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Evaluation of Fungicides and Bioagents against *Fusarium proliferatum* under *In vitro* by Spore Germination Inhibition Technique

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ABSTRACT

Background: Bottle gourd is a cucurbitaceous vegetable of culinary and medicinal importance cultivated in various tropical and sub-tropical regions of world. This crop is exposed to a wide variety of seed and soil mycoflora, out of which *Fusarium proliferatum* is utmost important as far as seed germination, viability and seedling vigour are concerned.

Methods: Study was taken up to evaluate different fungicides and bioagents for their efficacy against the fungus *Fusarium proliferatum* under *in vitro* through spore germination inhibition technique.

Result: Spore germination inhibition of 86.00%, 85.00% and 81.33% was recorded with hexaconazole (5% SC) @ 0.2% (C_3), mancozeb (75% WP) @ 0.3% (C_3) and *Pseudomonas fluorescens* (1% WP) @ 2% (C_3), respectively. The inhibition in spore germination by mancozeb (75% WP) and *Pseudomonas fluorescens* (1% WP) was upto 77.33% and it was 61.78% and 67.33% in treatments involving carbendazim (50% WP) and *Trichoderma harzianum* (1% WP) that could be exploited to devise integrated approach for disease management.

Key words: Bioagents, Bottle gourd, Cucurbits, Fungicides, Fusarium proliferatum.

INTRODUCTION

Bottle gourd is known to be an important vegetable crop grown globally in various tropical and sub-tropical regions including India, Srilanka, China and Bangladesh (Avinash and Rai, 2013; Saha *et al.,* 2016). The crop has high amount of protein, carbohydrates and water present in it and the aerial parts are usually consumed as vegetable (Abushaala *et al.,* 2016). Each and every part of crop is of medicinal importance and it is used particularly for its diuretic properties in India, Brazil and European countries (Minocha, 2015). Amongst large number of cucurbits grown, bottle gourd is mainly a crop in northern parts of India especially during summer and rainy seasons (Chauhan and Bhatia, 2013). The crop is exposed to a wide variety of fungal, bacterial and viral pathogens in addition to seed and soil borne pathogens (Maheshwari *et al.,* 2013). Seed borne pathogens present internally or externally on seed as a contaminant can reduce seed germination, viability and seedling vigour (Abdelwehab *et al.,* 2014). In the earlier studies, *Fusarium proliferatum* was found as major seed borne pathogen affecting seed germination, seedling vigour and seed viability of bottle gourd (Soni, 2018).

Disease development in any crop depends on various factors and is directly proportional to pathogen spore population (Mukund, 2006). In order to manage plant diseases, spore population must be minimized to a certain level for which farmers usually rely on agrochemicals as they offer a quick and effective solution to pests and diseases but chemical fungicides often result in problems of toxic residue, resistance development, environmental pollution and health hazards (Begum *et al.,* 2010; Anand and Bhaskaran, 2009). Considering the health benefits of bottle gourd and its domestic usage as a vegetable, it is high time to reduce fungicidal application in crop through feasible substitution with bioagents in order to reduce potential health hazards caused due to usage of synthetic pesticides (Pipliwal *et al*., 2015). Bioagents have been lately known for induction of systemic resistance against a number of plant diseases and are being widely used in agri-horticultural ecosystem as plant protectants (Ramamoorthy *et al.,* 2001; Tripathi *et al*., 2020). The most common bioagents include *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Gliocladium virens* which act as efficient antagonists against a wide variety of plant pathogens (Sultana and Ghaffar, 2010; Tripathi *et al*., 2020). Keeping this in view the impact of *Fusarium proliferatum* on seed germination, seedling vigour and seed viability, the present study was aimed at optimizing different concentrations of plant protectants (fungicides and bioagents)

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for their efficacy against the fungus under *in vitro* through spore germination inhibition technique and compare the efficacy of bioagents to synthetic chemicals so as to devise suitable eco-friendly alternative management strategies against *Fusarium proliferatum*.

MATERIALS AND METHODS

The fungal culture *Fusarium proliferatum* isolated from seeds and fruits of bottle gourd was used in the present investigation on evaluation of various fungicides and bioagents by spore germination inhibition technique. *Fusarium proliferatum* was maintained on potato dextrose agar slants and subcultured at regular intervals (fortnightly) (Plate 1).

The experiment on spore germination inhibition was conducted in Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana. Different fungicides and bioagents were evaluated under *in vitro* against the seed borne pathogen, *Fusarium proliferatum* by using spore germination inhibition method. Each fungicide and bioagent was used at three different concentrations, *viz.*, optimum concentration (C_2), concentration < optimum (C_1) and concentration > optimum (C_3) (Table 1). The apparatus used to perform the experiment consisted of Petri plates lined with moist filter paper on both the inside faces. Two glass rods were placed in each Petri plate with one of their ends coinciding with each other. The apparatus was made to support cavity slide and provide appropriate conditions for fungal spore germination. Each concentration of fungicides as well as bioagents was prepared in double the strength by using sterile distilled water. Equal volumes of standard spore suspension and fungicide/bioagent solutions were mixed under separate treatments, so that at the end, spore population was varied from 20-30 per low microscopic field. Each homogenous solution was placed in sterile cavity slides in three replicates @ 1 drop/replication (Plate 2). Three replications of control containing standard spore suspension mixed with equal volume of sterile distilled water were also maintained. A total of one hundred spores from each cavity slide were counted for presence or absence of germination with reference to check after incubation period of 24 h.

Per cent spore germination was calculated as per the formula given by Kiraly *et al.* (1974):

$$\% \text{ spore germination} = \frac{\text{No. of spore germinated}}{\text{Total no. of spore examined}} \times 100$$

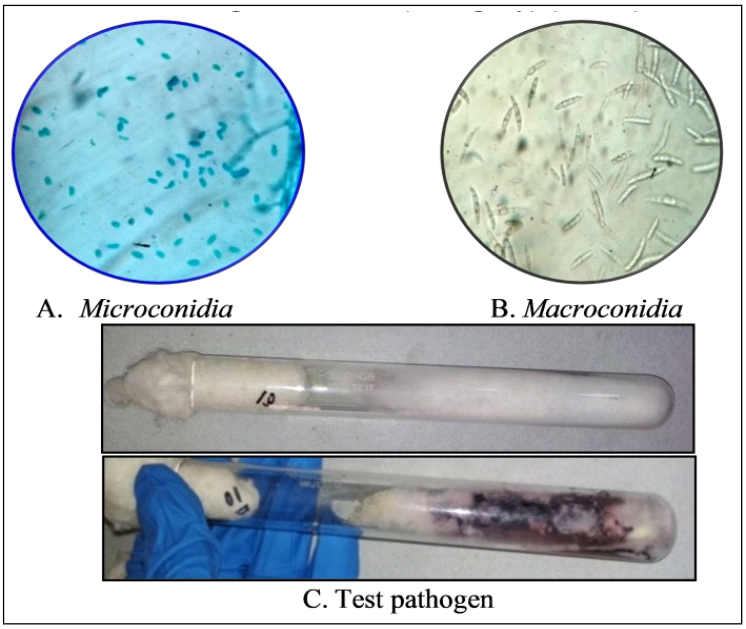

Plate 1: Macroconidia, microconidia and vegetative culture of *Fusarium proliferatum.*

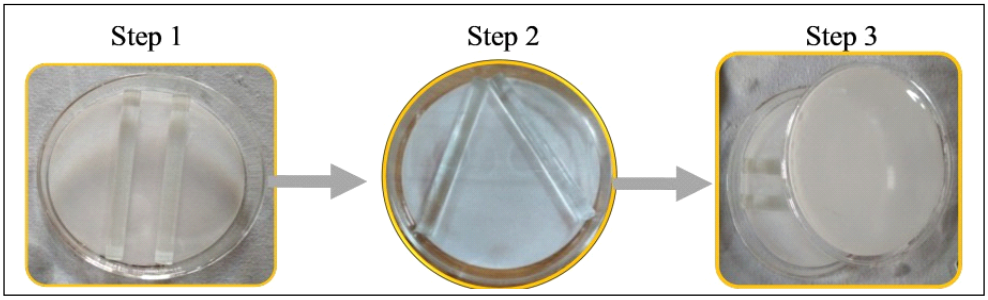

Plate 2: Spore germination inhibition assay under *in vitro* conditions by cavity slide technique.

Based on per cent spore germination, spore germination inhibition against each treatment was calculated as per Vincent, (1947):

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

Where:
I = Per cent inhibition.
C = Spore germination in control.
T = Spore germination in treatment.

RESULTS AND DISCUSSION

The evaluation of three systemic fungicides [Carbendazim (50% WP), Propiconazole (25% EC), Hexaconazole (5% SC)] and two non-systemic fungicides [Mancozeb (75% WP) and Copper oxychloride (50% WP)] alongwith two bioagents [*Trichoderma harzianum* (1% WP) and *Pseudomonas fluorescens* (1% WP)] for their efficacy against seed borne pathogen *Fusarium proliferatum* (Table 2) revealed that maximum inhibition in spore germination was 86.00%, 85.00% and 81.33% due to Hexaconazole (5% SC) @ 0.2% (C_3), Mancozeb (75% WP) @ 0.3% (C_3) and *Pseudomonas fluorescens* (1% WP) @ 2% (C_3), respectively (Table 2). These results were found at par with each other but were significantly different as compared to control. The lower spore germination inhibition of 56.00%, 59.67% and 64.33% was observed in Carbendazim (50% WP) @ 0.05%, Hexaconazole (5% SC) @ 0.05% and *Trichoderma harzianum* (1% WP) @ 0.3%, respectively. Maximum mean spore germination was observed in mancozeb (75% WP) and *Pseudomonas fluorescens* (1% WP) upto 77.33% with lower value of 61.78% and 67.33% in treatments involving carbendazim (50% WP) and *Trichoderma harzianum* (1% WP), respectively (Fig 1).

Among the systemic fungicides, significantly higher spore germination inhibition was observed in case of Propiconazole 25% EC whereas out of non-systemic fungicides evaluated, Mancozeb 75% WP was found to have higher efficacy. The efficacy of two non-systemic fungicides, Mancozeb 75% WP and Copper oxychloride 50% WP was however, significantly *at par* with each other. Among the bioagents evaluated for their efficacy on spore

Table 1: Evaluation of fungicides and bioagents against *Fusarium proliferatum* by spore germination inhibition technique.

Fungicides/ Bioagents	Concentration (%)		
	C_1	C_2	C_3
Carbendazim 50% WP	0.05	0.1	0.2
Mancozeb 75% WP	0.10	0.2	0.3
Propiconazole 25% EC	0.05	0.1	0.2
Hexaconazole 5% SC	0.05	0.1	0.2
Copper oxychloride 50% WP	0.10	0.2	0.3
Pseudomonas fluorescens 1% WP	0.50	1.0	2.0
Trichoderma harzianum 1% WP	0.30	0.4	0.5

Control [Equal volume of sterile distilled water (SDW)].

Table 2: Effect of fungicides and bioagents at different concentrations on spore germination of *Fusarium proliferatum* under *in vitro* conditions.

	C_1		C_2		C_3		Mean	
	I	G	I	G	I	G	I	G
Carbendazim 50% WP	56 .00 (50.99)*	44.00 (38.97)	64.67 (50.96)	35.33 (39.00)	64.67 (50.19)	35.33 (39.77)	61.78 (50.71)	38.22 (39.25)
Mancozeb 75% WP	69.00 (62.44)	31.00 (27.52)	78.00 (60.81)	22.00 (29.16)	85.00 (60.38)	15.00 (29.59)	77.33 (61.21)	22.67 (28.76)
Propiconazole 25% EC	74.67 (63.46)	25.33 (26.51)	76.67 (62.54)	23.33 (27.42)	77.00 (62.94)	23.00 (27.02)	76.11 (62.98)	23.89 (26.98)
Hexaconazole 5% SC	59.67 (59.35)	40.33 (30.62)	73.00 (58.94)	27.00 (31.03)	86.00 (57.83)	14.00 (32.13)	72.89 (58.71)	27.11 (31.26)
Copper oxychloride 50% WP	71.00 (61.18)	29.00 (28.78)	78.00 (60.69)	22.00 (29.29)	78.33 (60.82)	21.67 (29.14)	75.78 (60.89)	24.22 (29.07)
Pseudomonas fluorescens 1% WP	71.33 (61.26)	28.67 (28.70)	79.33 (61.87)	20.67 (28.09)	81.33 (60.59)	18.67 (29.37)	77.33 (61.24)	22.67 (28.72)
Trichoderma harzianum 1% WP	64.33 (54.53)	35.67 (32.77)	67.00 (55.14)	33.00 (30.96)	70.67 (55.75)	29.33 (31.89)	67.33 (55.14)	32.67 (31.87)
(Control) No fungicide	42.67 (36.85)	57.33 (53.11)	42.67 (41.54)	57.33 (48.43)	42.67 (34.44)	57.33 (55.53)	42.67 (37.61)	57.33 (52.35)
	Treatment					Concentration		
C.D. at 1%	(6.40)					N/A		
S.E. (m)	(2.30)					(1.40)		

*Angular transformed values; G: Spore germination (%); I: Spore germination inhibition (%); C_1, C_2 and C_3 are the respective concentrations of the fungicides and bioagents (Table 1).

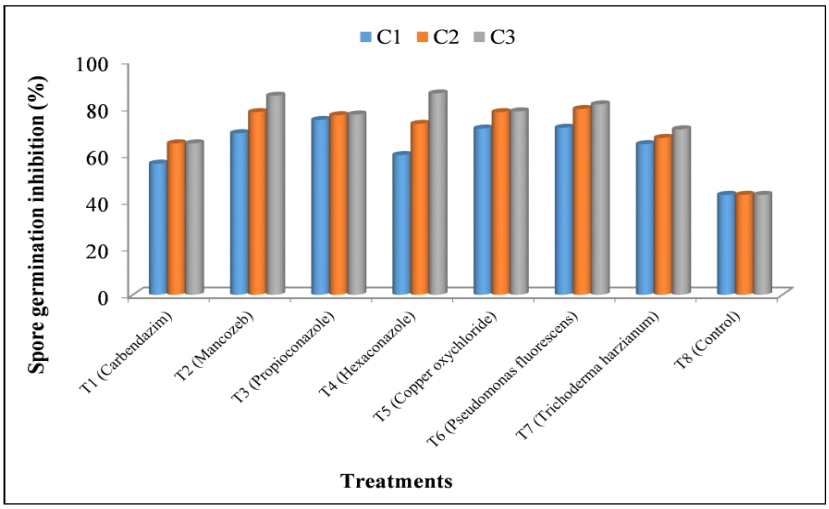

Fig 1: Effect of fungicides and bioagents on inhibition of spore germination of *Fusarium proliferatum.*

germination inhibition, *Pseudomonas fluorescens* 1% WP gave better results over *Trichoderma harzianum* 1% WP. On an average, the efficacy of Mancozeb 75% WP, *Pseudomonas fluorescens* 1% WP and Propiconazole 25% EC was the highest among all the fungicides and bioagents evaluated but significantly *at par* with each other. The results of present study are similar to the findings of other researchers. Anand and Bhaskaran (2009) studied eight antagonistic bioagents for ecofriendly management of chilli fruit rot caused by *Colletotrichum capsici* and *Alternaria alternata* and found *Pseudomonas fluorescens* and *Trichoderma* isolate 3 was effective in inhibiting pathogens. Similarly the results of various researchers are also corroborative to our observations regarding *Pseudomonas fluorescens* as effective bioagent (Leeman *et al*., 1995, Nayaka *et al*., 2009).

Nisa *et al.* (2011) tested systemic and non-systemic fungicides, *viz.*, carbendazim, myclobutanil, bitertanol, hexaconazole, mancozeb, captan and zineb for their effect on mycelial growth and spore germination of *Fusarium oxysporum* under *in-vitro* and observed significant germination inhibition with mancozeb followed by captan and zineb amongst non-systemic fungicides.

Dar *et al.* (2013) evaluated nine fungicides (carbendazim, hexaconazole, thiophanate methyl, triadimefan, metalaxyl, mancozeb, captan, copper oxychloride and chlorothalonil) and seven bioagents (*Trichoderma harzianum*, *Trichoderma virens*, *Lacaria laccata*, *Boletus edulis*, *Suillus placids* and *Russula lutea*) under *in vitro* for their efficacy towards inhibition of mycelial growth and spore germination in *Fusarium oxysporum f. sp. pini* through dual culture and culture filtrate technique. Maximum spore germination inhibition was shown by mancozeb 75% WP treatment among non-systemic fungicides and carbendazim 50% WP among systemic fungicides and *Trichoderma harzianum* 1% WP was observed to exhibit best results as compared to filtrates of other biocontrol agents.

CONCLUSION

The spore germination of *Fusarium proliferatum*, a prominent seed borne pathogen associated with seeds and fruits of bottle gourd was inhibited by mancozeb 75% WP and *Pseudomonas fluorescens* 1% WP upto 77.33% that was 61.78% and 67.33% in treatments involving carbendazim 50% WP and *Trichoderma harzianum* 1% WP that could be exploited to devise integrated approach for disease management.

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Conflict of interest

All authors declare that they have no conflict of interest.

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