viz., Vimala

and Suriachandraselvan (2008) evaluated 45 isolates of phylloplane *P. fluorescens in vitro* for their antagonistic potential against *E. cichoracearum*. Among the different isolates of *P. fluorescens*, I_{18} (74.90 %) and I_{36} (72.18 %) showed maximum inhibition of conidial germination. Siddappa (2012) studied *in vitro* efficacy of various bioagents against inhibition of conidial germination of *E. cichoracearum.* Results revealed that the irrespective of the concentration of the bioagents tested, maximum conidial inhibition was noticed in *P. fluorescens* (91.43 %) and was significantly superior over rest of the bioagents. *P. putida* (51.82%) was found to be least effective among the various bioagents tested. Elsisi (2019) conducted a study to investigate the role *B. subtilis, Paenibacillus polymyxa, T. harzianum*, *T. album, T. viride* and *T. hamatum* for controlling squash powdery mildew caused by *P. xanthii.* Results indicated that all treatments significantly inhibited the conidial germination of *P. xanthii* than control *in vitro*. Maximum inhibition of spore germination was noticed in *P. polymyxa* (88.0%) followed by *B. subtilis* (82.0%) and *T. viride* (79.20 %).

ACKNOWLEDGEMENT

I would like to acknowledge respected chairman of my advisory committee Dr. Amaresh Y. S., Senior scientist and Head, ICAR KVK Kawadimatti and my advisory committee members, Dr. Yenjerappa S. T., Professor and Head, Department of Plant Pathology, College of Agriculture, UAS, Raichur, Dr. Sunil Kulkarni, Professor of Plant Pathology and Head, Agricultural Research Station, Bidar, Dr. S. G. Hanchinal, Scientist (Entomology), AICRP on Cotton, MARS, UAS, Raichur and Dr. Shekhargouda Patil, Assistant Professor, Department of Horticulture, College of Agriculture, Raichur, for their fruitful and constant support, valuable suggestions and sensible criticism in animating and ameliorating this manuscript and also valuable counsel during the period of study.

REFERENCES

Elad Y (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot* 19 (10) : 709—714.

Elsisi AA (2019) Evaluation of biological control agents for managing squash powdery mildew under greenhouse conditions. *Egyptian J Biol Pest Cont* 29 (89) : 1—6.

Ganeshan G, Kumar AM (2005) *Psuedomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *J Pl Int* 1 (3): 123—134.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nat Rev Microb* 2 : 43—56.

Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC (2012) Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. *Ind J Microbiol* 52 (2) : 137—144.

Sastry KSM, Singh SJ (1974) Effect of yellow vein mosaic virus infection on growth and yield of okra crop. *Ind Phytopath* 27 : 294—297.

Siddappa B (2012) Investigations on powdery mildew of okra caused by *Erysipheo cichoracearum DC MSc* (*Agri*) *Thesis Univ Agric Sci Raichur.*

Vimala R, Suriachandraselvan M (2008) Influence of antagonistic agent, plant products and chemical agents on the powdery mildew disease of bhendi and its production. *J Biopest* 1 : 130—133.

Vincent JM (1947) Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 150 : 850.