INHIBITORY EFFECTS OF POWDERED CARAWAY AND PEPPERMINT EXTRACTS ON PEA ROOT ROT UNDER GREENHOUSE CONDITIONS

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Abstract: The effects of caraway and peppermint extracts was evaluated at concentrations of 2, 4, 6, 8 and 10%, respectively on the radial mycelial growth of *Sclerotium rolfsii*. High significant inhibitory effect on radial fungal growth was observed with different concentrations of each of plant extracts. Concentration of 6% and more of the two extracts in combination were able to cause complete growth inhibition of the tested fungus. In greenhouse, the efficacy of plant extracts in addition to the fungicide Rizolex-T as seed dressing on pea root rot incidence was evaluated in pot experiment using soil artificially infested with the disease agent. All treatments showed a significant reduction in disease incidence compared with the control treatment. Rizolex-T followed by combination of caraway and peppermint extracts as a mixture showed superior reduction effect on root rot disease incidence at pre-, and post-emergence growth stages than individual treatment with each of extracts. The usage of caraway and peppermint extracts for seed dressing before sowing might be applied as control measure for controlling root rot diseases.

Key words: control**,** fungi, pea, plant extracts, root rot**,** *Sclerotium rolfsii*

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important leguminous crops in many countries including Egypt. Pea proved vulnerable to root rot disease caused by certain soilborne fungi. Abada *et al.* (1992) reported that *Sclerotium rolfsii* Sacc, *Rhizoctonia solani* Kühn and *Macrophomina phaseolina* (Tassi) Goid. were the main soilborne pathogens responsible for causing damping-off and root rot diseases in grown pea plants. Plant fungal diseases have traditionally been controlled by chemical fungicides. The development of resistant strains of pathogens against various chemical fungicides (McGrath 1996; Brent and Hollomon 1998) and their toxic properties limited the use of these chemicals. The use of plants or plant materials as fungicides is of a great importance and needs more attention (Bodde 1982) and various plant products like gum, oil, resins etc. are used as fungicidal compounds (Daoud *et al.* 1990; Dwivedi *et al.* 1990). The biotic-control of plant diseases may have a minimum adverse effect on physiological processes of plants and less environmental hazards (Isman 1989). Bio-fungicides, being plant products are easily convertible into a common organic material and may create fewer health problems compared to their synthetic alternatives. Furthermore, several authors cited the antifungal and antitoxigenic activities of spices (Hitokoto *et al*. 1980; Azzous and Bullerman 1982; Farag *et al*. 1989).

The objective of the performed research work was to evaluate the antifungal activity of ethanol-water extracts of both caraway seeds (*Carum carvi* L.) and peppermint leaves (*Mentha piperita* L.) as well as their mixture against the growth of *Sclerotium rolfsii in vitro* and their protective effects against the fungal invasion of pea seeds and seedlings *in vivo.*

MATERIALS AND METHODS

The present work was preformed at the laboratory and greenhouse of Plant Pathology Department, National Research Centre (NRC), Giza, Egypt. An aggressive isolate of *Sclerotium rolfsii* was obtained from the same department. Plant extracts of caraway seeds and peppermint leaves were evaluated for their inhibitory effect on fungal radial growth using an *in vitro* test. The plant materials kindly obtained from Medicinal and Aromatic Plants Research Department, NRC, Egypt, were washed with distilled water and dried in shade. They were then finely grinded to powder. Fifty grams of each plant material in powder form was homogenized by laboratory blender in 200 ml of ethanol (96%) and distilled water (20:80 v:v) for 10 min, then left in dark glass bottles for 72 h for tissue maceration. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60°C in water bath for 30 min for ethanol evaporation. The collected extracts were then stored in a refrigerator at 5°C until needed. The plant extracts were added to conical flasks containing sterilized PDA before its solidifying to obtain

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the proposed concentrations of 2, 4, 6, 8 and 10% (v/v). Combination of the two plant extracts at the rate of 1:1 v:v of each at the same previous concentration were also tested. Twenty ml of amended media were poured into 9 cm diameter Petri dishes, and another set of untreated PDA plates was used as control. All plates were inoculated individually with 0.5 cm diameter discs of the tested fungus cultures, then incubated in the dark at 25±2°C, until the control plates reached full growth. Orthogonal measurements of colonies were taken using the control plates as a reference. The reduction in fungal growth was calculated in relation to the control treatment.

The effects of caraway and peppermint extracts on root rot disease incidence of pea was studied in a pot experiment. Loamy soil was artificially infested (at the rate of 5% w:w) with the inoculum of *S. rolfsii* previously grown for two weeks on sand barley medium (1:1 w:w and 40% water) at 25±2°C. Pea seeds (cv. Giza 3) were surface disinfected by immersing in sodium hypochlorite (2%) for 2 min, and washed several times with sterilized distilled water, then dried between two sterilized layers of filter paper. The disinfested pea seeds were soaked in previously prepared plant extractes at concentrations of 8, 10% as well as 6% of both extracts (1:1 v:v). Seeds were soaked for 12 h, then picked up and left for air drying onto plastic tray. A set of disinfected pea seeds were dressed with the fungicide Rizolex-T (Tolclofos-methyl 50% a.s.) at the recommended dose (3 g/kg) and used as a reference to treatments with extracts. Another set of disinfected seeds were used for control treatment. Plastic pots (30 cm in diameter) were filled with the infested soils and five treated pea seeds were sown in each of six replicated pots for a particular treatment. Percentage of disease incidence was calculated as pre- and post-emergence root rot after 15 and 30 days, respectively.

Statistical analysis

Two ways analysis of variance (ANOVA) was used to analyze differences in fungal radial growth on PDA plates amended with plant extracts, as well as to analyze differences between the incidence of root rot in the greenhouse pot experiment. General Linear Model option of the Analysis System SAS (SAS 1988) was used to perform the analysis of variance. Duncan's Multiple Range Test was used for separation of means (Winer 1971).

RESULTS AND DISCUSSION

Results presented in table 1 show the response of *S. rolfsii* to the tested plant extracts. Radial growth of the tested fungus decreased significantly with increasing concentrations of added extracts. A moderate inhibitory effect on radial fungal growth was observed at different concentrations of either caraway or peppermint when used individually to reach 78.8 and 82.2% at the maximum used concentration of 10%. A higher inhibitory effect was observed when the mixture of two plant extract (1:1 v:v) was applied. Caraway and peppermint extracts' mixture was able to cause a complete inhibition of *S. rolfsii* when added to the growth medium at the concentration of 6% and more.

The efficacy of *in vitro* highly effective concentrations of plant extracts in addition to Rizolex-T was evaluated as pea root rot incidence in the greenhouse pot experiment. Data in table 2 reveal that tested extracts significantly reduced the percentage of both pre- and post-emergence root rot of pea compared with the control treatment. Individual and/or combined plant extracts showed a lower significant effect on reducing disease incidence compared with the fungicide Rizolex-T.

The percentages of root rot incidence ranged between 3.3 and 10.0% and 17.2 and 18.5% while the fungicide treatment showed 0.0–10.0% in the corresponding with 13.3–23.1% in control treatment at both pre- and post-emergence stages, respectively. It is interesting to note that the plant extracts of caraway and peppermint as a mixture at a concentration of 8% gave a higher effect in reducing either pre- or post-emergence root rot incidence of pea although it was still lower as compared to the fungicide Rhizolex-T. The obtained results in the present study indicate that the plant extracts of either caraway or peppermint have an antifungal inhibitory effect which increased when combined to form a mixture.

Potentially active chemical constituents of caraway and peppermint are reported by several workers in different fields. Caraway fruits contain 1–6% of essential oils consisting of about 30 compounds, from which carvone and limonene represent the main portion, about 95% (Sedlakova *et al*. 2003). Peppermint contains menthol (40–70%), carvone (20–30%), cineol, limonene, menthone, pinene, thymol, among others (Anonymous 1990 and Fleming 1998). These registered active components could be

Table 1. Growth reduction[A] of *Sclerotium rolfsii* in response to different concentrations of caraway and peppermint extracts *in vitro*

Plant extracts	Growth reduction [%] in response to plant extract concentration [B]				
	2	4	6	8	10
Caraway	18.8 g	24.4 f	37.8 e	47.8 d	78.8 b
Peppermint	22.2 f	31.1 e	46.7 d	57.7 c	82.2 b
Caraway + peppermint (1:1 v:v)	47.8 d	75.6 b	100 a	100 a	100 a

[A] reduction in fungal growth at different treatment, calculated relatively to untreated control
[B] concentrations of plant extracts added as (v/v) to the growth medium
Mean values within columns followed by the same letter are not significantly different (p= 0.05)

Table 2. Incidence of pea root rot caused by *Sclerotium rolfsii* in response to seed dressing with extracts of spice plants[A] under greenhouse conditions

Treatment		Concentration [%]	Root rot %	
			pre-emergence [B]	post-emergence [C]
Spices plant extract	Caraway	8	10.0 b	18.5 b
		10	6.7 c	17.9 b
	Peppermint	8	10.0 b	18.5 b
		10	3.3 d	17.2 b
	Caraway + peppermint (1:1, v:v)	8	3.3 d	13.7 c
Fungicide	Rizolex-T at 3g/kg		0.0 e	10.0 d
Untreated control			13.3 a	23.1 a

[A] spice plant extracts concentrations were used for pea seeds soaking
[B] values are the incidence of infection rated as percentage of emerged plants relative to the number of seeds sown in soil artificially infested with pathogenic fungi
[C] values are the incidence of infection rated as percentage of infected plants relative to the number of emerged plants in soil artificially infested with pathogenic fungi
Mean values within columns followed by the same letter are not significantly different (p = 0.05)

dissolved and macerated during the procedures of water ethanol extraction of both caraway and peppermint in the present work. These phenolic compounds were reported to have antifungal inhibitory effect which was observed against the fungus *S. rolfsii* in the present findings. It is also interesting to note that the usage of both caraway and peppermint in a mixture showed synergistic superior inhibitory effect against both fungal growth *in vitro* and the invasion to pea plants *in vivo*. Several reports indicated that plant spices containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances (Kim *et al*. 1995). Alkaloids, flavonoids, isoflavonoids, tanins, cumarins, glycosides, terpens and phenolic compounds were synthesized by plants as secondary metabolites (Simões *et al*. 1999). In agricultural studies, these compounds have broad-spectrum activities against fungi, nematodes, and insects (Lee *et al.* 1997; Wilson *et al.* 1997; Calvet 2001). Plant spices offer a promising alternative for food safety and plant protection. Inhibitory activity of spices and their derivatives on the growth of bacteria, yeasts, fungi and microbial toxin synthesis was reported (Notermans and Hoogenboon-Verdegaal 1992; Sagdiç *et al*. 2003). Moreover, antifungal activity of spices and their derivatives were studied by viable cells count, mycelial growth and mycotoxin synthesis. However, there is little information on spices and their derivativesàction on/in a fungal cell. In general, inhibitory action of natural products on moulds involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intercellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating with mycelial growth inhibition (Cowan 1999). Also, it was reported that plant lytic enzymes act in the fungal cell wall causing breakage of *ß*-1,3 glycan, *ß*-1,6 glycan and chitin polymers (Brull and Coote 1999). Moreover, the mode by which microorganisms are inhibited by spices and their chemical compounds seems to involve different mechanisms. It was hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents (Juven *et al*. 1994).

Many authors emphasized that the antimicrobial effects of essential oil constituents are dependent on their hydrophobicity and partition in the microbial plasmatic membrane. The effect of specific ions due to their addition in/on plasmatic membrane had a great effect on the proton motive force, intracellular ATP content and overall activity of microbial cells, including turgor pressure control, solute transport and metabolism regulation (Lanciotti *et al*. 2004).

Hence, the objective of this study was to determine if spice plant extracts could provide protective effect against invasion by *S. rolfsii*. Considering their attribute and broad-spectrum activities, successful development of such compounds as antifungal would not only provide a potent tool for control of pea root rot, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of other plant diseases.

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POLISH SUMMARY

DZIAŁANIE INHIBICYJNE SPROSZKOWANYCH EKSTRAKTÓW KMINKU I MIĘTY PIEPRZOWEJ NA ZGNILIZNĄ KORZENI GROCHU W WARUNKACH SZKLARNIOWYCH

Badano działanie ekstraktów z kminku i mięty pieprzowej użytych odpowiednio w stężeniach 2, 4, 6, 8 i 10% oraz zaprawy nasiennej Rizolex-T na wzrost grzybni *Sclerotium rolfsii.* Zaobserwowano silny efekt inhibicyjny wymienionych kombinacji doświadczalnych. Stężenie 6% oraz wyższe powodowały całkowitą inhibicję wzrostu grzybni patogena. W warunkach szklarniowych badano dodatkowo w wazonach z ziemią zakażoną grzybem *S. rolfsii* efektywność zaprawy nasiennej Rizolex-T w zwalczaniu zgnilizny korzeni. We wszystkich kombinacjach wystąpiło istotne ograniczenie zgorzeli przedwschodowej i powschodowej grochu, w porównaniu do kombinacji kontrolnej. Najlepsze wyniki uzyskano stosując do zaprawiania nasion Rizolex-T, a następnie mieszaninę ekstraktów kminku i mięty. Użycie ekstraktów z kminku i mięty do zaprawiania nasion może więc być wykorzystane jako metoda zwalczania zgnilizny korzeni.