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Bioefficacy of botanicals 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 nine botanicals against pathogen *Colletotrichum gloeosporioides*, by applying Poisoned Food Technique. Experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. The results revealed that all of the nine botanicals significantly inhibited mycelial growth of *Colletotrichum gloeosporioides*, over untreated control. However, Average mycelial growth inhibition ranged from 23.00 (*A. squamosa*) to 85.55 (*A. sativum*) per cent. However, it was highest with *A. sativum* (85.55%), followed by *C. longa* (70.76%), *A. indica* leaves (69.37%), *Z. officinale* (56.92%), *C. annum* (55.82%), *D. metal* (53.00%), *A. cepa* (35.27%), and *O. sanctum* (24.87%).

Keywords: *Colletotrichum gloeosporioides,* onion, botanicals, poisoned food technique, completely randomized design

Introduction
The onion plant (Allium cepa), also known as the bulb onion or common onion, 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.

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) [5].

Therefore, present study on *in vitro* bioefficacy of Botanicals 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±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) [9].

In vitro evaluation of botanicals

A total of nine botanicals were evaluated *in vitro* against *C. gloeosporioides*, applying Poisoned Food Technique (Nene and Thapliyal, 1993) [12] and using Potato dextrose agar (PDA) as basal culture medium

Experiments, were planned in Completely Randomized Design (CRD), with nine treatments replicated thrice. Observations on radial mycelial growth/colony diameter were recorded at 24 hrs interval and continued till the untreated control plates were fully covered with mycelial growth of the test fungus. Per cent mycelial growth inhibition of *C. gloeosporioides*, over untreated control was calculated by applying following formula (Vincent, 1927) [17].

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = growth of the test fungus in untreated control plate

T = growth of the test fungus in treated plate

The data obtained was statistically analyzed (Panse and Sukhatme, 1978) [13].

Results and Discussion

The results (Fig.1 and Table.1) revealed that all of the nine test botanicals significantly inhibited mycelial growth of pathogen, over untreated control, At 10 per cent, mycelial growth inhibition ranged from 21.94 to 84.44 per cent. At 15 per cent, mycelial growth inhibition ranged from 24.07 to 86.66 per cent.

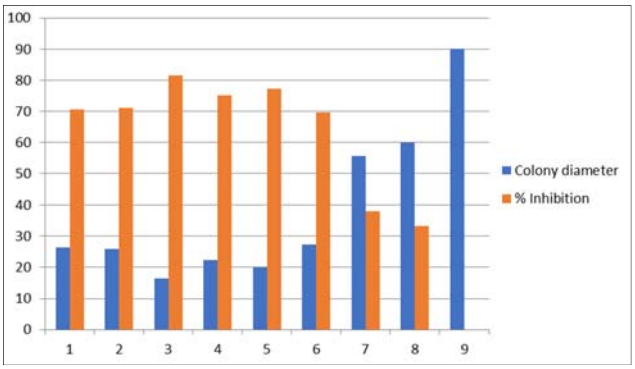

Fig 1: Percent inhibition of mycelium of *C. gloeosporioides* as influenced by plant extracts Treatment

T_1: *A. sativum*	T_2: *A. cepa*	T_3: *Z. officinale*
T_4: *D. metal*	T_5: *A. squamosa*	T_6: *O. sanctum*
T_7: *C. annum*	T_8: *A. indica* leaves	T_9: *C. longa*
T_{10}: Control (Untreated)		

Plate I

In vitro bioefficacy of plant extracts at various concentrations against *C. gloesporioides*, infecting onion

T_1 : *A.sativum*	T_2: *A. sepa*	T_3 : *Z.officinale*
T_4 : *D. metal*	T_5: *A.squamosa*	T_6: *O.sanctum*
T_7 : *C. annum*	T_8 : *A.indica* leaves	T_9: *C. longa*
T_{10}: Control (Untreated)		

Table 1: *In vitro* bioefficacy of botanicals against *C. gloeosporioides* (isolate Cg-1), infecting onion

Tr. No.	Treatments	Col. Dia.* (mm)		Av. (mm)	% Inhibition*		Av. (%)
		10 %	15 %		10 %	15 %	
T_1	*Allium sativum*	14.00	12.00	13.00	84.44 (66.76)	86.66 (68.57)	85.55 (67.65)
T_2	*Allium cepa*	60.50	56.00	58.25	32.77 (34.92)	37.77 (37.92)	35.27 (36.43)
T_3	*Zingiber officinale*	40.00	37.53	38.76	55.55 (48.18)	58.30 (49.77)	56.92 (48.97)
T_4	*Datura metal*	43.25	41.33	42.29	51.94 (46.11)	54.07 (47.33)	53.00 (46.71)
T_5	*Annona squamosa*	70.25	68.33	69.29	21.94 (27.93)	24.07 (29.38)	23.00 (28.65)
T_6	*Ocimum sanctum*	69.90	65.33	67.61	22.33 (28.18)	27.41 (31.57)	24.87 (29.91)
T_7	*Capsicum annum*	41.50	38.00	39.75	53.88 (47.22)	57.77 (49.47)	55.82 (48.34)
T_8	*Azadirachta indica leaves*	28.73	19.33	24.03	68.07 (55.59)	70.67 (57.20)	69.37 (56.39)
T_9	*Curcuma longa*	27.73	24.73	26.23	69.18 (56.27)	72.74 (56.27)	70.96 (57.39)
T_{10}	Control (Untreated)	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E. ±	**0.33**	**0.37**	-	**0.20**	**0.61**	-
	C.D. (P= 0.01)	**1.00**	**1.12**	-	**1.73**	**1.85**	-

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

At 10 per cent, mycelial growth inhibition ranged from 21.94 to 84.44 per cent. However, it was highest with *A. sativum* (84.44%), followed by *C. longa* (69.18 %), *A. indica* leaves (68.07%), *Z. officinale* (55.55%), *C. annum* (53.88%), *D. metal*, (51.94%), *A. cepa* (32.77%), *O. sanctum* (22.33%), and *A. squamosa* (21.94%).

At 15 per cent, mycelial growth inhibition ranged from 24.07 (*A. squamosa*) to 86.66 (*A. sativum*) per cent. However, it was highest with *A. sativum* (86.66%), followed by *C. longa* (72.74%), *A. indica* leaves (70.67%), *Z. officinale* (58.30%), *C. annum* (57.77%), *D. metal* (54.07%), *A. cepa* (37.77%), *O. sanctum* (27.41%) and *A. squamosa* (24.07%).

Average mycelial growth inhibition ranged from 23.00 (*A. squamosa*) to 85.55 (*A. sativum*) per cent. However, it was highest with *A. sativum* (85.55%), followed by *C. longa* (70.76%), *A. indica* leaves (69.37%), *Z. officinale* (56.92%), *C. annum* (55.82%), *D. metal* (53.00%), *A. cepa* (35.27%), and *O. sanctum* (24.87%).

These results of the present study are in agreement with the findings of several earlier workers. The phytoextracts viz., *A. sativum Z. officinale, A. indica, Annona squamosa, O. sanctum, A. indica* and *Capsicum annum* were reported against many *Colletotrichum* spp, including *C. capsici*, earlier by several workers (Mistry *et al*., 2008; Shovan *et al*., 2008; Watve *et al*., 2009; Gawade and Suryawanshi, 2009; Johney *et al*., 2011; Mukharjee *et al*., 2011; Rajmanickam *et al*., 2012; Jayalakshmi *et al*., 2012; Bediako, 2014; Kekuda *et al*., 2014; Harsha *et al*., 2014; Shinde and Gawai, 2014;) [10, 16, 18, 3, 7, 11, 14, 6, 2, 8, 4, 15].

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