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In-vitro evaluation of fungicides, botanicals and bio-agents against *Alternaria porri*

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Abstract
Onion (*Allium cepa* L.) is one of the major bulb crop in India, and it is widely cultivated species of genus *Allium* belongs to the family Alliaceae, Purple blotch of onion caused by *Alternaria porri* (Ellis) cif. is one of the most destructive disease causing heavy loss under field conditions. To know the efficacy of different fungicides, botanicals and bio-agents, were tested under *in-vitro* condition. Mancozeb75 WP was effective in controlling the mycelial growth of *A. Porri* with per cent mean inhibition of (97.68 %), followed by Difenconazole 25 EC (95.75 %). Among the five botanicals tested, Garlic (84.51%) was significantly superior over all other plant extracts evaluated. The next best treatment was Pongamia leaf extract (80.81%) and Neem (74.32%). *In-vitro* evaluation of bio-agents revealed that *T. harzianum* isolate 1 (83.10%) which was significantly superior over other isolate tested. Least inhibition was noticed in *Pseudomonas fluorescens* isolate 1 (51.52%).

Keywords: Onion, *Alternaria porri*, Fungicides, Botanicals and Bio agents

Introduction
Onion (Allium cepa L.) rightly called as "queen of kitchen" is one of the oldest and an important spice crop grown all over the world. It belongs to the family Alliaceae. According to Vavilov (1951) [1] the primary center of origin of onion lies in central Asia. The near east and Mediterranean are the secondary centers of origin. The genus Allium is very large comprising of more than 500 plant spp, usually perennial bulbous plants. Out of these, *Allium cepa* (onion) is the major cultivated spice grown all over the world. It is commonly used for cooking purposes by almost all the people. It ensures excellent taste to dishes and also exhibits a number of therapeutic properties such as antibacterial, antifungal, anthelminthic, anti-inflammatory, antiseptic and antispasmodic. In India, onion occupies an area of 1.20 million hectare area, with a production of 19.40 million tonnes and a productivity of 16.10 metric tonnes/ha in the year 2013-2014 (Anon., 2015) [2]. Onion crop is affected by many diseases, among them purple blotch disease caused by *Alternaria porri* (Ellis) Cif [3]. is a serious disease which affects both bulb and seed crop throughout India (Dhiman and Chadha, 1986) [4]. The disease is more severe in *kharif* season than in *rabi* season. The yield loss of onion in India due to this disease varies from 5.0 to 96.5 per cent (Gupta and Pathak, 1986) [5]. The disease causes heavy yield loss under severe epiphytotic conditions. In this regard a experiment was conducted during to known the efficacy of different fungicides, botanicals and bio agents against *Alternaria porri* under laboratory conditions.

Material and methods
In order to isolate a pathogen the leaves of onion showing typical symptoms of purple blotch were collected from fields and the standard tissue isolation method was followed to isolate the pathogen. The infected leaf bits were surface sterilized with 1% Sodium hypochlorite solution for 60 seconds and repeatedly washed with sterilized distilled water and then transferred to sterilized Petri plates (1-2 leaf bits per Petri dish) containing potato dextrose agar (PDA) under aseptic condition. Once the culture has been grown it is purified by single spore isolation technique. The purified culture was sub cultured on PDA slants and allowed to grow at 27±1°C for seven days and such slants were preserved in a refrigerator at 5°C and subcultured at 30 days interval. Identification of the fungus was made after examining conidia under microscope (under10x) from mature pure culture of the fungus obtained from infected leaves of onion. The culture is inoculated on onion seedlings to prove pathogenicity test.

The efficacy of non-systemic, systemic fungicides and combiprodcts were tested against *Alternaria porri* using poisoned food technique. Required quantities of individual fungicides were added separately into molten and cooled potato dextrose agar and 20 ml of the poisoned medium was poured into sterile petri plates. Mycelial discs of 5 mm size from actively growing 7 days old *A. porri* culture was aseptically placed at the centre of agar plate. Control was maintained without adding any fungicides to the medium. Each treatment was replicated thrice. The plates were incubated at room temperature for eight days and radial colony growth was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control was calculated by using the formula suggested by Vincent (1947) [6].

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

Where,
I = Per cent Inhibition, C = Radial growth in control plate, T = Radial growth in treated plate
The data were analysed statistically.

List of fungicides used

Fungicides	Formulation	Trade name
Carbendazim12% + Mancozeb 63%	75 WP	SAAF
Carbendazim 25 % + Iprodione 25 %	50 WP	Quintal
Tebuconazole 50% + Trifloxystrobin 25 %	75 WP	Nativo
Chlorothalonil	75 WP	Kavach
Mancozeb	75 WP	Dithane M-45
Azoxystrobin	23 EC	Amistar
Difenconazole	25 EC	Score
Hexaconazole	5 EC	Contaf
Zineb	75 WP	Dithane Z -78

To study the antifungal mechanism of plant extracts, poisoned food technique was used (Nene and Thapliyal, 1982) [7]. Two, five and ten ml of stock solution were mixed with 98, 95 and 90 ml of sterilized molten PDA media, respectively to obtain 2, 5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile Petri plates, mycelium of five mm size discs form periphery of actively growing seven day old culture were aseptically placed in the centre of agar plate. Control was also maintained by growing the pathogen on PDA plates. Inoculated plates were incubated at room temperature for eight days and radial growth was measured when the growth was observed in control plates. The efficacy of plant products or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the formula Vincent (1947) [6].

List of botanicals/plant extracts used

Common name	Parts used	Scientific name	Concentrations (%)
Neem	Leaf	*Azardirchta indica*	2, 5, 10
Pongamia	Leaf	*Pongamia pinnata*	2, 5, 10
Garlic	Clove	*Allium sativum*	2, 5, 10
Marigold	Leaf	*Tagetes* spp	2, 5, 10
Agave	Leaf	*Agave Americana*	2, 5, 10

TO evaluate different bio agents against *Alternaria porri*, *T. harzianum* and *Pseudomonas fluorescens* were obtained from Department of Microbiology, UAHS, Shivamogga, from UAS, GKVK and from UAS Dharwad, were tested *in vitro* against *Alternaria porri* by using dual culture technique (Dennis and Webster, 1971) [8]. Twenty ml of sterilized potato dextrose agar medium melted and cooled to 45°C was poured aseptically into sterilized petri dishes of nine cm diameter. Mycelial discs of five mm diameter cut from the edge of actively growing seven days old culture of pathogen and mycelial discs (5 mm) of *Trichoderma* spp. cut from actively growing colony of the respective fungal species were placed on the periphery about one cm from the edge of the Petri dish at opposite sides.

For bacterial antagonist's evaluation, the *P. fluorescens* was streaked at the centre of the Petri plate and two mycelial discs of the pathogen were placed at opposite ends. The Petri dish containing potato dextrose agar medium inoculated with the pathogen alone served as control. All the treatments were replicated thrice and the plates were incubated at room temperature (27 + 1oC). After incubation when the growth of the pathogen was 100 % in the control, the colony diameter of the pathogen was measured in each treatment and the per cent inhibition of the pathogen over control was calculated by adopting the formula given by Vincent (1947) [6]. The data were analyzed statistically.

Description of antagonists used

Trichoderma harzianum 1 (UAHS, Shivamogga)
Trichoderma harzianum 2 (UAS, GKVK)
Trichoderma harzianum 3 (UAS, DWD)
Pseudomonas fluorescens 1 (UAHS, Shivamogga)
Pseudomonas fluorescens 2 (UAS, DWD)

Result and Discussion

In-vitro* evaluation of fungicides against *A. porri

Three systemic, three contact and three combi products of fungicides were evaluated for their efficacy against *Alternaria porri* by poison food technique. The data are presented in Table (1). Present study revealed that, Mancozeb 75 WP with a higher per cent mycelial inhibition of 95.30, 96.53, 98.93 and 99.97 at 100, 200, 500 and 1000 ppm respectively followed by Difenconazole 25 EC with per cent mycelial inhibition of 93.43, 95.70, 96.57 and 97.33 at 100, 200, 500 and 1000 ppm respectively. Zineb 75 WP recorded least per cent mycelial inhibition of 9.53, 17.87, 24.53 and 64.30 at 100, 200, 500 and 1000 ppm respectively. The mean per cent inhibition of mycelial growth was more in case mancozeb 75

WP (97.68) followed by Difenconazole 25 EC (95.75 %). The lowest inhibition was recorded in case of Zineb 75 WP (29.05 %). Among three non-systemic fungicides tested *viz*., Zineb 75 WP, Chlorothalonil 75 WP and Mancozeb 75 WP, Mancozeb75 WP was effective in controlling the mycelial growth of *A. Porri* with percent mean inhibition of (97.68 %), followed by Chlorothalonil 75 WP (62.90 %). Among three Systemic fungicides tested *viz*., Difenconazole, Hexaconazole and Azoxystrobin, maximum per cent mean inhibition was recorded in case of Difenconazole 25 EC (95.75 %).Among three comb products tested *viz*., Saaf (Carbendazim + Mancozeb), Nativo (Tebuconazole + Trifloxystrobin) and Quintal(Carbendazim + iprodione). Maximum percent mean inhibition was observed in case of (Tebuconazole + Trifloxystrobin) (85.83 %) followed by (Carbendazim + iprodione) (79.14%). The best treatments was Mancozeb 75 WP (97.68%) and Difenconazole 25 EC (95.75%) whereas, Zineb 75 WP (29.05%) and Chlorothalonil 75 WP (62.90%) was least effective in reducing the fungal growth. The data presented in Table (1).

In-vitro evaluation of botanicals/ plant extracts against *A. porri*

To know the efficacy of botanicals on the inhibition of mycelial growth of the pathogen. The plant extracts were evaluated at three concentrations in the laboratory for their efficacy against the *A. Porri* by following Poisoned food technique. The data presented in Table (2). Among the five botanicals tested, all the botanicals showed inhibition of *A. porri* at varied level. Garlic (84.51%) was significantly superior over all other plant extracts evaluated. The next best treatment was Pongamia leaf extract (80.81%) and Neem (74.32%). Least inhibition was noticed in case of Marigold (56.51%). Irrespective of the plant species, botanicals were found to be most effective at 10 per cent which was significantly superior over 2 per cent and 5 per cent concentration. Interactions between botanicals and concentrations were significant. All the plant extracts reduced the mycelial growth with increase in concentrations. Maximum reduction of mycelial growth (99.73 %) was noticed in case of Garlic followed by pongamia leaf extract (92.89%) and neem (91.80%) at 10 per cent. Least reduction of mycelial growth was noticed in case of Marigold (30.53%) at 2 per cent concentration.

In-vitro evaluation of bio-agents against *A. porri* through dual culture technique

The antagonistic effects of all five isolates of bio agents *viz. Trichoderma harzianum* isolate 1 (UAHS, Shivamogga), *T. harzianum* isolate 2 (UAS, GKVK), *T. harzianum* isolate 3 (UAS, DWD), *Pseudomonas fluorescens* isolate 1(UAHS, Shivamogga) and *Pseudomanas fluorescens* isolate 2 (UAS, Dharwad) on growth of *A. porri* was studied *in vitro* by dual culture plate method as explained under. The results are presented in Table (3). The results revealed that, the antagonists significantly reduced the growth of *A. porri* either by competition (over growing) or by antibiosis (exhibiting inhibition zones). It was noticed that maximum reduction in colony growth of *A. porri* was observed in *T. harzianum* isolate 1 (83.10%) which was significantly superior over other isolate tested. Least inhibition was noticed in *Pseudomonas fluorescens* isolate 1 (51.52%).

Table 1: *In vitro* evaluation of fungicides against *Alternaria porri*

Fungicides	Inhibition of mycelial growth (%) Concentration (ppm)				Mean
	100	200	500	1000	
Systemic					
Difenconazole 25 EC (Score)	93.43 (75.24)	95.70 (79.22)	96.57 (79.47)	97.33 (80.33)	95.75 (78.56)
Azoxystrobin 23 EC (Amistar)	63.33 (52.74)	74.44 (59.64)	79.77 (63.27)	91.80 (73.38)	84.11 (62.25)
Hexaconazole 5 EC (Contaf)	76.57 (61.05)	77.80 (61.89)	79.20 (62.87)	92.18 (73.38)	81.43 (64.89)
Non systemic					
Zineb 75 WP (Dithane Z-78)	9.53 (17.97)	17.87 (24.99)	24.53 (29.68)	64.30 (53.31)	29.05 (31.48)
Chlorothalonil 75 WP (Kavach)	53.87 (47.22)	55.83 (48.35)	64.4 (53.37)	77.53 (61.71)	62.90 (52.66)
Mancozeb 75 WP (Dithane M-45)	95.3 (77.51)	96.53 (79.3)	98.93 (84.08)	99.97 (89.40)	97.68 (82.57)
Combiproducts					
Mancozeb 63%+ Carbendazim 12%75 WP (Saaf)	74.40 (59.63)	74.83 (59.90)	76.40 (60.94)	87.83 (69.60)	78.36 (62.51)
Tebuconazole 50% + Trifloxystrobin 25% 75WP (Nativo)	77.7 (61.93)	83.37 (65.98)	86.69 (68.62)	95.58 (77.95)	85.83 (68.61)
Carbendazim 25% + Iprodione 25% 50 WP (Quintal)	66.87 (54.86)	72 (58.06)	83.5 (66.04)	94.20 (76.10)	79.14 (63.76)
	S. Em ±			CD at 1%	
Fungicides	0.40			1.49	
Concentration	0.33			0.991	
F ×C	0.99			2.98	

* Figures in parentheses are arcsine transformed values

Table 2: *In-vitro* evaluation of botanicals against *Alternaria porri*

Botanicals	Botanicals name	Plant part used	Inhibition of mycelial growth (%) Concentration (%)			
			2	5	10	Mean
Pongamia	*Pongamia pinnata*	Leaves	66.17(54.43)	83.37(65.93)	92.89(74.53)	80.81(64.96)
Agave	*Agave americana*	Leaves	44.25(41.70)	76.5(61.00)	88.48(70.16)	69.74(57.62)
Garllic	*Allium sativum*	Clove	58.27(49.76)	95.55(77.82)	99.73(87.04)	84.51(71.53)

Marigold	*Tagetes spp*	Leaves	30.53(33.54)	50.53(45.31)	85.33(67.48)	55.46(48.77)
Neem	*Azadirachta indica*	Leaves	45.83(42.61)	85.34(67.48)	91.80(73.36)	74.32(61.15)
			Concentration	Botanicals	C *B	
	SEm±		0.50	0.6643	1.11	
	CD at 1%		1.49	1.993	3.320	

* Figures in parentheses are arcsine transformed values

Table 3: *In vitro* evaluation of bio-agents against *Alternaria porri*

Bio agents	Inhibition of mycelial growth (%)
Trichoderma harzianum 1 (UAHS Shivamogga)	83.10(65.90)
Trichoderma harzianum 2 (UAS GKVK)	81.32(64.44)
Trichoderma harzianum 3 (UAS DWD)	81.51(64.61)
Pseudomonas flouroscens 1 (UAHS Shivamogga)	51.52(45.87)
Pseudomonas flouroscens 2 (UAS DWD)	55.18(47.97)
SEm±	1.10
CD at 1%	3.31

* Figures in parentheses are arcsine transformed values

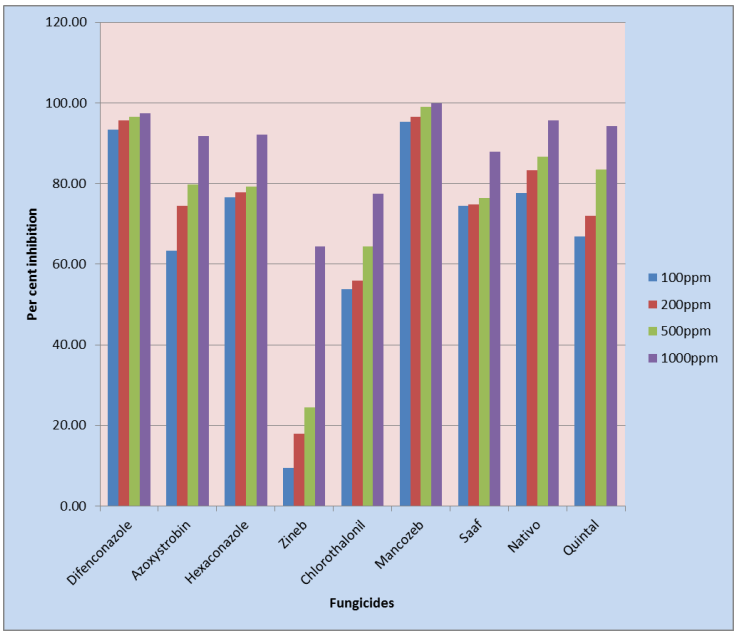

Fig 1: *In vitro* evaluation of fungicides against *Alternaria porri*

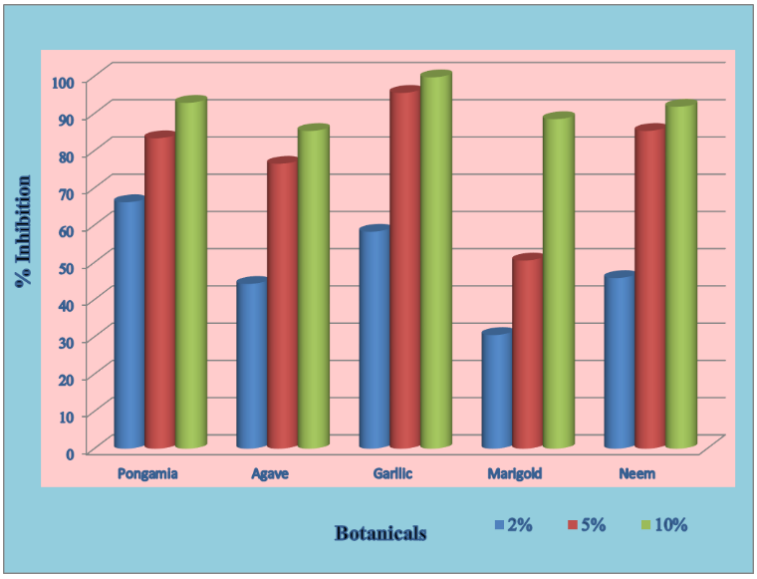

Fig 2: *In vitro* evaluation of botanicals against *Alternaria porri*

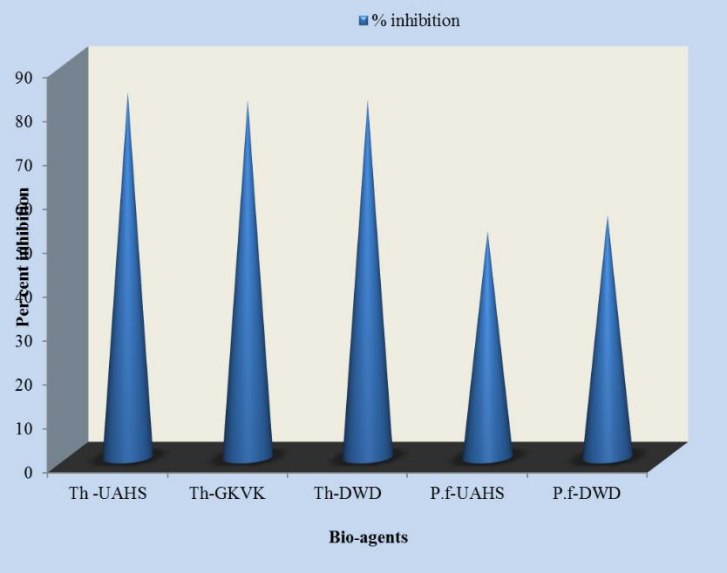

Fig 3: *In vitro* evaluation of bio-agents against *Alternaria porri*

Th- UAHS: *Trichoderma harzianum* 1 (UAHS, Shivamogga)
Th –GKVK: *Trichoderma harzianum* 2 (UAS, GKVK)
Th- DWD: *Trichoderma harzianum* 3 (UAS, DWD)
P.f- UAHS: *Pseudomonas fluroscens* 1 (UAHS, Shivamogga)
P.f- DWD: *Pseudomonas fluroscens* 2 (UAS, DWD)

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