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RESEARCH ARTICLE

Antifungal Evaluation of Some Plant Extracts in Controlling *Fusarium solani*, the Causal Agent of Potato Dry Rot *In vitro* and *In vivo*

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ABSTRACT

Five, ten and fifteen percent methanolic extracts (ME) and aqueous extracts (AE) of six plants namely, Lavender, Eucalyptus, Artemisia, Thyme, Savory and Datura were evaluated for their antifungal effect against *Fusarium solani*, the causal agent of potato dry rot under lab. condition and also for their efficacy in reducing dry rot development in potato tubers during 2013. Methanolic extracts of all tested plants exhibited better antifungal activity compared to their corresponding aqueous extracts against *F. solani in vitro & in vivo*. Artemisia (ME), 15, 10 and 5% followed by Eucalyptus (ME), 15, 10 and 5% performed best antifungal activity in inhibiting the mycelial growth of *F. solani* but in respect to spore germination inhibition, 15% methanolic extracts of Artemisia, Thyme and Eucalyptus exhibited best effect compared to control respectively (P=0.01). Methanolic extract of Artemisia (15%) followed by methanolic extract of Eucalyptus (15%) performed best activity for reducing dry rot development in potato tubers (P=0.05).

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INTRODUCTION

Dry rot is one of the most important post-harvest diseases of potato tubers, causing significant economic losses worldwide (Stevenson *et al.,* 2001).The disease is caused by different species of *Fusarium*, including *Fusarium solani* (Mart.) Sacc. (Lib. ex Sacc.) C. Booth. Control of dry rot has been achieved primarily by post-harvest applications of thiabendazole (Secor and Gudmestad, 1999). However, strains of some *Fusarium* spp. have become resistant to thiabendazole (Desjardins *et al.,* 1993; Holley and Kawchuk, 1996; Platt, 1997), thus resulting in increased incidence and severity of dry rot. Although cultural practices such as crop rotation, use of disease free seed, wound healing of stored potatoes and minimizing wounds during harvesting and handling can help to reduce dry rot (Secor and Gudmestad, 1999), alternative control strategies are needed. The use of synthetic pesticides has shown major drawbacks such as their lack of long-term efficacy due to the development of resistance by plant pathogens. In the present context of safe and sustainable disease control, great effort toward the use of alternatives for plant disease control has been made (Avis, 2007). High costs of chemical fungicides and the problems of environmental pollution are other reasons that have stimulated investigations on alternative strategies for the control of pests and pathogens (Lyon *et al.,* 1995). Natural plant extracts/products have been found effective against a wide range of plant pathogens (Amodioha, 2003; Feng and Zheng, 2007; Wilson *et al.,* 1997; Zaker and Mosalanejad, 2010). Studies on the mechanisms of disease control by plant extracts/products have revealed that their biologically active constituents 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). Badar *et al*. (2012) reported that several medicinal plant gums had antifungal activity against phytopathogenic fungi including *Fusarium equiseti and F. oxysporum.* In another study, extract of different plants such as *Azardiachta indica*, *Artemesia annua*, *Eucalyptus globulus*, *Ocimum sanctum* and *Rheum emodi* were tested for controlling the brinjal wilt pathogen *Fusarium solani* f. sp. *melongenae* under lab. condition, *Azardiachta indica* extract (20%) was most effective against this pathogen followed by *Rheum emodi, Eucalyptus globulus, Artemesia annua* and *Ocimum sanctum* (Joseph *et al.,* 2008). Hur *et al.* (2000) screened methanol extracts of three cold-tolerant eucalyptus species (*Eucalyptus darlympleana, E. gunnii* and *E. unigera*) for their antimicrobial activity against twenty two phytopathogenic fungi and reported that *E. unigera* extract had the antagonistic activity against all the tested pathogens and among the tested fungal pathogens,

Pythium species were highly sensitive to the leaf extracts, specially *P. vanterpoolii*, a causal agent of leaf blight in creeping bentgrass (*Agrostis palustris*) was completely inhibited by the extracts. The eucalyptus extracts were also effective in inhibiting the fungal growth of *Botrytis cinerea* and *Phomopsis* sp. isolated from the lesions of kiwifruit soft rot during post-harvest storage. In Iran, Farzaneh *et al*. (2006) tested Artemisia oil against four soil born plant pathogenic fungi and reported that the oil was slightly effective against *Tiarosporella phaseolina, Fusarium moniliforme* and *F. solani* whereas against *Rhizoctonia solani* exhibitted high antifungal activity. 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 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% respectively. Shirzadian *et al*. (2009) evaluated extracts of 23 plants obtained by ethanol, water and petroleum ether solvents against 7 pathogenic fungal pathogens 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 their aqueous extracts.

The objectives of this study were (1) to evaluate the effect of different plant extracts on the in vitro development of *Fusarium solani* and (2) to evaluate the efficacy of the plant extracts for reducing dry rot severity caused by the pathogen in potato tubers.

MATERIALS AND METHODS

Fungal isolate

Potato tubers showing dry rot symptoms were collected from different stores. Small pieces of diseased specimens were grown on Fusarium selective medium (Nash and Snyder, 1965). After purifications, isolates were identified according to the morphological characteristics with the help of standard key (Nelson *et al.,* 1983). Pathogenicity tests were conducted on healthy potato tubers (var. Agria) and a pathogenic isolate of *F. solani* was selected for further studies. The fungus was maintained on Potato Dextrose Agar (PDA) slants. The agar slants were stored at 4°C and served as stock cultures.

Preparation of plant extracts

Solvent extraction

Matured leaves or young flowering shoots of lavender (*Lavandula officinalis*, family: Lamiaceae), eucalyptus (*Eucalyptus camaldulensis*, family: Myrtaceae), sweet wormwood (*Artemisia annua*, family: Asteraceae), thyme (*Tymus vulgaris*, family: Lamiaceae), Savory (*Satureja mutica*, family: Labiatae) and datura (*Datura stramonium*, family: Solanaceae) were thoroughly washed in running water and kept in shade to dry. Dry materials were then ground finely by a blender to prepare plant powders. Five hundred ml of pure methanol (96%) was added to 50 g of dry mater of each plant and homogenized for 20 min in a homogenizer. Fifty ml sterilized disstilled water was added and mixture was centrifuged at 447 × g. for 10 min to obtain clear extract. The methanol was completely removed from the clear solution in a rotary evaporator. Final supernatant was passed through 0.2 µ seitz filters to remove any unwanted bacteria and final extracts were used as pure extracts (Wilson *et al.,* 1997).

Aqueous extraction

Fifty g leaf powder of above mentioned plants were dissolved in 50 ml of sterilized distilled and homogenized for 20 min in a homogenizer. Mixture was centrifuged at 447 × g. for 10 min to obtain clear extract. The supernatant was passed through 0.2 µ seitz filters to remove any unwanted bacteria and reached to the original volume equal to methanolic extracts by adding sterilized distilled water.

Effect of plant extracts on mycelial growth of *F. solani*

The poisoned food technique was used for evaluating the effect of different plant extract concentrations on mycelial growth of *F. solani* (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. Petri plates were filled with PDA containing known percentage of extracts. Five mm plugs of 7 days old culture of *F. solani* were kept in the center of plates. Plates were incubated at 25-27°C for 7 days and there after the smallest and largest diameters of mycelial growth of the pathogen in plates were measured and recorded. Three plates were kept for each treatment.

Effect of plant extracts on spore germination of *F. solani*

The spore germination assay was used for evaluating the effect of different extract concentrations on spore germination of *F. solani* (Suprapta and Kalimi, 2009). Exact extract concentrations (5, 10 and 15%) were prepared in sterilized distilled water. Twenty five µL of these preparations was transferred into each pit of pitted glasses and kept as such to dry. One drop of spore suspension of *F. solani* (5×10^5 spores/ml) was then transferred into these pits. Pitted glasses were kept inside desiccators at room temperature with above 80% relative humidity. After 48 hrs, germinated spores were counted under microscope with the help of a hemocytometer and recorded. Three pits were kept for each treatment.

Effect of plant extracts on dry rot development of potato tubers

Healthy potatoes tubers of the cultivar Agria were stored in the dark at 4°C until use. Selected tubers were thoroughly washed in running water. After drying and surface sterilization of tubers with ethyle alcohol (96%), four wounds (4 mm deep) were performed on each tuber using a cork borer. Twenty five µl of a suspension of *F. solani* conidia (5×10^5conidia/ ml) prepared from a 7 days old culture grown on PDA were injected into each wound. Tubers were incubated in the dark at 24°C for 24 h. They

were then dipped (10 min) in the different extract solutions (5, 10 and 15%) and incubated individually in the dark at 24°C in plastic chambers containing a humidified towel. Disease severity was evaluated after 7 days of incubation. Dry rot severity was assessed by lesion area (cm2) as described by Satyaprasad *et al.* (1997). Lesion area is a mean of four inoculation sites per tuber.

Statistical method

The factorial design, based on completely randomized design was used for analyzing the data of this study. Results were analyzed in MSTAT-C and means were compared by Duncan's multi range test.

RESULTS AND DISCUSSION

Effect of extraction method on antifungal activity of plant extracts against *F. solani*

An interesting alternative to fungicide application involves the use of plant extracts/products that can widely be used as antimicrobial agents in plant disease control. Plant extracts/products can even be used in food preservations. These compounds have shown broad-spectrum antimicrobial activity with low mammalian toxicity, possess biocompatibility and are generally recognized as safe (GRAS) (Bhat *et al.,* 2012; Negi, 2012). 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).

The methanolic extracts of all tested plants showed higher antifungal activity against *F. solani* in comparison to their aqueous extracts and in overall significant differences were observed between these two groups of extracts in inhibiting the mycelial growth (Fig. 1), spore germination (Fig. 2) and dry rot development of the pathogen in potato tubers (Fig 3). Ignoring the percentages of plant extracts, Artemisia (ME) and Eucalyptus (ME) showed highest mycelial growth inhibition, Artemisia (ME) followed by Eucalyptus (ME), Thyme (ME) and Savory (ME) exhibited best inhibitting effects on spore germination of the fungus and finally Artemisia (ME) and, Eucalyptus (ME) showed best inhibitory effect in reducing dry rot development of potato tubers compared to control. Datura and Lavender extracts found to be less effective in all three tests, even their methanolic extracts (Fig 1, 2 and 3).

Several reports are in favour of our findings specially of those who had used different extraction methods. This might be due to the fact that some active compounds are less soluble in water and are not separated in the final aqueous extracts, whereas, such compounds are soluble in chemical solvents and are separated in the supernatant. For instance, Damián-Badillo *et al.,* (2008) reported that the ethyl acetate and methanol-chloroform extracts of 4 medicinal plants including *Artemisia ludoviciana* were effective against *Colletotrichum lindemuthianum, Candida albicans*, *Mucor circinelloides*, *Saccharomyces cerevisiae*, and *Sporothrix schenckii* while their aqueous extracts showed proper growth inhibitory effects only against *C. albicans*, and a weak or no effect against the

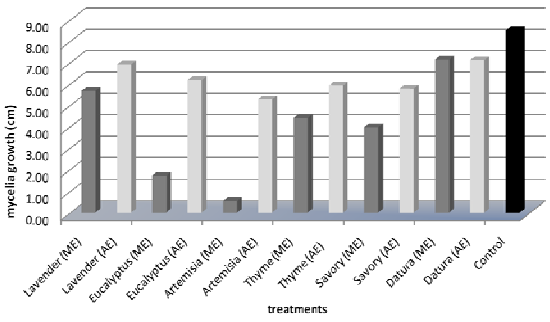

Fig. 1: Effect of different treatments on mycelial growth of *F. solani*

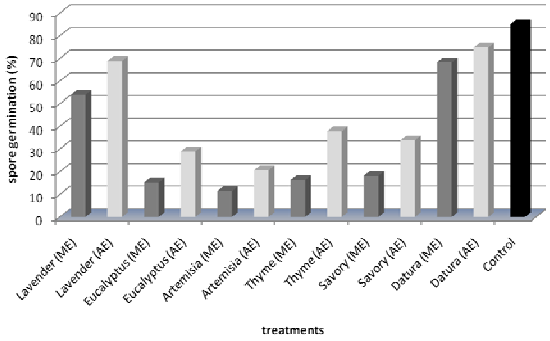

Fig. 2: Effect of different treatments on spore germination of *F. solani*

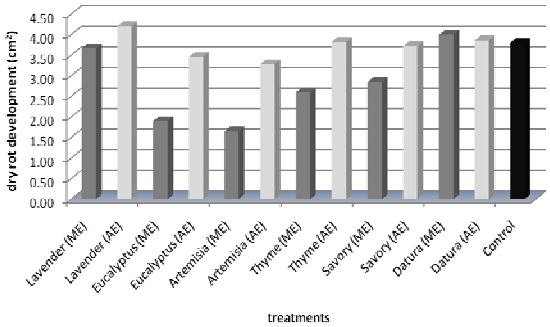

Fig. 3: Effect of different treatments on dry rot development in potato tubers

rest of the fungi tested. Hassanein *et al.* (2008) also reported that neem and chinaberry extracts derived by ethanol and ethyl acetate were more effective against *Fusarium oxysporum* and *Alternaria solani* than their aqueous extracts. In another study Shirzadian *et al*. (2009) reported that plant extracts derived by ethanol had more antifungal activity against some plant pathogenic fungi including *Fusarium solani* and *F. oxysporum* than same extracts performed by water. Although some researchers who only used the aqueous extracts in their studies reported antifungal effectiveness of this form of extracts against some fungi (Bhardwaj, 2012; Yasmin *et al*., 2008) but much documents are available in favour of present findings from researchers who compared antifungal properties of chemically derived and aqueous extracts (Alizadeh Behbahani *et al.,* 2013; Ambikapathy *et al*., 2011; Ashraf *et al*., 2011; Jat and Agalave, 2013; Moorthy *et al.,* 2013).

Table 1: Effect of different plant extracts on *F. solani* under *in vitro* condition

Treatment	Mycelial growth (cm.)			Spore germination (%)		
	5%	10%	15%	5%	10%	15%
Lavender (ME)	6.30 efgh	5.70 ghij	5.00 jkl	58.33 e	55.67e	48.00 f
Eucalyptus (ME)	2.40 o	1.53 p	1.16 pq	20.00 jkl	15.33 lmn	10.67 no
Artemisia (ME)	0.86 pqr	0.53 qr	0.23 r	14.67 mn	10.67 no	9.00 o
Thyme (ME)	5.10 jkl	4.50 lm	3.60 n	25.00 i	14.67 mn	9.66 o
Savory (ME)	4.63 kl	3.80 mn	3.46 n	24.00 ij	18.33 klm	12.33 no
Datura (ME)	7.50 b	7.20 bcd	6.70 cdef	71.00 c	68.67 cd	65.33 d
Lavender (AE)	7.40 bc	6.93 bcde	6.36 efgh	81.33 a	67.33 cd	58.00 e
Eucalyptus (AE)	5.40 ijk	6.67 cdef	6.43 defg	34.67 h	27.33 i	25.00 i
Artemisia (AE)	6.08 fghi	5.06 jkl	4.66 kl	24.33 ij	20.00 jkl	17.67 lm
Thyme (AE)	6.70 cdef	6.00 fghi	5.06 jkl	48.00 f	43.00 g	22.67 ijk
Savory (AE)	6.53 def	5.60 hij	5.23 jkl	43.00 g	36.00 h	22.67 ijk
Datura (AE)	7.70 b	7.06 bcde	6.56 def	81.67 a	75.67 b	67.33cd
Control	8.50 a	8.50 a	8.50 a	85.00 a	85.00 a	85.00 a
LSD		0.7006			4.351	

Values in a column followed by the same letter are not significantly different ($P \leq 0.01$) according to Duncan's multiple rang test.

Table 2: Effect of different plant extracts on dry rot development under *in vivo* condition

Treatment	rot development in potato tuber (cm^2)		
	5%	10%	15%
Lavender (ME)	3.86 cdefg	3.66 cdefghi	3.40 hijkl
Eucalyptus (ME)	2.16 pq	2.00 qr	1.53 st
Artemisia (ME)	1.90 qrs	1.76 rs	1.30 t
Thyme (ME)	2.90 mno	2.66 o	2.23 pq
Savory (ME)	3.16 jklmn	2.86 no	2.53 op
Datura (ME)	4.30 ab	4.00 abcd	3.70 cdefghi
Lavender (AE)	4.30 ab	4.40 a	3.93 bcdef
Eucalyptus (AE)	3.76 cdefgh	3.50 fghijkl	3.13 klmn
Artemisia (AE)	3.46 ghijkl	3.30 ijklm	3.10 lmn
Thyme (AE)	4.33 ab	3.66 cdefghi	3.46 ghijkl
Savory (AE)	3.96 bcde	3.56 defghij	3.63 defghi
Datura (AE)	4.10 abc	3.96 bcde	3.53 efghijk
Control	3.80 cdefgh	3.80 cdefgh	3.80 cdefgh
LSD		0.3665	

Values in a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple rang test.

Effect of plant extracts on mycelial growth and spore germination of *F. solani*

Among different plant extracts, methanolic extract of Artemisia (15%) demonstrated highest antifungal activity against mycelial growth of *F. solani* followed by its 10 and 5% in comparison to control after 7 days of incubation at 25-27°C (p= 0.01). Methanolic extracts of eucalyptus (15, 10 and 5%) were the next to also show acceptable activity in controlling the pathogen's mycelial growth but 5% methanolic extracts of Datura, Lavender, Thyme and Savory showed lowest antimycelial activity. The aqueous extracts of all tested plant extracts showed few or no effect in controlling pathogen's mycelial growth (Table 1). The spore germination of *F. solani* was reduced by 15% methanolic extracts of Artemisia, Thyme and Eucalyptus with 9.00, 9.66 and 10.67% of spore germination compared to control (85.00%) and was placed in same statistical group but Thyme (ME) was not as effective as two others in respect of mycelial inhibitory effect on *F. solani*. Fifteen percent aqueous extracts of Artemisia, Thyme and Eucalyptus respectively with 17.67, 22.67 and 25% performed better activity in inhibiting spore germination of *F. solani* than other aqueous extracts in comparison to control (85.00%). Therefore according to results of lab. tests, Artemisia (ME) at 15% concentration had best antifungal activity against *F. solani*, the causal agent of potato dry rot.

Extracts of Artemisia and Eucalyptus have been shown to possess antifungal property against different plant pathogens (Farzaneh *et al*., 2006; Hur *et al*., 2000; Jat and Agalave, 2013; Liu *et al*., 2001; Soylu *et al*., 2005; Suresh *et al*., 2010). Several reports are in agreement with our results approving the antifungal efficacy of methanolic extracts of these two plants. Yan *et al*. (2009) tested inhibitory activity of the extracts from three Artemisia species (*Artemisia annua*, *Artemisia capillaris*, *Artemisia argyi*) against *Fusarium oxysporum* and *F. moniliforme* under lab. Condition and reported that the extracts of all the three species showed strong antifungal activity against the tested pathongenic fungi, especially the antifungal activity of the extract of *Artemisia argyi* was stronger than two others. Cosoveanu *et al*. (2012) in their studies reported that the ethanol extract of *Artemisia thuscula* Cav. showed an interesting activity against the phytopathogenic fungi *Fusarium monilforme*, *F. solani* and *F. oxysporum* and antibiotic activity against 2 Gram-positive bacteria: *Bacillus cereus* and *Streptomyces griseus*.

Effect of plant extracts on dry rot development of potato tubers

Disease severity in potato tubers treated with 15% Artemisia extract (ME) followed by 15% Eucalyptus extract (ME), with 1.53 and 1.53 cm^2 were found to be the best treatments in reducing dry rot development in comparison to control (3.80 cm^2) after 7 days of incubation. Other concentrations of these two extracts also showed reasonable antifungal activity against potato dry rot but Thyme (ME) was not as effective as these two. The dry rot development in tubers treated with other plant extracts (datura (ME), 5 and 10%; Lavender (AE), 10, 5 and 15%; Lavender (ME), 5% and datura (ME), 5 and 10%) were found to be higher than the control (Table 2). In the lab. condition, different inhibiting rates on mycelial growth and spore germination of *F. solani* between plant extracts were quite sharp while in the case of *in vivo* condition these differences were milder. Overall results of *in vitro* and *in vivo* tests indicated superiority of Artemisia (ME) over Eucalyptus (ME).

Our results showed that under lab. condition, mycelial growth of *F. solani* was best reduced by 15% methanolic extracts of Artemisia followed by Eucalyptus while its spore germination was best affected by 15% methanolic extracts of Artemisia, Thyme and Eucalyptus respectively (Table1) and under *in vivo* condition, 15% methanolic extracts of Artemisia and Eucalyptus had best performance in reducing dry rot development of tested potato tubers (Var. Agria) and Thyme (ME) was not as effective as these two treatments and therefore results were focused on Artemisia and Eucalyptus extracts. Extract of Artemisia had shown a wide antimicrobial activity even against protozoan parasite *Plasmodium falciparum* causing malaria in human being resulted for production of a medicine called Artemisinin (White, 1997). Belabid *et al*. (2010) evaluated the antifungal activity of powders and essential oil formulations of some medicinal plants including *Artemisia herba* and *Eucalyptus* sp. against *F. oxysporum* population, the causal agent of lentil Fusarium wilt in the soil and found that essential oil formulations in all treatments significantly reduced the soil population densities of the pathogen and the disease incidence on lentil.

Conclusion

The present report seems to be the first study for evaluating antifungal efficacy of plant extracts against dry rot development in potato tubers. According to results of this study, the methanolic extracts of Artemisia and Eucalyptus can be incorporated for biofungicide formulations as less hazardous natural plant product in controlling this disease, however, additional research is required before we can consider industrial application of these plant extracts, including the evaluation of their efficacy at a larger scale and the evaluation of potential health and environmental risks associated with their application.

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