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EPIDEMIOLOGY, SYMPTOMATOLOGY AND MANAGEMENT OF THE FRUIT ROT OF BOTTLE GOURD (*LAGENARIA SICERARIA* STANDL.) CAUSED BY *ALTERNARIA ALTERNATA* (FR.) KEISSLER

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ABSTRACT
The disease epidemiology and symptomatology of black fruit rot of bottle gourd caused by *Alternaria alternata* (Fr.) Keissler was studied under both *in vitro* and *in vivo* conditions. The results revealed that, *A. alternata* can grow at wide range of temperatures between 15-30ºC; with 26-28ºC as the most favoured temperature range. The pathogen required high relative humidity (> 90 %) for optimum growth and sporulation. Microscopic observations revealed that the pathogen bear hyaline, septate and irregularly branched mycelia, hyphae of an average width 3.50 μm (range 1.50-7.30 μm), short or long conidiophores arising singly or in groups of 2-6 from host tissue, and 48 x 14 μm sized conidia borne in long chains of 10 or more bearing 2-10 septa. Artificially inoculated healthy fruits of bottle gourd expressed the typical symptoms of water soaked lesions which turned brown within 2-3 days. The fungicide mancozeb (0.25 %) was found most effective with 100 % inhibition of pathogen, *in vitro*. Among the two antagonistic fungi, *Trichoderma hamatum* with 87.77 % inhibition of pathogen growth under *in vitro* was more promising than *Trichoderma harzianum* (77.77 %). The findings of the present study are crucial in understanding the field epidemiology and severity of *A. alternata* incidence. The knowledge on efficacy of fungicides and antagonistic fungal bioagents in suppressing the pathogen growth will aid in implementing effective management strategy for *A. alternata*.

INTRODUCTION

The bottle gourd (*Lagenaria siceraria* Standl., F: Cucurbitaceae), is a tropical vegetable of Afro-Asian origin and is cultivated in India throughout the year for its young and tender fruits eaten as popular domestic vegetable called '*Lauki*'or '*Dudhi*'. The serious diseases like downy mildew, powdery mildew, root rot and fruit rot that attack the crop during various stages of growth and also in post-harvest are among the major limiting factors responsible for low productivity of bottle gourds (Neeraj and Verma, 2010). The black fruit rot caused by *Alternaria alternata* (Fr.) Keissler is the most damaging disease of bottle gourd, that causes rotting of fruits at blossom end stage. This result in huge economic losses thereby causing great set back to the bottle gourd growers. The fundamental aim of the present study was to understand the disease epidemiology and symptomatology of fruit rot in bottle gourd so as intervene its spread by suggesting eco-friendly management strategy.

The prevalence of fruit rot disease in bottle gourd was first time reported by Singh and Chouhan (1980) from North India. The infection of the rot pathogen causes extensive decay of the fruits in the form of blackish brown rotting which leads to arrested fruit growth and dropping of fruits at blossom end. This causes great set back to the growers as yield of the crop is affected severely and also the infected fruits lose their market quality. The rot disease in bottle gourd is a serious destroyer and disease development is so fast that whole crop is lost in a few days (Singh and Majumdar, 2004; Singh *et al.,* 2006). Besides the bottle gourds, the pathogen *A. alternata* attacks many other vegetables and ornamentals of economic importance and cause huge yield losses, e.g. summer squash (Gangopadhyay and Kapoor, 1973; Hellan, 1985), tomato (Mehta and Saxena, 1976; Kumar *et al.*, 2012), water melons (Narain *et al*., 1985), mango (Mohsan *et al*., 2011) and chrysanthemum (Kumar *et al*. 2011).

The earlier studies on fruit rots caused by *A. alternata* involved only reports of field observations on disease occurrence and its symptoms. Few workers have cultured a pathogen in laboratory and observed the colony morphology. However, there has been no systematic study involving disease epidemiology, effects of abiotic factors like temperature and relative humidity on growth and development of pathogen and efficacy of different commonly used fungicides and biological control agents in suppressing this pathogen, especially in case of bottle gourd. Considering the wide host range and damage potential of *A. alternata,* the problem deserves immediate and effective measures of control so as to minimise the yield losses. The present study aimed to investigate the disease epidemiology, symptomatology and to intervene the disease spread by suggesting eco-friendly management strategy for fruit rot in bottle gourd.

MATERIALS AND METHODS

Sample collection

The samples of bottle gourd fruits (variety: Samrat) infected with fruit rot were collected from experimental field of Regional Fruits and Vegetable Research Station (Mahatma Phule Krishi Vidyapeeth), Ganeshkhind, Pune (Fig. 1). The samples were kept in sterile polythene bags and were brought to the laboratory for isolation and further studies on rot pathogen.

Isolation of fruit rots pathogen

The isolation of pathogen associated with bottle gourd fruit rot was carried out by following a detached tissue method (Loladze *et al*., 2005; Park *et al*., 2008). The infected tissues were cut into small bits of size 2-3 mm and were surface sterilized in 0.001% mercuric chloride solution for two minutes followed by washing with sterile water for three times. The bits of disease infected tissues were then transferred aseptically on solidified potato dextrose agar medium in sterilised petriplates (PDA, composition for 1 litre medium: peeled potatoes 200 g; dextrose 20 g; agar agar 15 g; double distilled sterile water 1000 mL; p^H adjusted to 7.0). The inoculated plates were incubated for 7days at 27 ± 1°C temperature and 65-70% RH.

Preparation and maintenance of pure culture

After incubation for a week, the growth of the fungus obtained on culture medium was recultured. The spores were collected from the pure colonies and single spore culture was prepared on PDA by following the technique developed by Choi *et al*. (1999). The purified culture was held in refrigerator at 10-15ºC for further studies. To maintain viability of the culture, it was revived by periodical subculturing on PDA slants.

Identification of isolated microorganism

A seven days old culture of the isolated organism was examined under microscope for morphological observations like shape, size, septation and mycelial growth. The microscopic measurements were recorded with the help of filar micrometer. Based on morphological characters, the microorganism was identified by referring standard books on mycology (Elliot, 1971; Barnett *et al*., 1972), and an identification manual prepared for *Alternaria* sp. (Simmons, 2007). Additionally, the culture was identified from '*Fungus Identification Service, National Fungal Culture Collection of India*', Agharkar Research Institute, Pune.

Effect of culture media on growth and sporulation

The growth characteristics and sporulation ability of *A. alternata* on five different growth media (Table 1) were studied according to Masangkay *et al*. (2000). Petri plates containing 20mL of following media: Potato dextrose agar, Czapek's agar, Richard's agar, Sach's agar and Nutrient agar separately, were inoculated with 5 mm mycelial disc cut from seven days old culture of *A. alternata*. The inoculated plates were incubated at 27 ± 1ºC for seven days in dark. Three replicates were maintained for each media. The observations were recorded on mean colony diameter, sporulation, colour and growth characters.

Effect of abiotic factors on growth and sporulation

The growth characteristics and sporulation ability of *A. alternata* were studied at 11 constant temperatures (0, 5, 10, 15, 20, 25, 26, 28, 30, 35 and 40ºC) and six constant RH levels (35, 50, 65, 75, 90 and 100 %). *A. alternata* was grown on sterilized PDA plates at above temperatures in incubators for a period of seven days. The observations were recorded on colony diameter, growth characters and sporulation at respective temperatures.

Similarly, the desired levels of humidity were artificially created in moist chambers by addition of concentrated sulphuric acid diluted in water (Soloman, 1951). Healthy fruits of bottle gourd were surface sterilised with the help of 0.001 % $HgCl_2$ solution followed by washing in sterilised water for three times. The slight injury was made to the fruits using sand paper and a thin suspension of fully grown fungus culture prepared in distilled water was applied on the injured portion. The inoculated fruits were then kept in moist chamber at respective humidity levels and 27 ± 1 °C for a period of one week. The observations were recorded on colony diameter, growth characters and sporulation at respective temperatures.

Pathogenicity test

The pathogenicity test was carried out with the isolated fungus on both the detached healthy whole fruits (*in vitro)* and the healthy fruits on the plants in the field itself (*in vivo*) according to the method suggested by Ash and Lanoiselet (2001).

For *in vitro* assay, the fruits were surface-sterilized with 0.001% $HgCl_2$ solution for two minutes followed by washing with sterile water and drying the fruits with sterile filter papers. A slight injury was made to the fruits from floral end using sand paper. The spore suspension of seven days old fungal pathogen prepared in sterile water was applied on the injured portion using sterilised cotton wool. The inoculated fruits were securely placed in a moist chamber at 27 ± 1ºC temperature for one week.

Similarly, for *in vivo* assay, the bottle gourd fruits on the plant itself in the field were surface sterilised and slightly injured from blossom end. The injured fruits were dipped into the spore suspension of seven days old fungal culture. The injured portion of the fruits was covered with cotton wool soaked in sterile water to prevent moisture loss and subsequent drying of inoculated fruit portion. The typical symptoms expressed were recorded a week post inoculation.

The pathogen was reisolated from the artificially inoculated fruits from both *in vitro* and *in vivo* treatments. The culture obtained from reisolation was purified and transferred on to the PDA slants for comparison with the original culture.

Fungicide bioassay

Fungicides *viz*., copper oxychloride, mancozeb, carbendazim, chlorothalonil, difenconazole and propiconazole were evaluated at recommended concentration by poison food technique (Dhingra and Sinclair, 1995). Accurately weighted quantities of each fungicide were mixed with autoclaved PDA medium in 500mL Erlenmeyer flasks. The fungicide mixed medium was then poured into sterilised petriplates. The mycelial discs (5 mm diameter) cut from seven days old *A. alternata* culture were put at the centre of the petriplates. The inoculated plates were then incubated at 27 ± 1ºC for one week. The radial growth of the fungal colony was recorded

after seven days incubation when maximum growth was observed in untreated control plates. The per cent inhibition of mycelial growth over control was calculated using the formula of Vincent (1947).

$$I = \frac{C-T}{C} 100 \quad \text{.....(1)}$$

Where,

I = inhibition of mycelial growth (%)

C = radial growth of fungus in control (mm)

T = radial growth of fungus in treatment (mm).

Bioefficacy of antagonistic fungi

The antagonistic effects of two bioagents *viz*., *Trichoderma harzianum* and *Trichoderma hamatum* were tested against *A. alternata* by dual culture technique (Morton and Stroube, 1955). The mother cultures of the bioagents obtained from Biocontrol Laboratory (Department of Plant Pathology), College of Agriculture, Pune were maintained on PDA medium. The bioagents and the test pathogen *A. alternata* were inoculated equidistant on PDA medium by putting 5 mm discs of each of them on PDA plates. The inoculated petriplates were incubated at 27 + 1°C for one week. In each case four replications were maintained along with untreated control. The observations on radial growth of pathogen was taken and the per cent inhibition in growth of *A. alternata* due to bioagent treatment was calculated by following formula (Vincent, 1947)

$$I = \frac{C-T}{C} 100 \quad \text{.....(2)}$$

Where,

I = inhibition of mycelial growth due to treatment of antagonistic fungi (%)

C = radial growth of fungus in control (mm)

T = radial growth of fungus in treatment of antagonistic fungi (mm).

RESULTS AND DISCUSSION

Epidemiology

The profuse mycelial growth of the test fungus *A. alternata* was obtained on PDA medium. At initial stage, the mycelia were hyaline, septate and irregularly branched which turned brown at later stage (Fig. 2). The average width of the hyphae was 3.50 µm (range 1.50-7.30 µm). Conidiophores arising singly or in groups of 2-6 from host tissue were short or long, olivaceous brown in colour, straight to slightly curved and swollen at the apex having terminal scars indicating the point of attachment. Conidia were borne in long chains of up to 10 or more, golden yellow or dark brown in colour, ellipsoidal, tapering at apex with distinct beak, bearing 2-10 septa and slightly constricted at septation. The conidia inclusive of beak measured 48 x 14 µm in length. They were muticelled with 10-15 transverse and 1-2 longitudinal septa. Our results are largely in agreement with those reported by earlier workers for *A. alternata* affecting different crops. Prasad and Upadhyay (2010) reported that the conidiophores of *A. alternata* f. sp. *lycopersici* isolated from infected tomato leaves were brown, straight, bearing light brown conidia with a short obovate beak at the tip. Conidia were produced in chains and showed 3-8 transverse and longitudinal septa. Mmbaga *et al*. (2011) reported that, the colonies of *A. alternata* causing leaf blight in *Syringa* sp. were dark to grey-black and conidiophores arising singly or in small groups produced spores in chains. Conidiophores were large with longitudinal and transverse septa and a short beak typical for *Alternaria* sp. Wagh *et al*. (2013) reported that the microscopic observations of seven days old *A. alternata* culture on PDA revealed hyaline, septate and branched mycelia, conidiophores were obclavate to obpyriform with average width of 3-6µ, conidia with short conical beak arranged in acropetal fashion. Thus, it was confirmed that the isolated pathogen is *Alternaria alternata*.

Effect of culture media on growth and sporulation

An excellent mycelial growth and sporulation of *A. alternata* was obtained on PDA medium followed by Czapek's medium. Very poor colony growth and sporulation was obtained on Sach's agar medium (Fig. 3, Table 2). Our results are in line with the reports of Prasad and Kulshrestha (1999) who found PDA supplemented with $CaCO_3$ and Sabourd's agar as the best culture media for *Alternaria helianthi* affecting sunflower. In our results, besides variation in growth ability, the *A. alternata* also exhibited variation in colony colour with different media. The colonies appeared grey coloured with yellowish tinge on Czapek's agar, white coloured on Richard's agar, light violet coloured on Sach's agar, purplish coloured on nutrient agar and dark brown coloured on PDA (Fig. 3). These results are confirmed by the findings of Hubbali *et al*. (2010) who reported colonies of *A. alternata* as blackish grey coloured on Czapek's agar, white coloured on Richard's agar and dark brown coloured on PDA.

Effect of abiotic factors on growth and sporulation

The results revealed that, *A. alternata* can grow at wide range of temperature between 15-30ºC, however, best growth was observed at 26-28ºC. The lower and upper threshold temperatures observed were 5ºC and 40ºC, respectively at which no growth was observed (Table 3). Our results are supported by Hubbali *et al*. (2010) who reported that temperatures ranging from 25 - 30°C are better for the growth of *A. alternata* and there was very less growth at 5ºC. Martin and Fernandez (2006), Garibaldi *et al*. (2007) and Balai and Ahir (2013) recorded optimum growth of *A. alternata* at 27ºC.

The results on effect of RH levels indicated that, the pathogen required high relative humidity of > 90% for optimum growth and sporulation, whereas least growth was observed at 50% RH. No growth was observed at 35% RH (Table 4), indicating that drier climatic conditions are unfavourable for development of *A. alternata*. Our results are supported by the findings of earlier researchers. Pruski *et al*. (1993) reported that, RH of > 80% has caused frequent infections of *A. alternata* in mango fruits at harvest. Balai and Ahir (2013) also reported similar trend stating that, *A. alternata* growth was observed best at RH between 90-100% and least at RH 50%. Kumar *et al*. (2013) reported maximum incidence of *A. alternata* on ber (*Zizyphus mauritiana* Lamk) at 100 % RH and temperatures between 25-30ºC.

Table 1: Composition of growth media used for culturing of *Alternaria alternata*

Composition(g/ or ml)	Growth media Potato dextrose agar medium	Czapek's medium	Richard's medium	Sach's agar medium	Nutrient agar medium
Peeled potatoes	200	-	-	-	-
Dextrose	20	-		-	-
Sucrose	-	30	50	-	-
Sodium nitrate	-	2		-	-
Potassium phosphate	-	1	5	0.025	-
Potassium nitrate	-	-	10	-	-
Magnesium sulphate	-	0.5	2.5	0.25	-
Potassium chloride	-	0.5	-	-	-
Ferrous sulphate	-	0.01	-	-	-
Iron chloride	-	-	0.02	trace	-
Agar agar	15	15	15	20	20
Calcium nitrate	-	-	-	4	-
calcium carbonate	-	-	-	4	-
Beef extract	-	-	-	-	3
Peptone	-	-	-	-	5
Distilled water	1000	1000	1000	1000	1000
pH	7.0	7.0	7.0	7.0	7.0

Table 2: Epidemiology of *Alternaria alternata* on different growth media

S.no.	Growth media	Mean colony diameter (mm) *	Sporulation	Growth characters
1.	Capek's Agar Medium	85	+ + + +	Profuse growth Colonies greenish gray in colour and circular with entire margin Profuse growth of mycelium at center Prominent concentric rings showing abundant sporulation
2.	Richards Agar Medium	75	+ + +	Good growth and sporulationColonies yellowish gray in colour and circular with entire marginProfuse growth of mycelium at periphery
3.	Sach's Agar Medium	20	+	Poor growthColonies dark gray in colour, irregular with entire marginPoor sporulation
4.	Nutrient Agar Medium	61	+ + +	Good growth and sporulationColonies grayish white with circular surfaceMycelium with velvety growth
5.	Potato Dextrose Agar Medium	90	+ + + +	Excellent growth and abundant sporulationColonies grayish white, circular with entire marginProfuse white mycelial growth at periphery

* Mean of four replicates; Sporulation pattern: + Poor, + + Moderate, + + + Good, + + + + Excellent/ abundant

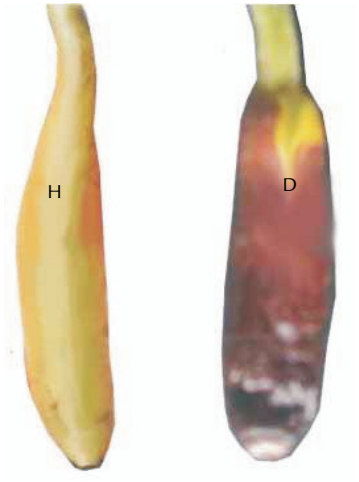

Figure 1: Samples of bottle gourd fruits collected from experimental field of Regional Fruits and Vegetable Research Station, Ganeshkhind, Pune. Healthy bottle gourd fruit (H) and Bottle gourd fruit infected with fruit rot disease (D)

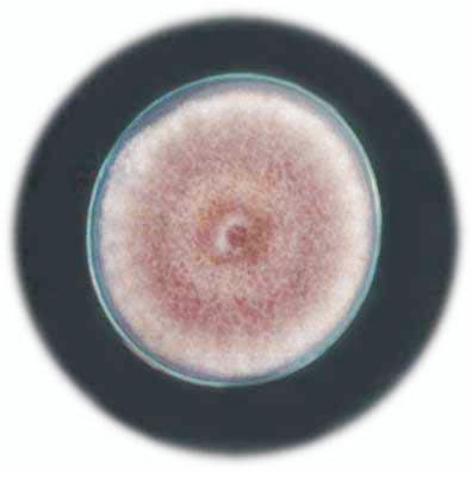

Figure 2: Culture of the fungus *Alternaria alternata* isolated from rotted fruits of bottle gourd on PDA medium

Table 3: Effect of temperatures on growth and sporulation of *Alternaria alternata*

Sr. No.	Temperature (°C)	Mean colony diameter (mm) *	Sporulation	Growth characters
1.	0	-	-	No growth and sporulation
2.	5	-	-	No growth and sporulation
3.	10	10	-	Poor growth and no sporulationMycelium white, thread like
4.	15	20	+	Poor growth and sporulationColonies light pink in colour, circular with serrated margin
5.	20	52	+ +	Moderate growth and sporulationColonies olive green in colour, circular with entire margin
6.	25	70	+ + +	Good growth and sporulationColonies pale green in colour, circular with entire margin
7.	26-28	85	+ + + +	Abundant growth and sporulationColonies dark green to black in colour, circular with entire margin
8.	30	70	+ + +	Good growth and sporulationColonies pale green in colour, circular with entire margin and raised at the center and are compact
9.	35	35	+ +	Moderate growth and sporulationColonies dark green coloured at center and light green towards periphery, circular with entire margin
10.	40	-	-	No growth and sporulation

* Mean of four replicates; Sporulation pattern: + Poor, + + Moderate, + + + Good, + + + + Excellent/ abundant

Table 4: Effect of RH on growth and sporulation of *Alternaria alternata*

S. No.	RH (%)	Growth	Sporulation
1.	35	-	-
2.	50	+	-
3.	65	+	-
4.	75	+ +	+
5.	90	+ + +	+ +
6.	100	+ + + +	+ + + +

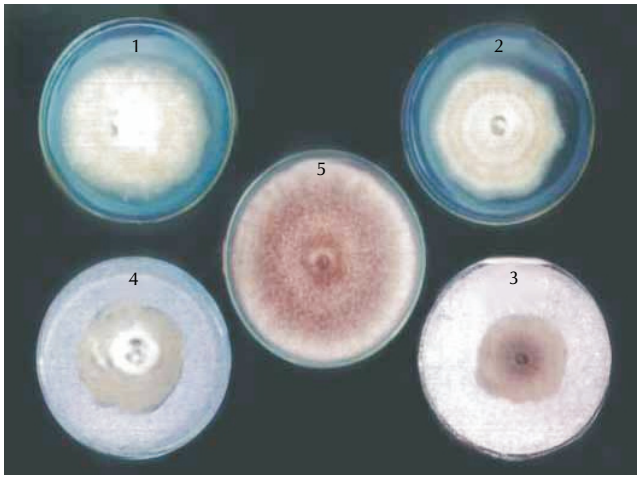

Figure 3: Effect of different media on growth and colony characters of *Alternaria alternata*. Czapek's Agar Medium (1), Richard's Agar Medium (2), Sach's Agar Medium (3), Nutrient Agar Medium (4) and Potato Dextrose Agar Medium (5)

Pathogenicity and symptomatology

Typical symptoms as water soaked areas were developed on artificially inoculated healthy fruits of bottle gourd. The infected portion turned brown coloured within 2-3 days post infection. The infection spread rapidly to cover the healthy portion under high relative humidity. The brown spots showed presence of white mycelial growth with conidia and conidiophores, when examined under microscope. Affected portion was later shrunk and mummified (Fig. 4). Under *in vivo* conditions, young tender fruits were rotten with formation of water soaked areas. Infected fruits were seen loosely hanging on the plants (Fig. 5). Expression of symptoms was rapid in *in vitro* compared to *in vivo* conditions which may be due to variable impact of environmental conditions affecting disease development. We have successfully re-isolated the pathogen from artificially inoculated fruits which further confirmed the identity of the pathogen as *A. alternata* causing fruit rot in bottle gourd. Similar kind of symptoms have been described due to *A. alternata* causing fruit rot of summer squash (Gangopadhyay and Kapoor, 1973; Hellan, 1985), tomato (Mehta and Saxena, 1976), and water melon (Narain *et al*., 1985). Prasad and Upadhyay (2010) described the symptoms of *A. alternata* f. sp. *lycopersici* causing leaf blight in tomato as brown to black sunken necrotic lesions with typical concentric rings on the leaf surface. Abeer *et al*. (2014) reported that the symptoms of *A. alternata* causing leaf spot disease in *Avicennia marina* (Forski) initially appear as bright to pale yellow spots on the upper leaf surface surrounded by water soaked areas. The

Figure 4: Bottle gourd fruits showing symptoms of fruit rot disease under *in vitro*

Table 5: Efficacy of different fungicides against *Alternaria alternata*

S. No.	Fungicide	Concentration (%)	Mean colony diameter (mm) *	Growth inhibition (%)	Sporulation
1.	Copper oxychloride	0.25	30.0c	66	-
2.	Mancozeb	0.25	0.00[a]	100	-
3.	Carbendazim	0.1	11.00[b]	87	-
4.	Chlorothalonil	0.2	40.6[e]	54.88	+
5.	Difenconazole	0.1	59.00[f]	34.44	+
6.	Propiconazole	0.1	37.00[d]	58.88	-
7.	Control(water spray)	-	90.00[g]	0.00	+ + + +
	SE (m)	0.76			
	CD (@ 0.05 %)	2.18			

Table 6: Efficacy of two antagonistic fungi against *Alternaria alternata*

S. No.	Bioagents	Mean colony diameter (mm)*		Growth inhibition (%)	
		7 DAT*	10 DAT	7 DAT	10 DAT
1.	*Trichoderma harzianum*	25.00	20.00	72.22	77.77
2.	*Trichoderma hamatum*	15.00	11.00	83.33	87.77
3.	Control	90.00	90.00	0.00	0.00

*Days after treatment

Figure 5: Rot infected fruits loosely hanging on the bottle gourd plant in field (under *in vivo*)

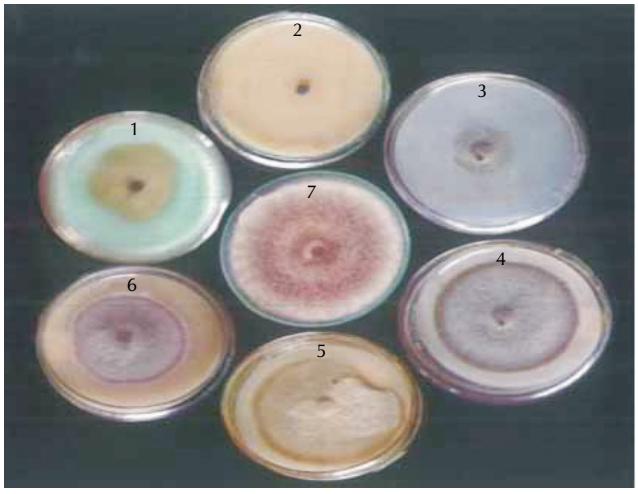

Figure 6: Efficacy of chemical fungicides against *Alternaria alternata*. Copper oxychloride (1), Mancozeb (2), Carbendazim (3), Chlorothalonil (4), Difenconazole (5), Propiconazole (6) and Control (7)

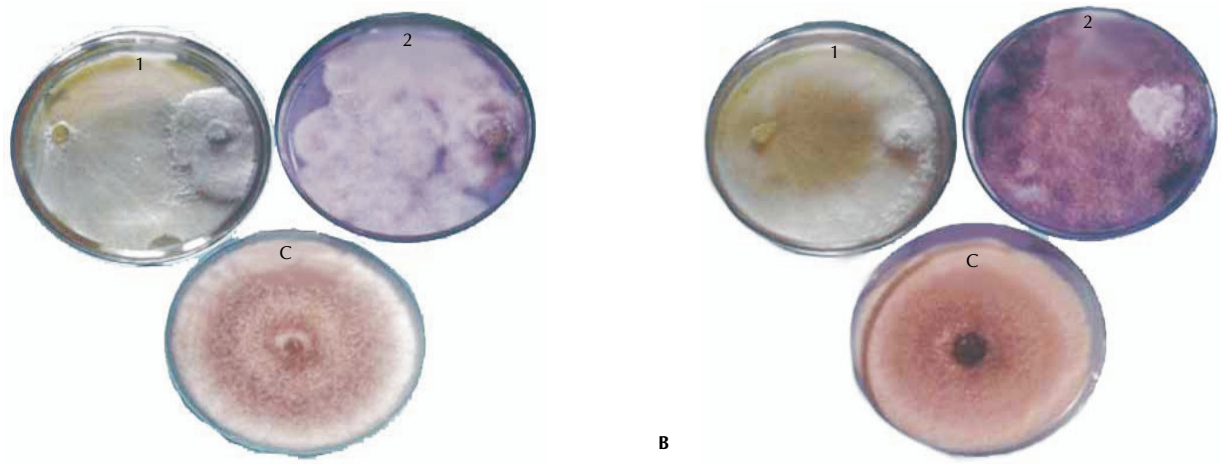

Figure 7: Biological control potential of antagonistic fungi against *Alternaria alternata* at seven days after inoculation (A) and ten days after inoculation (B). *Trichoderma harzianum* (1), *T. hamatum* (2) and Control (C).

older spots are somewhat circular to irregularly lobed and are light brown to black coloured.

Fungicidal bioassay

The fungicide mancozeb (0.25 %) was found most effective with 100% inhibition of growth and sporulation of *A. alternata*. It was followed by carbendazim (0.1 %), copper oxychloride (0.25 %), propiconazole (0.1 %) and chlorothalonil (0.2 %). The chemical difenconazole (0.1 %) was observed as least effective fungicide with 34.44 % inhibition of pathogen growth and development. All the fungicides tested were effective in suppressing the sporulation of *A. alternata* however; mancozeb (0.25 %) was the only fungicide wherein complete inhibition of fungal growth was observed (Fig. 6, Table 5). Mancozeb has been reported as promising fungicide for controlling *A. alternata* causing black spot in mango (Mohsan *et al*., 2011), chrysanthemum leaf blight (Kumar *et al.,* 2011) and tomato blight (Kumar *et al*., 2012). Sahu *et al*. (2013) reported that mancozeb reduced the incidence of *A. alternata* causing early blight in tomato by 40.39% and increased the yield by 40.66%.

Bioefficacy of antagonistic fungi

Both of the antagonistic fungi tested against *A. alternata* were effective in restricting the growth and sporulation of the pathogen. *T. hamatum* with 87.77 % growth inhibition was more promising compared to *T. harzianum* (77.77 %). There was progressive increase in growth inhibition from seven to 10 days after treatment (Fig. 7, Table 6). Our results are in line with earlier reports. Pandey (2010) reported 67.07 and 66.67 % growth inhibition by *T. harzianum* and *T. viride, respectively* of *A. alternata,* a destructive pathogen of *Capsicum frutescens*. Gveroska and Ziberoski (2011) reported a strong antagonistic effect of *T. harzianum* on *A. alternata*. *T. viride* has been reported as potential biocontrol agent against *A. alternata* causing tomato leaf blight (Kumar *et al*., 2012). Rajput *et al*. (2013) reported that, the fungal bioagents *viz*., *T. viride* and *T. harzianum* caused maximum inhibition of *A. alternata* causing leaf spot disease in brinjal under South Gujarat conditions.

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