IN-VITRO EVALUATION OF SOME PLANT EXTRACTS AND FUNGICIDES AGAINST *COLLETOTRICHUM CAPSICI* CAUSING ANTHRACNOSE OF CHILLI

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ABSTRACT

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Chilli (*Capsicum annuum*)**,** an important commercial spice crop with export potential, suffered from many diseases of which anthracnose caused by *Colletotrichum capsici* was one of the major diseases causing severe yield loss due to both pre- and post-harvest fruit decay. Seven aqueous plant extracts @ 5, 10 and 15% and seven chemical fungicides @ 100, 200, 300 ppm were evaluated under *in-vitro* condition at the plant pathology laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during 2013-2014to know their efficacy against anthracnose pathogen *Colletotrichum capsici* of chilli using poison food plate technique. Among the seven aqueous plant extracts, 15% aqueous Ginger (*Zingiber officinale*) was most effective against anthracnose under *in-vitro* condition followed by Turmeric (*Curcuma longa*) extract where mycelial growth inhibition was 72.66 % and 61.25 %. Among the seven fungicides, Conja 5 EC (Hexaconazol) @ 200 ppm, Tilt 250 EC (Propiconazol) @ 300 ppm and Provax 200 WP (Caboxin + Thiram @ 300 ppm were effective against the fungus under *in-vitro* conditions where the mycelial growth was inhibited completely. The plant extracts of Ginger and Turmeric @ 15 % or Conja (Hexaconazol) @ 200 ppm, Tilt and Provax 300 ppm might be used to control the anthracnose pathogen (*Colletotrichum capsici*) of Chilli.

Key word: Plant extracts, fungicides, *Colletotrichum capsici*, anthracnose, chilli

INTRODUCTION

Anthracnose caused by *Colletotrichum capsici* is one of the major diseases of chilli (*Capsicum annuum*) in tropical and subtropical regions of the world including Bangladesh. It incurs extensive pre- and post-harvest damage to fruits causing anthracnose lesions that reduces market value of chilli (Manandhar *et al.* 1995). It is mainly a problem on mature fruits causing severe losses due to both pre- and post-harvest fruit decay (Hadden and Black 1989, Bosland and Votava 2003). Chilli (*Capsicum capsici*), an important economic crop worldwide is severely infected by anthracnose which causes yield losses up to 50% (Pakdeevaraporn *et al*. 2005). *Colletotrichum* species reduces 10% to 80% yield of marketable fruits (Poonpolgul and Kumphai 2007).

Availability of commercial chilli cultivars resistant to diseases are still very limited, this may cause farmers to rely mostly on fungicides to control this disease. Chilli farmers usually apply different fungicides indiscriminately, resulting accumulation of harmful chemical residues in the soil, water and fruits which breakdown the ecological balance by killing the beneficial and/or antagonistic microorganisms (Rajathilagam and Kannabiran 2001). Hence, efforts need to keep the pathogen activity below economic threshold level and minimize environmental pollution by judicial use of more effective fungicides.

Plant extracts with toxic properties against the phytopathogen are now being explored. Antifungal compound has been tested from *Allium sativum* that inhibits fungi (Yoshida *et al*. 1987). Several plant species have been screened for antifungal activity (Grayer and Harborne 1994) and to control pre-harvest (Tewari 1995) and post-harvest diseases of several plant species (Mishra and Dubey 1994). The plant extracts exhibited a marked effect on germination of fungal spores as well (Islam *et al*. 2003) and inhibited the fungal growth (Khair *et al.* 1995).

Plant extracts deserve a special attention to develop a strategy for ecologically safe method of plant

disease management. Several higher plants and their constituents have shown success in plant disease control and proved to be harmless and non-phytotoxic unlike chemical fungicides (Singh *et al*. 1986, Singh and Dwivedi 1990, Dubey 1991, Bisht and Kamal 1994). Using extracts from plants containing natural antifungal compounds for plant disease control is considered to be one of the desirable methods for plant protection in agriculture (Kim *et al*. 2002).

Occurrence of anthracnose disease of chilli is very common and recently it increased manifold in Bangladesh. No effective control strategies with botanicals or chemicals are available in Bangladesh. Therefore, the present investigation was undertaken to find out an effective botanical and/or fungicide against anthracnose pathogen of chilli under *in-vitro* condition.

MATERIALS AND METHODS

In-vitro evaluation of aqueous plant extracts and fungicides

An *in-vitro* experiment was conducted for preliminary screening of seven aqueous plant extracts (@ 5, 10 and 15 %). Water extracts of leaves of Neem, Mahogany, Mehandi and Tulsi; rhizome extract of Turmeric and Ginger rhizomes; and clove extract of Garlic were evaluated *in-vitro* against *C. capsici* following poisoned food technique (Nene and Thapliyal 1993) on potato dextrose agar (PDA) medium. To prepare plant extract, healthy parts of selected plant species were washed with sterile distilled water and chopped into small pieces with sterilized sharp knife. Each sample was separately grounded and homogenized in mechanical grinder with equal quantity of sterile distilled water 1:1 (W/V). The homogenate obtained was strained through double layered cheese cloth and the filtrate collected was again filtered through Whatman No. l filter paper. The clear leaf extracts were considered as the stock solution of 100 percent concentration. An appropriate quantity of each leaf, bulb and rhizome extract was mixed separately with melted PDA medium in conical flask (250 ml capacity) to get desired concentrations (5, 10 and 15 %) of each extract and autoclaved.

Another *in-vitro* experiment was conducted to screen seven selected fungicides following poison food technique on PDA medium (Nene and Thapliyal 1993). The selected fungicides were Provax 200 WP (Carboxin + Thiram), Tilt 250 EC (Propiconazole), Conja 5 EC (Hexaconazol), Contaf 5 EC (Hexaconazol), Dithene M 45 (Mancozeb), Metataf 25 WP (Metalaxyl) and Ridomil gold 68 WP (Metalaxyl + Mancozeb). PDA medium was amended with individual fungicides @ 100, 200, 300 ppm. The PDA amended with plant extract or fungicides were poured separately into sterilized petridishes @ 20 ml per plate. After solidification, the plates were inoculated with 0.5 cm mycelium block of *C. capsici* cut from 3 days old culture of the pathogen. Plates containing un-amended PDA were also inoculated with the pathogen, which served as control. The colony diameter of the test fungus grown on PDA was recorded when the plates under control were fully covered with the mycelium of the test fungus. The inhibition of mycelium growth was measured based on growth of the fungus on control plate and also that on treatment plate following the formula of Vincent (1927) as percent inhibition (I) = $\frac{C-T}{C}$ x 100, where, C = growth of test fungus (mm) in control plate, T = growth of test fungus (mm) in treatment plates. The percent data were converted into arcsine transformation values. All data were analyzed statistically and the means were separated by least significance test (LSD) at p=0.05% level.

RESULTS AND DISCUSSION

In vitro evaluation of plant extracts

The radial mycelial growth of *Colletotrichum capsici* varied significantly at different concentrations of plant crude extracts (Table 1). Radial growth of mycelia was reduced with the increase of concentration of plant extracts. Significantly highest colony diameter of 88.00 mm was found in untreated control plate and the lowest colony diameter of 24.06 mm was recorded in plate treated with 15% extract of Zinger (*Zingiber officinale*). All the plant extracts treated plate showed reduced colony diameter as compared to control. In case of every extract, the highest colony diameter was observed at 5% concentration and lowest at 15% concentration (Table 1, Fig. 1).

The mycelial growth inhibition at different concentrations of plant extracts differed significantly over untreated control (Table 2 and Fig. 1). The mycelial growth inhibition was increased with the increase of concentration of plant extracts. None of the plant extracts caused complete inhibition of the mycelial growth but considerable amount of inhibition was noticed in all extracts. Among seven plant extracts tested Zinger extract showed the lowest colony diameter (24.06 mm) and the highest mycelial growth inhibition (72.66 %) at 15 % concentration which was followed by Turmeric, Mahogany, Mehendi, Garlic and Tulsi with growth inhibition of 61.45, 56.11, 51.00, 45.03 and 38.5 %, respectively at 15 % concentration. Neem extract was least effective and caused minimum growth inhibition (36.86 %) of the test pathogen at the highest concentration (15%) of extract.

Table 1. *In-vitro* evaluation of plant extracts on mycelial growth of *Colletotrichum capsici* at different concentrations after 10 days of incubation at 25 ± 2^0C

Plant extracts	Radial growth of mycelium (mm)		
	5 % extract	10 % extract	15 % extract
Neem (*Azardiachta indica*)	71.31 b	61.31 c	55.56 b
Garlic (*Allium sativum*)	63.63 c	58.62 d	48.38 c
Zinger (*Zingiber officinale*)	53.94 e	41.25 g	24.06 g
Termaric (*Curcuma longa*)	51.16 e	44.06 f	33.92 f
Tulsi (*Oscimum sanctum Linn.*)	68.83 b	63.88 b	54.15 b
Mahogoni (*Swietenia mahogoni)*	58.38 d	49.41 e	38.65 e
Mehendi	59.30 d	44.43 f	43.13 d
Control	88.00 a	88.00 a	88.00 a
CV	1.55 %	1.49 %	2.41 %

*****The Means followed by same letters in row and column are not significantly different at 1% level of significance.

All the plant extracts tested at 5%, 10% and 15% concentration were inhibitory to colony growth of the test pathogen and 15 % concentration was most effective in every cases. However, higher concentration (15 %) of tested plant extracts caused 36.86 to 72.66 % inhibition of mycelial growth compared to lower concentration (5 %). Botanicals *viz*., Neem, Garlic, Ginger, Turmeric, Tulsi, Mahogany, Mehendi, etc. were reported as toxic against several *Colletotrichum* species causing anthracnose, blights and leaf spots in many crops including chilli (Gomathi and Kannabiran 2000, Chandrasekaran and Rajappan 2002, Swamy and Kulkarni 2003, Gorawar *et al*. 2006, Alam *et al*. 2006 and Tasiwal *et al.* 2009). Jagtap *et al.* (2012) reported Garlic extract @ 15 % concentration as most effective for inhibiting mycelial growth of *C. truncatum* under *in-vitro* evaluation. But in the present investigation, Ginger rhizome extract at 15% concentration was found most effective against *C. capsici* in Petri plates which might be due to species difference of *Colletotrichum* and therefore, the results were in agreement with the findings of Mistry *et al*. (2008) and Jagtap *et al.* (2013).

Table 2. *In-vitro* effect of different plant extracts on mycelial growth inhibition of *Colletotrichum capsici* at different concentrations after 10 days of incubation

Plant extracts	Mycelial growth inhibition (%)		
	5 % extract	10 % extract	15 % extract
Neem (*Azardiachta indica*)	18.96 d (25.81)	30.33 e (33.41)	36.86 f (37.38)
Garlic (*Allium sativum*)	27.70 c (31.75)	33.38 d (35.29)	45.03 e (42.15)
Zinger (*Zingiber officinale*)	38.71 a (38.47)	53.13 a (46.79)	72.66 a (58.48)
Turmeric (*Curcuma longa*)	41.86 a (40.32)	49.93 b (44.96)	61.45 b (51.62)
Tulsi (*Oscimum sanctum Linn.*)	21.79 d (27.82)	27.42 f (31.57)	38.50 f (38.35)
Mahogany (*Swietenia mahogoni)*	33.67 b (35.46)	43.85 c (41.47)	56.11 c (48.51)
Mehendi (*Lawsonia innerucis*)	32.62 b (34.83)	49.52 b (44.72)	51.00 d (45.57)
Control	00	00	00
CV	2.21 %	1.45 %	1.53 %

*The Means followed by same letters in row and column are not significantly different at 1% level of significance. Figures within parentheses are arc sin transformed values

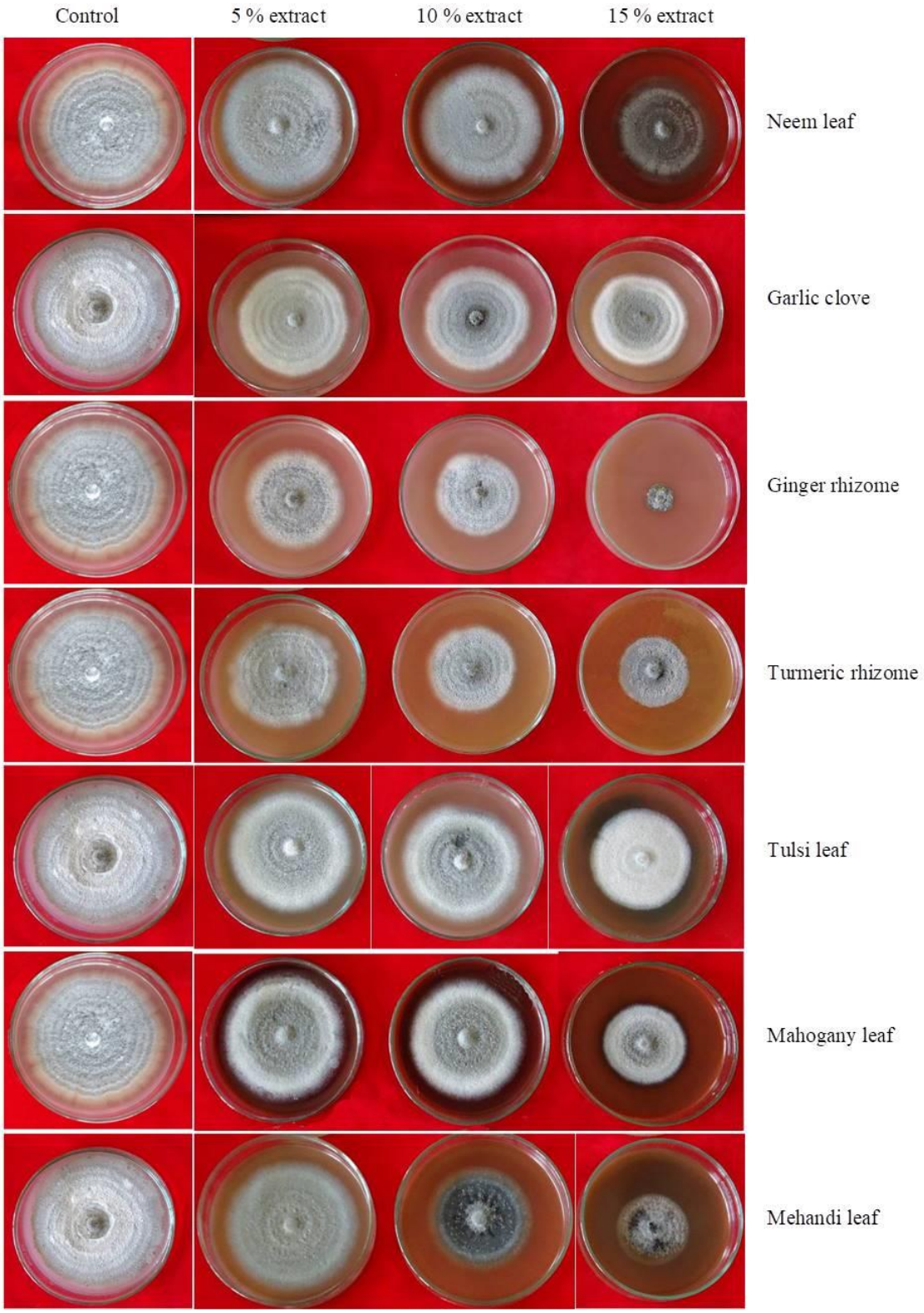

Figure 1. Effect of plant extracts at different concentration on radial growth of *Colletotrichum capsici*.

***In-vitro* evaluation of fungicides**

Radial mycelial growth of *Colletotrichum capsici* varied significantly at different concentrations of fungicides (Table 3). The increase of concentration of all fungicides caused sharp decline in the radial growth of mycelium (Fig. 2). Significantly the highest colony diameter of 88.50 mm was found in untreated control. No mycelial growth was found in the treatments of Conja at 200 ppm, Provax and Tilt at 300 ppm concentrations. Highest colony diameter was observed at 100 ppm concentration and lowest at 300 ppm in case of all fungicides (Table 3 & Fig. 2).

The mycelial growth inhibition of *C. capsici* at different concentrations of fungicides was remarkably different (Table 4 and Fig. 2). The mycelial growth was sharply increased with the increase of concentration of all fungicides. The complete inhibition (100 %) of mycelial growth was observed at 200 ppm of Conja (Hexaconazole) and 300 ppm of Provax (Carboxin + Thiram) and Tilt (Propiconazole).

Table 3. *In-vitro* evaluation of different fungicides on mycelial growth of *Colletotrichum capsici* at different concentrations after 10 days of incubation

Fungicides	Radial growth (mm)		
	100ppm	200ppm	300ppm
Provax 200 WP(Caboxin+ Thiram)	39.63 d	20.25 e	0.00 f
Tilt 250 EC (Propiconazol)	15.70 f	4.95 f	0.00 f
Conja 5 EC (Hexaconazol)	16.75 f	0.00 g	0.00 f
Contaf 5 EC (Hexaconazol)	36.38 e	29.75 d	10.05 e
Dithene M45(Mancozeb)	74.75 b	69.63 b	62.08 b
Ridomil gold (Metalaxyl + Mancozeb)	66.75 c	58.80 c	15.61 d
Metataf 25 WP (Metalaxyl)	64.81 c	57.75 c	44.83 c
Control	88.50 a	88.50 a	88.50a
CV	1.55 %	1.49 %	2.41 %

*****The Means followed by same letters in row and column are not significantly different at 1% level of significance.

Table 4. *In-vitro* effect of different fungicides on mycelial growth inhibition of *Colletotrichum capsici* at different concentrations after 10 days of incubation

Fungicides	Mycelial growth inhibition (%)		
	100ppm	200ppm	300ppm
Provax 200WP(Carboxin+ Thiram)	55.23 c (48.00)	77.12 c (61.43)	100.0 a (87.97)
Tilt 250 EC (Propiconazol)	82.26 a (65.10)	94.41 b (76.40)	100.0 a (87.97)
Conja 5 EC (Hexaconazol)	81.08 a (64.22)	100.0 a (87.97)	100.0 a (87.97)
Contaf 5 EC (Hexaconazol)	58.90 b (50.13)	66.39 d (54.57)	88.65 b (70.33)
Dithane M45(Mancozeb)	15.54 e (23.21)	21.33 f (27.50)	29.86 e (33.12)
Ridomil gold MZ 68 WP Metalaxyl + Mancozeb)	24.58 d (29.72)	33.40 e (35.30)	82.36 c (65.18)
Metataf 25 WP (Metalaxyl)	26.77 d (31.1)	34.86 e (36.19)	49.35 d (44.63)
CV	1.33 %	1.52 %	1.25 %

*****The Means followed by same letters in row and column are not significantly different at 1% level of significance. Figures within parentheses are arc sine transformed values.

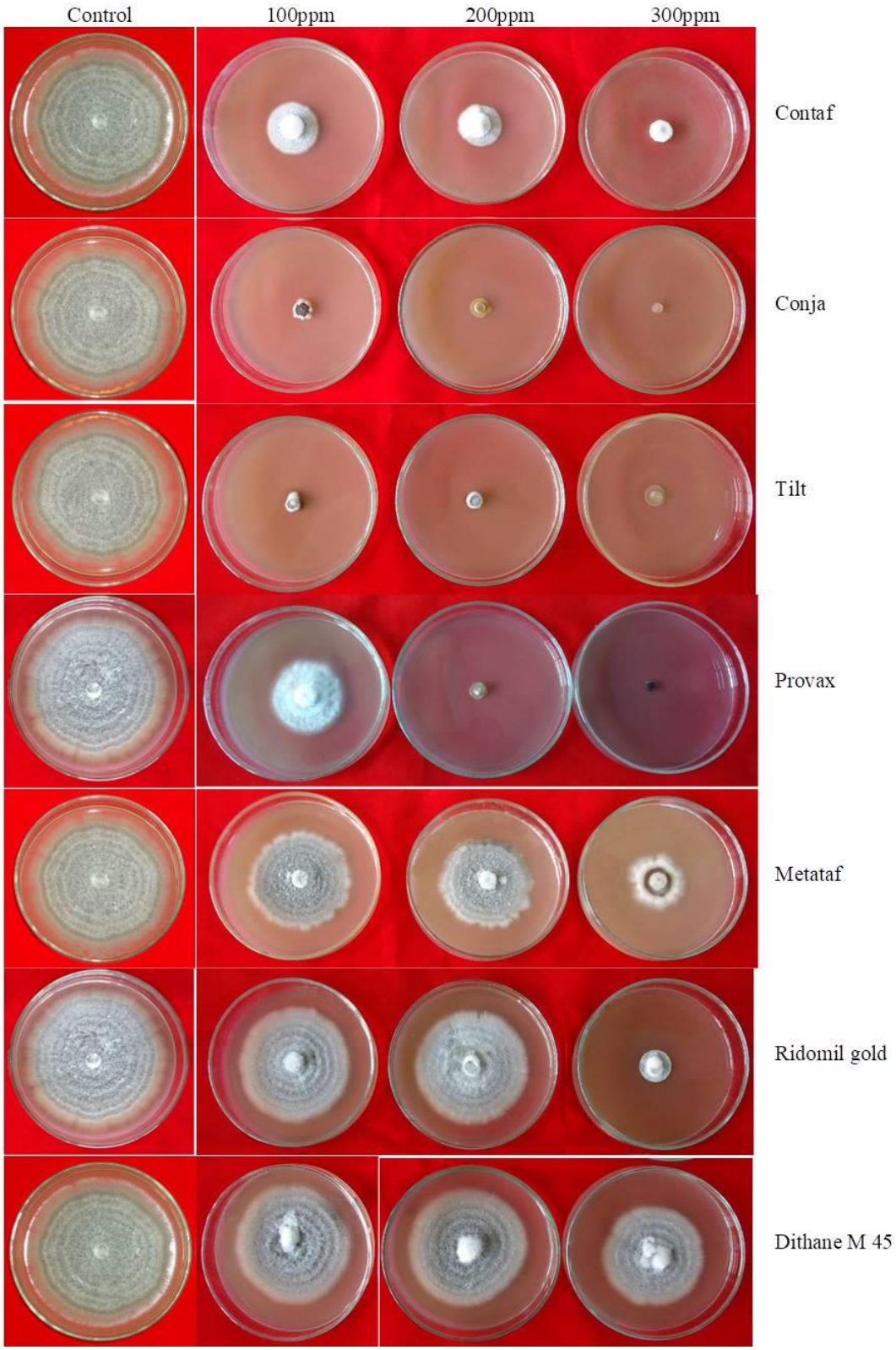

Figure 2. Effect of fungicides at different concentration on radial growth of *Colletotrichum capsici*

Among seven fungicides tested @ 100, 200 and 300 pm, Conja was the best in inhibiting mycelial growth where inhibition was 100 % at 200 ppm followed by the fungicides, Provax and Tilt which gave 100 % mycelial growth inhibition at 300 ppm. The fungicides like Contaf and Ridomil gold caused 88.65% and 82.36% reduction of mycelial growth of the test fungi at 300 ppm. Dithane M 45 and Metataf were comparatively less effective showing 29.86 and 49.35% mycelial growth inhibition at 300 ppm.

All the fungicides tested at various concentrations significantly inhibited the mycelial growth of *C. capsici*. However, Conja (Hexaconazole) was found most effective followed by Provax (Carboxin+ Thiram) and Tilt (Propiconazoe). Hexaconazole and Propiconazle were reported inhibitory to the fungi *C. gleosporioides*, *C. capsici*, *C. truncatum* and *C. lindemuthianum* (Swamy and Kulkarni 2003, Kumar *et al*. 2003 and Gorawar *et al*. 2006). Jagtap *et al.* (2012) reported that Propiconazole and Hexaconazole at 200 ppm inhibited 85.47 and 85.10% mycelial growth of *C. truncatum*. In the present study, the mycelial growth inhibition was 94.41 and 100% at the same concentration which might be due to the species difference of pathogen and also formulation of Hexaconazole products. Results of the present study are in conformity with the findings of Jagtap *et al.* (2013). Thus, Hexaconazole (Conja), Propiconazole (Tilt) were the most effective fungicides against C. *capsici* in *in-vitro*.

The present investigation reveals that some botanicals and fungicides were effective against *C. capsici* under *in-vitro* conditions. It was also observed that, the fungicides were more effective than plant extracts. However, Conja (Hexaconazol) and Tilt (Propiconazol)) can be effectively used to control *C. capsici* of chilli. Besides, Ginger, Turmeric and Mahogany extracts may be possible alternatives to synthetic fungicides to control *C. capsici* after availability of marketable formulation especially for organic production of chilli.

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