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Bioefficacy of bioagents against *Colletotrichum gloeosporioides* of onion

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Abstract
Onion (*Allium cepa*) (Latin 'cepa' = onion), also known as the bulb onion or common onion, is used as a vegetable and is the most widely cultivated species of the genus *Allium*.) crop is affected by number of pathogenic fungi, causing significant qualitative and quantitative losses. Therefore, present *in vitro* study was conducted to asses bioefficacy of eight bioagents against pathogen *Colletotrichum gloeosporioides*, by applying Dual Culture Technique. Experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. The results revealed that all of the eight test bioagents significantly inhibited mycelial growth of *Colletotrichum gloeosporioides*, over untreated control. However, *T. hamatum* was found most effective with least mycelial growth (16.33 mm) and highest mycelial inhibition (81.11%), followed by *T. virens* (20.00 mm and 77.77%), *T. koningii* (22.33 mm and 75.55%), *T. harzianum* (26.00 mm and 71.11%), *T viride*, (26.50 mm and 70.00%), *A. niger* (27.33 mm and 69.63%), *P. fluorescens* (55.66 mm and 38.15%) and *B. subtilis* (60.00 mm and 33.33%).

Keywords: *Colletotrichum gloeosporioides*, onion, bioagents, dual culture technique, completely randomized design

Introduction
Onion (*Allium cepa*) (Latin 'cepa' = onion), also known as the bulb onion or common onion, is used as a vegetable and is the most widely cultivated species of the genus *Allium*. The name "wild onion" is applied to a number of *Allium* species but *A. cepa* is exclusively known from cultivation. The onion is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season.

About 20 Percent of total area is under *kharif* season. *Kharif* season onions are cultivated mainly in major countries i.e. China, India, USA, France, Japan, Korea, Brazil, Spain, Pakistan and in Maharashtra Satara, Nashik, Manmad, Nifad and in the district of Ahmadnagar, Sangamner, Rahuri, Parner, Shrigonda and Pathardi. The crop is grown in the area where rainfall is 500-550 mm; and for growth of onion temperatures during night at about 17-18 ^{0}C and during day time temperature at about 30-33 ^{0}C are required.

In India Area under onion is 579.9 thousand hectares, Production 7158.4 million tones and Productivity 12357 kg/ha; and In the state of Maharashtra area, production and productivity of onion are 359.0 thousand hectare, 5036.0 million tonnes, 14.03 t/ha respectively (Anonymous, 2015) [1].

Sevaral diseases have been reported on onion, Bacterial flower stalk and leaf necrosis (*Pantoeaagg lomerans*) fungal diseases are: Anthracnose (*Colletotrichum gloeosporioides*) Purple blotch (*Alternaria porri* [*Ellis*] Cif) and Stemphylium leaf blight (*Stemphylium vesicarium*) viral diseases are: Yellow dwarf (Yellow dwarf virus,) and nematode diseases are: Stem and bulb nematode (*Ditylenchus dipsaci*) and root knot nematode (*Meloidogyne incognita*). Among these diseases the Anthracnose (*Colletotrichum gloeosporioides*) is one of the major constrains in onion cultivation. The pathogen is polyphagous infecting crop like onion, Garlic, Shallot and other *Allium* crops. High relative humidity (80 to 90%) and optimum temperature (24±1 ^{0}C) are needed for further development of Anthracnose disease symptoms causing considerable yield losses and is seed borne pathogen causing up to 20-60 percent loss in bulb yield and extent of loss depend on time of infection and stage of crop growth (Hegde *et al*., 2012) [7].

Therefore, present study on *in vitro* bioefficacy of bioagents against Anthracnose of onion was planned and conducted at the Department of Plant Pathology, College of Agriculture, Latur, during 2016-17

Materials and Methods

Isolation, identification and pathogenicity of fungi

The diseased specimens were washed gently in running tap water; blot dried and cut with sharp sterilized blade into small bits (5mm), keeping half healthy and half diseased portion intact. Such leaf and bulb bits were surface sterilized with 0.1% aqueous solution of sodium hypochlorite in glass petriplates for two minutes, washed by giving three sequential changes with sterile distilled water in petriplates to remove traces of sodium hypochlorite, blot dried and separately inoculated these bits aseptically on autoclaved and cooled Potato dextrose agar (PDA) medium in Petri plates, Laminar-air-flow cabinet and incubated in BOD incubator at 28 $\pm$ 2 °C temperature. Within a week of incubation, profuse fungal mycelial growth was obtained. Applying hyphal-tip technique, the test isolates of the test pathogen were aseptically sub-cultured, purified and maintained the pure cultures separately on agar slant tubes in refrigerator for further studies.

The pathogenicity of fungi was proved by Spore-cum-mycelial suspensions techniques, from artificially diseased/anthracnosed leaves of the onion seedlings, the pathogen was re-isolated on PDA medium and incubated at 28±2^{0}C. After a week of incubation, the cultural and morphological characteristics of the test pathogen were observed and compared the same with the characteristics (cultural and morphological) of the test pathogens pure culture isolated from naturally anthracnosed foliage of onion plant specimens collected from various locations.

Observations on incubation period (days required to appear first symptoms), number of lesions per plant, lesion diameter, per cent defoliation and per cent disease intensity/index (PDI) etc. were recorded. For recording foliage anthracnose disease intensity, 0 to 9 grade rating scale was used (Mayee and Datar, 1986) [11]

In vitro evaluation of bioagents

A total of eight bioagents were evaluated *in vitro* against *C. gloeosporioides*, applying Dual Culture Technique (Dennis and Webster, 1971) [5].

Separate experiments was planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. Observations on linear mycelial growth of the test fungi and test bio-agents were recorded separately at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungi. Per cent mycelial growth inhibition of the test fungi with the test bioagents, over untreated control was calculated by applying following formula (Arora and Upadhyay, 1978) [2].

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

The data obtained was statistically analyzed (Panse and Sukhatme, 1978) [13] and the results were interpreted thereof.

Results and Discussion

The results (Fig.1 and Table.1) revealed that all of the eight test bioagents significantly inhibited mycelial growth of pathogen, over untreated control, which was ranged from 33.33 – 81.85 per cent.

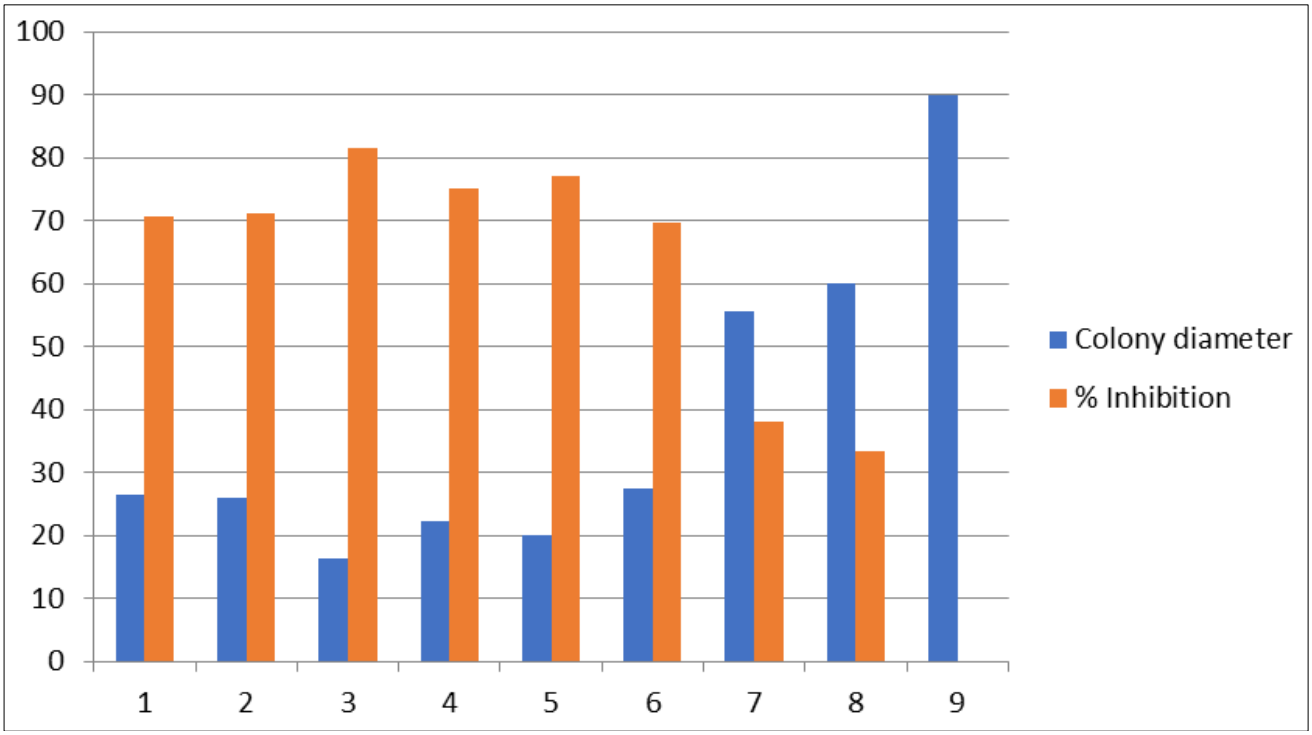

Fig 1: *In vitro* efficacy of bioagents on mycelial growth and inhibition

Treatment

T_1: Trichoderma viride	T_2: T. harzianum	T_3: T. hamatum
T_4: T_4: koningii	T_5: T. virens	T_6: A. niger
T_7: P. fluorescens	T_8: B. subtilis	T_9: Control (Untreated)

Table 1: *In vitro* bioefficacy of bioagents against *C. gloeosporioides*, infecting onion

Tr. No.	Treatments	Col. Dia.* of test pathogen (mm)	% Inhibition*
T_1	*Trichoderma viride*	26.50	70.55 (57.13)
T_2	*T. harzianum*	26.00	71.11 (57.48)
T_3	*T. hamatum*	16.33	81.85 (64.78)
T_4	*T. koningii*	22.33	75.18 (60.11)
T_5	*T. virens*	20.00	77.77 (61.86)
T_6	*Aspergillus niger*	27.33	69.63 (56.55)
T_7	*Pseudomonas fluorescens*	55.66	38.15 (38.14)
T_8	*Bacillus subtilis*	60.00	33.33 (35.26)
T_9	Control (untreated)	90.00	00.00 (00.00)
	S.E. ±	0.62	0.69
	C.D. (P=0.01)	1.85	2.06

*: Mean of three replications, Dia.: Diameter, Figures in parentheses are arcsine transformed values

However, *T. hamatum* was found most effective with least mycelial growth (16.33 mm) and highest mycelial inhibition (81.11%), followed by *T. virens* (20.00 mm and 77.77%), *T. koningii* (22.33 mm and 75.55%), *T. harzianum* (26.00 mm and 71.11%), *T viride*, (26.50 mm and 70.00%), *A. niger* (27.33 mm and 69.63%), *P. fluorescens* (55.66 mm and 38.15%) and *B. subtilis* (60.00 mm and 33.33%).

Various species of *Trichoderma*, *Aspergillus niger*, *P. fluorescens* and *B. subtilis* are most commonly and commercially exploited bioagents/ antagonists to combat several seed borne and / or soil borne plant pathogens. Fungicidal / fungistatic effects of these bioagents have been attributed to various mechanisms exerted such as antibiosis, lysis, mycoparasitiam, competition, production of volatile / non- volatile compounds etc. In present study also, various species of *Trichoderma*, *Aspergillus niger*, *P. fluorescens* and *B. subtilis* were found as efficient antagonists against pathogenic fungi.

These results of the present study are in agreement with previous findings of several workers Bioagents *viz*., *T. hamatum*, *T. virens*, *T. koningii*, *T. harzianum*, *T. viride*, *A. niger*, *P. fluorescens* and *B. subtilis* were reported as efficient antagonists against many *Colletotrichum* spp. including *C. capsici* by several earlier workers (Pathania *et al*., 2004; Kaur *et al*., 2006; Kumar and Dubey, 2006; Mistry *et al.*, 2008; Tiwari *et al*., 2008: Jadhav *et al.*, 2009; Gawade *et al*., 2009; Watve *et al*., 2009; Pardhi and Raut, 2011; Barhate *et al*., 2012a; Shilpa and Gokulapan, 2015; Bhujbal *et al*., 2015b) [15, 9, 10, 12, 17, 8, 6, 18, 14, 3, 16, 4].

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