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In vitro evaluation of plant extracts against *Alternaria brassicae* (Berk.) Sacc. causing leaf spot of mustard and *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato

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Extracts of five plants viz. *Azadirachta indica, Lantana camara, Ocimum sanctum, Eucalyptus globulus* and *Calotropis gigantea* were evaluated *in vitro* by poison food technique (PFT) @ 3, 5, 7 and 9% concentrations against *Alternaria brassicae* and *Fusarium* oxysporum f. sp. *lycopersici* causing blight of mustard and wilt of tomato respectively. *In vitro* study on *A. brassicae* revealed that all five plant extracts at all four concentrations significantly inhibited the mycelial growth of this pathogen as compared to control. However *O. sanctum* was found most efficacious with growth inhibition of (31.85%) followed by *E. globulus* (28.97%) and *L. camara* (23.60%). While in *in vitro* study of *F. oxysporum* f. sp. *lycopersici A. indica* extract was found most efficacious with growth inhibition of (29.33%) followed by *E. globulus* extract (28.72%). Therefore more evaluations in the green house of field experiments may validate their antifungal activity.

Key words: *Alternaria brassicae*, plant extracts, poison food technique, *Fusarium oxysporum,* tomato.

INTRODUCTION

Indian mustard (*Brassica juncea* (Linn.) Czern. and Coss.) is an important oil seed crop, grown both in tropical and sub tropical regions of the world. It yields important edible oil, which cannot be easily replaced. The major constraints in growing mustard are diseases, aphid pest, frost injury, non-availability of high yielding varieties suitable for high input conditions and fluctuations in weather conditions (Kumar, 1999). Among all these, diseases, *Alternaria* blight (*Alternaria brassicicola* (Schw.)) Wiltshire plays a prominent role in reducing the yield of mustard. *Alternaria* is an important polyphagus fungus and occurs most frequently as a saprobe on dead and decaying organic material, on / in seed and is responsible for causing leaf spot diseases (Mehrotra and Aneja, 2003). *Alternaria* leaf blight of mustard is a serious disease in India (Chahal, 1986; Kolte, 1985). Besides, the quantitative losses in yield (Kolte et al., 1987), it also affects the quality of seed and its germination (Ansari et al., 1988). Leaf spot of mustered caused by *Alternaria brassicae* causes severe damage up to 35% (Kolte et al*.,* 1987).

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Table 1. *In vitro* growth inhibition(%)of *Alternaria brassicae* by plant extracts.

Treatment \ Concentration (%)	3	5	7	9	Overall Mean
Azadirachta indica	5.88(12.69)	20.58(11.73)	25.49(11.23)	37.25(10.30)	**22.30(11.48)**
Lantana camara	8.04(12.65)	18.58(11.89)	30.07(11.01)	37.73(10.38)	**23.60(11.48)**
Ocimum sanctum	17.69(12.45)	26.54(11.75)	36.28(10.93)	46.90(9.97)	**31.85(11.27)**
Eucalyptus globulus	10.61(12.98)	25.66(11.82)	36.28(10.93)	43.36(10.30)	**28.97(11.51)**
Calotropis gigantea	8.82(12.45)	19.60(11.68)	24.50(11.31)	32.50(10.70)	**21.35(11.53)**
Overall Mean	**10.20(12.64)**	**22.19(11.77)**	**30.52(11.08)**	**39.54(10.33)**	

CD (P = 0.05); Treatments: 0.12; Concentration: 0.12; Treatments × Concentration: 0.26; * Figures in parenthesis are arc sine transformed values.

Fusarium oxysporum is one of the common soil inhibiting plant pathogenic fungus causing wilt of pigeon pea, tomato, gram, guava and many other crops. Several other species of the fungus are responsible for causing huge loses to their respective hosts (Mehrotra and Aneja, 2003). Besides being the cause of wilt diseases, species of this fungus are also found associated with seedling blight and damping off (Agrios, 2005). A number of plant species have been reported to possess some natural substances which are toxic to many fungi causing plant diseases (Mishra and Dixit, 1977). Some of the toxic substances obtained from various plant species have been reported to manage a number of fungal diseases of crop plants (Chary et al., 1984).

Control of plant diseases by chemicals has some limitations such as development of resistant strains of pathogens, toxicity towards both plants and animals etc; moreover use of chemicals through drenching contaminates the running and sub soil water (Richardson, 1991). An estimate by World Health Organization (WHO) put the annual number of acute poisoning by pesticides at 3 million with 0.22 million deaths (Hoddy, 1993). Extracts of several plants, viz.,*Allium cepa* L., *Allium sativum* L., *Ocimum sanctum* L. and Mont., *Mentha piperata* L. and *Beta vulgaris* L. were found inhibitory to *Alternaria tenuis* (Shekhawat and Prasada, 1971). In view of increasing awareness about pesticide hazards, some commonly available plant extracts (leaves) were evaluated under laboratory conditions against *A. brassicae* and *F. oxysporum* f. sp. *lycopersici.*

MATERIALS AND METHODS

The plant extracts were evaluated *in vitro* against both selected pathogenic fungi through poisoned food technique (Carpenter, 1942; Nene and Thapliyal, 1993). The botanicals used were *Azadirachta indica* (Neem)*; Lantana camara* (Wild sage)*; Ocimum sanctum* (Tulsi)*; Eucalyptus globulus* (Eucalyptus) and *Calotropis gigantea* (Madar). Plant parts were washed with sterilized distilled water and air dried. Weighed plant materials were ground in pestle and mortar using 1:1 w/v. The materials were homogenized for 5 min, filtered through double layered muslin cloth followed by Whatman's filter paper No. 41 and filtrates were considered as standard extract (100%) (Kamlesh and Gurjar, 2002; Prasad and Barnwal, 1994). The standard leaf extract solution (100%) and Potato Dextrose Agar (PDA) medium was mixed at required quantities to get 3, 5, 7 and 9% concentrations. 3-mm culture discs of *A. brassicae* and *F. oxysporum* f. sp. *lycopersici* isolated from mustard and tomato leaves respectively were taken from seven days old colony and placed in the centre of the Petri dishes containing potato dextrose agar and appropriate percentages of plant extracts, without extract medium served as control. Three replications were maintained. The radial growth of the mycelium was measured after 10 days of incubation at 21 ± 1°C and per cent inhibition was calculated using the formula;

$$\text{Per cent inhibition} = \frac{\text{Growth in check} - \text{Growth in treatment}}{\text{Growth in check}} \times 100$$

The data was subjected to statistical analysis as per the methods described by Panse and Sukhatame (1985). The software used for analysis was Minitab.

RESULTS AND DISCUSSION

Extracts of all five botanicals significantly inhibited mycelial growth of *A. brassicae* at all the tested concentrations (Table 1). Data revealed that extract of O*. sanctum* demonstrated maximum mycelial growth inhibition of *A. brassicae* (31.85%) followed by *E.globulus* extract (28.97%) and were significantly superior to all other tested extracts. *A. indica* extract (22.30%) also inhibited its mycelial growth followed by *C. gigantea* extract (21.35%) as compared to control. However all the treatments at 9% concentration showed maximum inhibition compared to other tested concentrations.

The present study indicated that *O. sanctum* leaf extract reduced the growth of *A. brassicae* at (3, 5, 7 and 9%) concentrations as compared to control within 10 days after incubation. Daya and Ram (1997) also reported that leaf extracts of *O. sanctum* was found most effective against *A. brassicae* as compared to other tested extracts. Similar results on the efficacy of plant extracts against *Alternaria* sp. have been reported by Shivpuri et al. (1998), Fawzi et al. (2009), Taskeen et al. (2010), Baraka et al. (2011), Nashwa and Abo-Elyousr (2012), Al-Askar (2012), Mishra and Gupta (2012), and Ravikumar and Garampalli (2013). A number of plants have been reported to possess antifungal property

Table 2. *In vitro* growth inhibition (%)of *Fusarium oxysporum* f. sp. *lycopersici* by plant extracts.

Concentration (%) / Treatments	3	5	7	9	Overall Mean
Azadirachta indica	14.28 (12.65)	25.00 (11.85)	33.92 (11.09)	43.75 (10.19)	**29.33 (11.43)**
Lantana camara	10.71 (12.92)	22.14 (12.06)	30.17 (11.41)	41.07 (10.46)	**26.02 (11.71)**
Ocimum sanctum	11.11 (12.65)	24.07 (11.68)	32.40 (11.01)	40.74 (10.30)	**27.08 (11.41)**
Eucalyptus globulus	12.28 (12.92)	24.56 (11.96)	32.45 (11.31)	45.61 (10.14)	**28.72 (11.58)**
Calotropis gigantea	3.70 (13.18)	22.22 (11.82)	33.33 (10.93)	39.81 (10.38)	**24.76 (11.57)**
Overall Mean	**10.41 (12.86)**	**23.59 (11.86)**	**32.45 (11.15)**	**42.19 (10.29)**	

CD (P = 0.05); Treatments: 0.08; Concentration: 0.08; Treatments × Concentration: 0.18 ; * Figures in parenthesis are arc sine transformed values.

(Shekhawat and Prasada, 1971; Dixit and Tripathi, 1975). These include bulb extracts of garlic and and leaf extracts of datura and mentha (Shivpuri et al*.,* 1998; Shivpuri and Gupta, 2001; Chattopadhyay et al*.,* 2002; Singh et al., 2003). Several workers while studying the *in vitro* effect of different plant extracts on *Fusarium solani* and other *Fusarium* spp. have reported almost similar findings (Arya et al*.,* 1995; Lolpuri, 2002). The presence of essential oils in garlic (*A. sativum*) and tulsi (*O. sanctum*) fixed oils in datura (*D. alba*) and canabinol in cannabis (*Canabis sativa*) are also considered responsible for such inhibitions (Anonymous, 1972). Vanitha (2010) reported that EC formulation of winter green oil exhibited 100% inhibition of mycelial growth of *Alternaria chlamydospora.* Similar results on the efficacy of different plant extracts against *Alternaria alternata* have also been reported by Wu and Zheng (2007), Raghavendra et al*.* (2009), and Zaker and Mosallanejad (2010). In the present study, tested plant extracts showed antifungal activity against the test pathogens. The study emphasizes the need for evaluation of extracts of common flora for their antimicrobial activity. Our observations are in tune with Zaker (2013) who reported that among tested extracts, methanolic extracts of peppermint (15%) and eucalyptus (15%) were best in preventing the spore germination of *Alternaria sesame*. The plant extracts shall have to be tested extensively *in vivo* before formulating any general recommendation.

The *in vitro* study of *F. oxysporum* f. sp. *lycopersici* revealed that all five tested botanical extracts significantly inhibited its mycelial growth at all tested concentrations (Table 2). Irrespective of treatments, *A. indica* extract (29.33%) demonstrated maximum mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici* followed by *E. globulus* (28.72%). However *C. gigantea* extract (24.76%) was least effective in inhibiting its mycelial growth. But all the treatments at 9% concentration exhibited maximum mycelial growth inhibition as compared to other concentrations (3, 5 and 7% against the tested pathogen.

The present study indicated that *A. indica* leaf extract restricted the growth of *F oxysporum* f. sp. *lycopersici* at (3, 5, 7 and 9%) concentrations in comparison to control within 10 days after incubation. Similar observations were reported by Pal and Kumar (2013) and Pattnaik et al. (2013). Gupta et al. (1996) reported that extracts of *C. gigantea and A. indica* were most effective against *F. oxysporum* Schlecht inhibiting the mycelial growth by 78.5 and 73.2% respectively. Antifungal properties of *A. indica* and *A. sativum* extracts against *F. oxysporum* have also been reported by Thakur et al. (1995). The variation in inhibition among test extracts could be due to variation in the components of antifungal chemicals in different plant species (Gautam et al., 2003).

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