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Screening Some Medicinal Plant Extracts Against *Alternaria sesami*, the Causal Agent of Alternaria Leaf Spot of Sesame

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Abstract

Natural plant extracts / products have the potential as safe alternatives for chemical fungicides in plant disease management. Methanol and pure methanol:water (50:50 v/v) extracts of peppermint, lavandula, eucalyptus, datura and nettle were screened for their antifungal activity against *Alternaria sesami*, the causal agent of Alternaria leaf spot of sesame at 5, 10 and 15% concentrations in Shahrood Agricultural Research Center, Shahrood, Iran, during 2010. Poisoned food technique and spore germination assay were used to evaluate the antifungal potential of plant extracts against the pathogen. Fungicide mancozeb 80WP (0.2%) was used for better comparison. Results indicated that methanolic extracts of peppermint (15 and 10%), lavandula (15%) and eucalyptus (15%) were more effective than methanol: water extracts and completely inhibited the growth of the pathogen. Among tested extracts, methanolic extracts of peppermint (15%) and eucalyptus (15%) were best in preventing the spore germination of the pathogen (P =0.01).

Keywords: *Alternaria sesami,* Antifungal, Control, Plant extracts.

INTRODUCTION

Alternaria leaf spot of sesame caused by *Alternaria sesami* (Kawamura) Mohanty and Behera appears mainly on leaf blades as small, brown, round to irregular spots and is responsible for losses in grain yield of the crop. The disease is usually controlled by conventional fungicides applied as foliar spray. High costs of fungicides and the problems of environmental pollution have stimulated investigations on alternative strategies for the control of pests and pathogens (Lyon *et al.,* 1995).

Natural plant extracts/products have the potential as safe alternatives for chemical fungicides in plant disease management and apart from conventional fungicides and microbial biocontrol agents, these have been found effective against a wide range of plant pathogens (Amodioha, 2003; Bowers and Locke, 2004). Furthermore, plant product based biofungicides are non-phytotoxic and have poor environmental pollution (Singh, 1994). Studies on the mechanisms of disease control by plant extracts/products have revealed that the biologically active constituents present in them may have either direct antimicrobial activity (Amodioha, 2000; Ansari, 1995) or induce host plants defence response resulting in reduction of disease development (Schneider and Ullrich, 1994). During recent decades several researches have been conducted on plant extracts and oils to find out such alternatives and valuable results have been achieved (Singh, 1994; Ayoub and Niazi, 2001; Bowers and Locke, 2000; Suprapta and Kalimi, 2009). Khallil (2001) tested 20 plant extracts against *Alternaria solani* and *Saprolegnia parasitica* under laboratory condition and found that extract of clove (Eugenia aromatic) completely inhibited the spore germination of *A. solani* and exerted a highly significant depressive effect on the mycelial growth of both tested species. He also reported that extracts of garlic and onion bulbs, eucalyptus leaves and pepper fruits could exhibit remarkable inhibitory effects against them.

Feng and Zheng (2007) studied the antifungal activity of essential oils of thyme, sage, nutmeg, eucalyptus and cassia against *Alternaria alternata* at different concentrations (100-500 ppm) and reported that the cassia oil completely checked the growth of *A. alternata* at 300-500 ppm, thyme oil exhibited a lower degree of inhibition (62% at 500 ppm) and cassia oil also reduced the percentage of decayed tomatoes at 500 ppm. Singh (1994) in a study evaluated five plant extracts including garlic and eucalyptus extracts against artificially induced early blight of Indian mustard (*Alternaria brassicae*) at 10, 20 and 30% concentrations and found the garlic extract as best biocide in controlling the disease. Abo-El-Seoud *et al*. (2005) tested essential oils of fennel, peppermint, caraway, eucalyptus, geranium and lemongrass for their antimicrobial activities against *Fusarium oxysporum*, *Alternaria alternata*, *Penicillium italicum* and *Botrytis cinerea* and on the basis of their effectiveness, selected essential oils of fennel, peppermint and caraway as active ingredients for formulating biocides. Hassanein *et al*. (2008) tested leaf extracts of neem (*Azadirachta indica*) and chinaberry (*Melia azedarach*) extracted by ethanol, ethyl acetate and water against two tomato fungal pathogens at 5, 10, 15 and 20% concentrations and found that both ethanol and ethyl acetate extracts of neem leaves assayed at a concentration of 20%, completely suppressed the growth of *F. oxysporum* and inhibited *A. solani* by ratios between 52.44 and 62.77%. Shirzadian *et al*. (2009) evaluated extracts of 23 plants including 21 moss species and 2 leafy liverwort species obtained by ethanol, water and petroleum ether solvents against 7 pathogenic fungal pathogens including *Alternaria alternata* and found that ethanolic extracts of 6 moss species (*Philonotis marchica*, *Grimmia pulvinata, Plagiomnium rugicum*, *Haplocladium* sp. *Bryum pallens* and *Drepanocladus aduncus*) followed by two liverworts (*Pellia epiphylla* and *Dumortiera hirsute*) had more antifungal activity than aqueous extracts. Hadizadeh *et al*. (2009) working on antifungal effect of essential oils of nettle, thyme, eucalyptus and common yarrow (medicinal plants of Iran) against *A. alternata* of potato found that both the nettle and the thyme oils exhibited proper antifungal activity against this pathogen and its spore germination and germ tube elongation in potato dextrose broth was strongly reduced in the presence of 1500 ppm of the nettle oil.

The objective of this study was to find out effective medicinal plant extracts against *Al-*

ternaria sesami, the causal agent of Alternaria leaf spot of sesame for their further evaluations and formulations.

MATERIALS AND METHODS

Preparation of pathogenic isolate of the pathogen

Several sesame plants showing prominent leaf spots were collected from local fields. Some samples with disease symptoms were cultured on acidified potato dextrose agar (PDA) and incubated at 25-27° C. After proper growth, colonies were purified by single spore method and according to morphological characteristics their identification was done with the help of standard key (Simmones, 2007). Pathogenicity tests were done on potted sesame plants and according to standard assessment key, the isolate causing highest disease severity was used for further studies (James, 1971).

Preparation of plant extracts

Matured leaves or young flowering shoots of peppermint (*Mentha piperita*, family: Lamiaceae), eucalyptus (*Eucalyptus camaldulensis*, family: Myrtaceae), datura (*Datura stramonium*, family: Solanaceae), nettle (*Urtica dioica*, family: Urticaceae) and lavandula (*Lavandula officinalis*, family: Lamiaceae) were thoroughly washed in running water and kept in shade to dry. Dry materials were then grinded finely by a blender. Methanol (96% purity) was used as solvent but in two separate forms, pure methanol and pure methanol: water (50:50 v/v). Five hundred ml of each solvent was added to 50 g of dry mater of each plant and homogenized for 20 min in a homogenizer. Mixtures were centrifuged at 447 × g. for 10 min to obtain clear extracts. The methanol was completely removed from the clear solutions in a rotary evaporator. Final extracts were passed through 0.2 µ seitz® filters to remove any unwanted bacteria and were used as base extracts (100% purity) (Wilson *et al*., 1997).

Inhibitory tests on mycelial growth

For evaluating the effect of different plant extract concentrations on mycelial growth of *A. sesami*, poisoned food technique was used (Schmitz, 1930). Sufficient amounts of acidified PDA were poured into 100 ml erlenmeyers, autoclaved for 20 minutes and kept under sterilized hood to cool up to 50° C. Exact amounts of pure extracts were then added to erlenmeyers and shacked gently to prepare PDA containing 5, 10 and 15% of extracts. In the case of control, instead of extracts, sterilized distilled water was added. Petri plates were filled with PDA containing known percentage of extracts. Five mm plugs of 7 days old culture of *A. sesami* were kept in the center of plates. Mancozeb 0.2% was used in the same manner for better comparison. Plates were incubated at 25-27° C for 7 days and there after the smallest and largest diameters of mycelial growth of the pathogen of plates were measured and recorded. Three plates were kept for each treatment.

Inhibitory tests on spore germination

The spore germination assay (Suprapta and Kalimi, 2009) was used for evaluating the effect of different plant extract concentrations on spore germination of *A. sesami*. Exact extract concentrations (5, 10 and 15%) were prepared in sterilized distilled water. One drop of these preparations was transferred into each pit of pitted glasses and kept as such to dry. After that one drop of spore suspension of *A. sesami* (5×10^5 spores/ml) was transferred into these pits. Pitted glasses were kept inside desiccators at room temp. with above 80% relative humidity. After 48 hrs, germinated spores were counted under microscope with the help of a hemocytometer and recorded. Mancozeb 0.2% was used in the same manner for better comparison. Three pits were kept for each treatment and in the case of control only sterilized distilled water was used.

Statistical method

The split plot design was used in this experiment. Main factors were two forms of methanol as solvents [pure methanol and methanol: water (diluted)] and sub factors were as: peppermint 5, 10 and 15%, eucalyptus 5, 10 and 15%, lavandula 5, 10 and 15%, nettle 5, 10 and 15%, datura 5, 10 and 15%, mancozeb 80WP (0.2%) and control. Results were analyzed in MSTAT-C and means were compared by Duncan's multi range test.

RESULTS AND DISCUSSION

Selection of pathogenic isolate of *A. sesami*

Out of several Alternaria isolates collected from different sesame fields, five identified as A. sesami (Simmones, 2007) were used for Pathogenicity tests. According to standard assessment key (James, 1971) isolate sh-4 caused highest disease severity on potted plants and was used for plant extract tests.

Effect of solvents on antifungal constituents of plant extracts

Our findings showed that in overall inhibitory effect of methanolic extracts against A. sesami were more effective than their corresponding extracts derived by diluted methanol. In this connection significant differences were observed between pure methanol extracts (m) and those derived by methanol:water (mw) solvent in inhibiting the mycelial growth (Fig. 1) as well as spore germination (Fig. 2) of the pathogen (P= 0.01). These results are in agreement with findings of several researchers approving that plant extracts performed by pure solvents such as ethanol, methanol and ethyl acetate demonstrate more antifungal activity in comparison to those of aqueous extracts, for instance Shirzadian *et al*. (2009) compared some plant extracts derived by ethanol, petroleum ether and water for testing against some plant pathogenic fungi including *Alternaria alternata* and reported that ethanolic extracts had more antifungal activity than extracts performed by water against these plant pathogens. Hassanein *et al*. (2008) also reported that neem and chinaberry extracts derived by ethanol and ethyl acetate solvents were more effective against *Fusarium oxysporum* and *Alternaria solani* than their aqueous extracts.

Inhibitory effect of plant extracts on mycelial growth of *A. sesami*

Among extracts of five tested plants, methanolic extracts of peppermint, lavandula and eucalyptus extracts performed promising potential against *A. sesami* and totally inhibited the mycelial growth of the pathogen at 1% level of significance in comparison to other treatments including mancozeb (0.2%) (Table 1). Extract of these plants contain antibacterial and antifungal active ingredients which inhibit fungal plant pathogens. Their active ingredients are menthol, lavender and eucalyptol respectively. Reviewing of several articles approve the effectiveness of eucalyptus extract as natural antifungal agent. Our results can be compared with several findings. Abo-El-Seoud *et al*. (2005) reported that peppermint and eucalyptus essential oils were effective against several plant pathogens including *A. alternata* and could be incorporated for biocide formulations. In another study although Singh (1994) after evaluating some plant extracts against early blight of Indian mustard (*Alternaria brassica*) reported garlic extract as most effective in controlling the disease, but also mentioned eucalyptus extract as acceptable extract in controlling the disease. But Hadizadeh *et al*. (2009) reported that eucalyptus essential oil was not so effective in controlling *A. alternata* of potato which is against our results. In our study datura extract did not demonstrate any antifungal property while Ayoub and Niazi (2001) found it effective in controlling wheat rust (*Puccinia recondita*) under controlled condition.

Inhibitory effect of plants extracts on spore germination of *A. sesami*

Significant differences could be noticed between various treatments in inhibiting the spore

germination of *A. sesami* specially between pure methanolic extracts and extracts derived by diluted methanol (Table 1 and Fig. 2). Although, in this experiment Mancozeb 0.2% with 4.66% spore germination showed best inhibitory effect but had no significant difference with methanol extracts of peppermint (15%) and eucalyptus (15%) and these two treatments with 11.00 and 11.67% spore germination were placed in the same group with mancozeb ($P \leq 0.01$). Findings of Khallil (2001) are in agreement with our results because he tested several plant extracts in preventing the spore germination of *Alternaria solani* the causal agent of early blight of potato and reported that eucalyptus extract was highly effective in this respect. But Hadizadeh *et al*. (2009) reported that nettle and thyme oils were more effective than eucalyptus, rute and common yarrow essential oils against spore germination of *A. alternata* while in our experiments nettle extract was not effective in preventing spore germination of *A. sesami* of sesame.

CONCLUSION

On the basis of obtained results methanolic extracts of peppermint exhibited superiority in comparison to other treatments against *A. sesami* in both tests but lavandula and eucalyptus also exhibited acceptable antifungal activity against this pathogen, therefore it may be stated that peppermint extract can be incorporated for biofungicide formulations as less hazardous natural plant product in controlling this disease, thus reducing the dependence on the synthetic fungicides

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Tables

Table 1. Effect of different treatments on *A. sesame*.

	Mean mycelial growth (cm)*			Mean spore germination (%)*		
Treatment	**5%**	**10%**	**15%**	**5%**	**10%**	**15%**
Mancozeb (0.2%)		0.76mn			4.66m	
Peppermint(m)	1.50klmn	0.00n	0.00n	24.00ijk	17.00kl	11.00lm
Lavandula(m)	3.13hijk	0.96lmn	0.00n	34.00h	20.67jkl	15.00kl
Eucalyptus(m)	2.50jkl	0.66mn	0.00n	29.00hij	15.33kl	11.67lm
Nettle(m)	4.96defg	3.00ijk	1.16lmn	64.67f	65.00f	36.33h
Datura(m)	6.56bcd	3.50ghij	2.16jklm	76.67de	53.33g	31.33hi
Pepermint(mw)	8.43a	6.16bcde	3.73fghij	84.67abcd	76.00de	74.00e
Lavandula(mw)	5.16defg	4.50efghi	4.836defgh	87.67abc	84.67abcd	81.67bcde
Eucalyptus(mw)	5.3def	5.16defg	4.43efghi	92.33a	82.33abcd	82.33abcd
Neetle(mw)	7.73ab	7.23abc	6.50bcd	88.00ab	77.33de	77.67cde
Datura(mw)	7.40abc	5.33def	5.73cde	89.00ab	82.67abcd	80.00bcde
Control		**8.00a**			**90.33ab**	

*According to Duncan's multiple rang test, values of each column followed by same letters are not significantly different (P= 0.01).

Figures

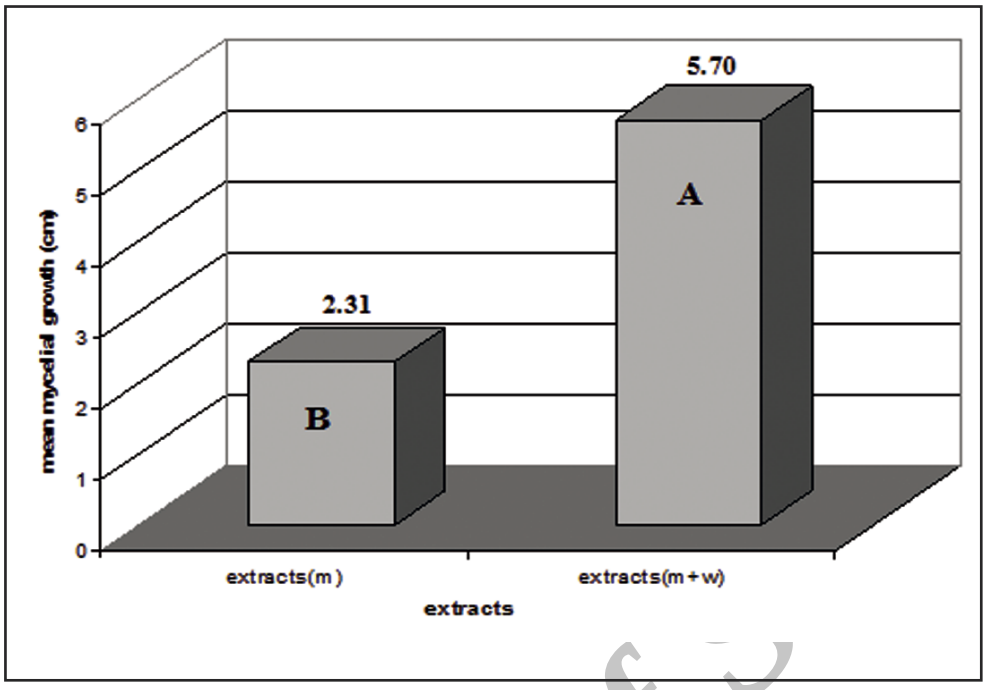

Fig.1. Effect of two solvents on antifungal constituents of extracts regarding the mycelial growth of *A. sesame*.

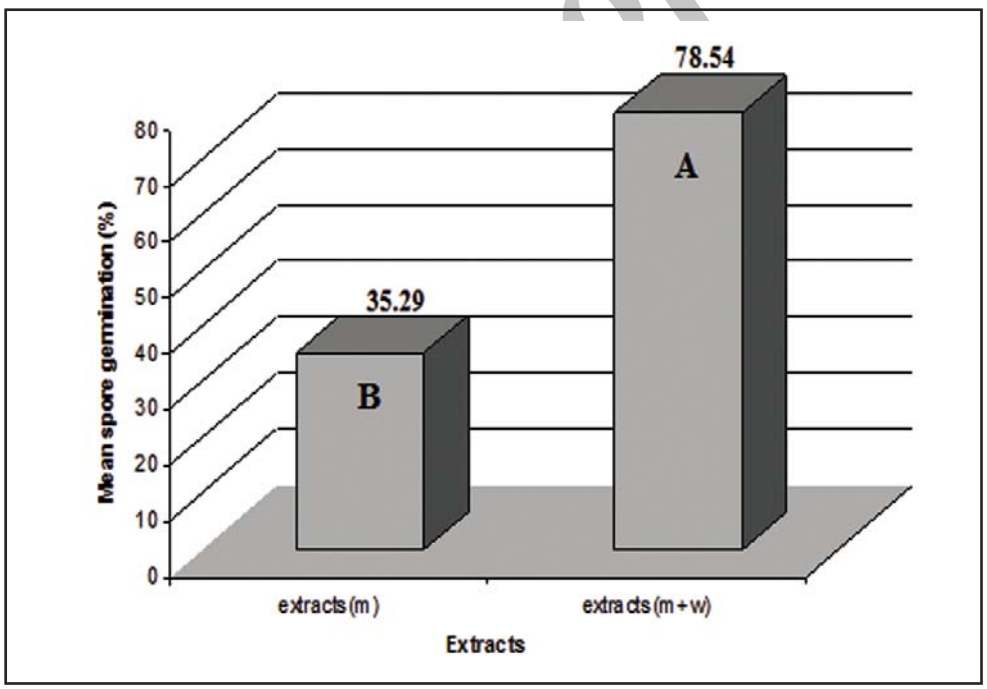

Fig.2. Effect of two solvents on antifungal constituents of extracts regarding the spore germination of *A. sesame*.