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Control of *Beta vulgaris* Pathogens using *Lantana camara* Linn. Essential oil *in vitro*

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Abstract: *Rhizoctonia solani* Kühn (A022), *Scierotium rolfsii* Sacc. and *Fusarium solani* (Mart.) Sacc were isolated from sugar beet *Beta vulgaris* in Egypt. The antimycotic behavior of *Lantana camara* Linn. flowers essential oil was investigated *in vitro.* including its effect on mycelial growth, conidia and/or sclerotia germination. 10-40 μl mL^{-1} essential oil had a significant inhibitory effect upon conidia/sclerotia development and germination when compared to the control, where, *R. solani* exhibited the fastest growth. Growth tolerance towards the essential oil was highest for *F. solani* and lowest for *R. solani.* The sclerotia of *S. rolfsii* were most sensitive to the essential oil stress with regard to their germination and the produced number. Maximum inhibition of *F. solani* macro-conidia was recorded at 40 μl mL^{-1}, as no macro-conidia was observed at a higher concentration.

Key words: *Beta vulgasis*, *Lena camara* Linn., essential oil, germination percentage

INTRODUCTION

Since fungicides are very expensive and cause serious environmental pollution. Control strategies are today directed towards replacing the use of hazardous chemical fungicides by environmentally friendly natural products (Mamdouh and Eweis, 2007).

Essential oils of several plants are frequently used in the preparation of cosmetics, perfumes, antiseptics and as active ingredients in certain medicines. Essential oils are used for purification of environment since, they possess antimicrobial properties. Anti-microbial activity of essential oils has been reported by various workers and has been reviewed time to time (Singh *et al*., 1980; Reuveni *et al*., 1984; Dubey, 1991; Baruah *et al*., 1996; Zambonelli *et al*., 1996; Lachoria *et al*., 1999; El-Shazly, 2000; Kamboj, 2000; Deena and Thoppil, 2000; Soylu *et al*., 2006; Haikal *et al*., 2008; Cleveland *et al*., 2009; Jia *et al*., 2010; Wanner *et al*., 2010; Louis *et al*., 2011).

The shrub *Lantana camara* Linn. belongs to the family Verbenaceae. It is well described by Grewal (2000) and Lachoria *et al.* (2004). The plant is commonly believed to have acquired poisonous properties since its fruit, leaf and flowers when consumed by animals cause certain disorders in their health (Sharma *et al*., 1981). Its air-dried leaves and flowers have been reported to possess 0.2 and 0.07(%v/w) essential oil, respectively (Chopra *et al*., 1965). The plant contains 80% of a Caryophyllene-like sesquiterpene and 1-α- phellandrene with some aldehyde and free alcohol. The fresh leaves of this weed have yielded 0.069% of an essential oil containing citral. From the distillation of whole plant, 0.053% of aromatic oil containing aterpinene (10%), pymene (6%), sesquiterpenes of caryo-phyllene type (43%) and of cadinene-type (21%) have been recovered (Chopra *et al*., 1965; Lachoria *et al*., 2004).

Sugar-beet *(Beta vulgaris* L., Chenopodiaceae) is one of the most important crops grown mainly in the areas of temperate climate conditions for sugar production. It has great economic importance for Egypt, since it is the second crop plant for the sugar production after sugarcane (Eweis, 2007). Sugar-beet is attacked by several root-rot diseases, the most serious of which are caused by *Rhizoctonia solani* Kühn, *Sclerotium rolfsii* Sacc. And also a wilt disease caused by *Fusariurn* species (Eweis *et al*., 2006; Eweis, 2007; Haikal *et al*., 2008). Taking this economic importance into consideration, the present work was designed to investigate the *in vitro* effect of essential oil derived from the flowers of *Lantana camara* Linn on the growth activities of the sugar-beet pathogens: *R. solani*, S. *rolfsii* and *Fusarium solani.*

MATERIALS AND METHODS

The pathogens: *Rhizoctonia solani* (AG22) Kühn, *Scierotiurn rolfsii* Sacc. and *Fusarium solani* (Mart.) Sacc. were isolated from diseased sugar- beet roots and were identified (Eweis, 2002; Eweis *et al*., 2006).

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Maintenance and cultivation media

Slants were maintained on the medium composed of (g L^{-1}): dextrose, 30, KH_2PO_4, 1; $MgSO_4.7H_20$, 0.5; KC1, 0.5; KNO_3, 2 and 1 mL of each of stock solutions (1 g L^{-1}) of $FeSO_4.7H_2O$, $MnSO_4.7H_2O$, $ZnSO_4.7H_20$ and thiamine; agar, 20 (Johnson and Curl, 1972).

Czapek-Dox and Potato dextrose (PDA) agar and broth were used for germination of pathogens conidia/sclerotia. Slants were kept by sub-culturing every two weeks and were maintained at 4°C.

Plant material: The fresh flowers of *Lantana camara* Linn. (Family-Verbenaceae) were collected from the shrubs cultivated in El Orman gardens Giza-Egypt.

Extraction of essential oil: Flowers of *Lantana camera* Linn. (1500 g) were semidried and subjected to hydro distil lation for 12h to obtain essential oil according to the method described by Lachoria *et al.* (2004). It was treated with anhydrous sodium sulfate to remove water molecules. A light yellowish colored essential oil having a characteristic smell was obtained, stored in dark bottle and kept in a refrigerator (5±1°C) until needed.

***In vitro* experiments**

Germination experiments: Sclerotia of *R. solani* and *S. rolfsii,* produced on potato dextrose agar (PDA) and Czapek-Dox agar, respectively, were collected and surface disinfected by soaking them for 5 min in 1:400 (w/v) bromine in water to kill hyphal extension, washed thoroughly with distilled water and dried (Abeygunawardena and Wood, 1957). Ten sclerotia/Petri dish for either pathogen were plated on the surface of tap water agar (1.5% w/v) supplemented with the relevant amounts the extracted fungicide in concentrations ranging from (10-40 µl mL^{-1} medium The dishes were incubated at 27±1°C for 12 h for *R. solari* and 30 h. For *S. rofsii* the percentage of germinated sclerotia and average lenghth of hyphal extension were determined and five plates were prepared for each treatment and mean were compared.

For *F. solani* microscope slides were covered, each, with 1 mL of the micro-conidial suspension of *F. solani* in aqueous solution of the desired essential oil concentrations in Petri dishes and then incubated at 27±1°C for 9 h in complete darkness. The percentage of germination and the average length of the germ-tubes were assessed according t o the method described by El-Abyad and Saleh (1971). Five plates were prepared for each treatment and the means were compared.

Growth experiments:

Mycelia dry mass: The extracted essential oil was mixed aseptically with Czapek-Dox broth medium to produce concentration ranging from 10, 20, 30 and 40 µl mL^{-1} medium and dispensed in 50 mL aliquots into 250 mL Erlenmeyer flask. A 6 mm diameter agar disk bearing hyphae of either *R. solani*, *S.* rolfsii or *F. solani.* From 7-days-old colonies grown on Czapek-Dox agar was transferred to each flask and incubated at 27±1°C for 9 days. The mycelia were harvested, dried to constant weight at 80±1°C, the dry mass yield and final pH value were recorded. Five flasks were prepared for each treatment and the means were compared (Mamdouh and Eweis, 2007).

Production of sclerotia: PDA was used for *R. solani* and Czapek-Dox agar for S. *rofsii.* The extracted essential oil was mixed aseptically with the medium to produce the required concentrations and poured in Petri dishes. The fungi were inoculated to the dishes and incubated at 27±1°C for 9 days. For *R. solani,* 1 mL of hyphal suspension was added to each dish. This was prepared by inoculating two 6 m diameter potato dextrose agar (PDA) disks bearing hyphae into potato dextrose broth (PDB) in 250 mL Erlenmeyer flasks, each containing 50 mL medium. Flasks were incubated at 27±1°C for 3 days, filtered and the mycelia mats were washed with sterile distilled water. This mycelium was homogenized with 100 mL sterile distilled water in a sterile micro-blender for 3 mm to form a heavy suspension (Manning *et al.*, 1970). For S. *rolfsii:*One 6 mm diameter Czapek-Dox agar disk bearing hyphae of the fungus was transferred to each dish. the number of sclerotia produced /plate in each treatment was visually counted. Five plates were prepared for each treatment and the means were compared.

For *F. solani,* Production of macro-conidia was assessed according to the method described by El-Abyad and Saleh (1971). Modified Czapek-Dox agar was mixed aseptically with the extracted essential oil in amounts calculated to produce the required concentrations and poured into Petri dishes. The dishes were incubated with a 6 mm disk of mycelia of *F. solani,* incubated for 9 days at 27±1°C and the number of spores produced was counted by a haemocytometer. Five dishes were prepared for each treatment and the means were compared

Statistics: All measurements are the means of five replica; the results obtained were processed by analysis of variance and the significance was determined at the Least Significant Difference (LSD) levels of 1 and 5% (Snedecor and Cochran, 1967).

Table 1: Effect of the extracted essential oil on (%)germination and average length of hyphal extension of *R . solani*, *S. rolfsii* and macro-conidia of *F. solani* at 27°C

Essential oil concentration	(%) germination			Average lenghth of hyphae µm		
(µL mL^{-1})	*R. solani*	*S. rojfsi*	*F. solani*	*R. solani*	*S. rojfsi*	*F. solani*
0	56.1	53.1	63.2	843.3	662.4	12.4
10	52.4	32.7	61.4	816.2	503.2	12
20	18.4	13.2	55.3	202.7	270.1	11
30	4.7	3.2	37.5	49.1	45.2	8.7
40	2	0	29.2	6	0	6.3
LSD						
1%	6.1	5.7	7.2	10.7	12.4	2.5
2%	4.2	3.5	5	7.9	7.5	1.6
1.6	7.5	7.9	5	3.5	4.2	2%

(%) germination and average length of the hyphal extension of sclerotia were assessed after 12 h for *S. solani* and 30 h for *S. rolfsii*. *F solani* macro-conidia (%)germination and averge length of germ tubes were assessed after 9 h

RESULTS AND DISCUSSION

In vitro experiments

Sclerotial germination and hyphal extension: Studies on spore germination represent and integral part of the ecological studies of the fungi pathogenic to sugar-beet as spores are the specialized structures capable of initiating new growth. Once germination had occurred, the ensuring mycelia growth rate may be of prime importance in determining the degree of virulence of the fungus concerned. The antifungal efficacy of the extracted essential oil derived from the flowers of *Lantana camara* towards the three phytopathogenic fungi was studied *in vitro* and the results are presented in Table 1 which reveals that the extracted essential oil had fungicidal effects on the three phytopathogenic fungi.

The data indicated in Table 1 showed that the germination (%) of sclerotia o*f R. solani* and S. *rolfsii* significantly decreased with increasing the concentration of the essential oil. The inhibitory effect was proportional with the applied concentration as well as the nature of the bioactive compounds found in an essential oil. Germination was prevented at 40 µl mL^{-1} for *S. rolfsii.* The average length of µm hyphal extensions was affected similarly to germination, decreasing effect proportionally to essential oil concentration.

Effect of the extracted essential oil on mycelial growth: Table 2 showed that another important physiological parameter to be examined is the determination of growth under various concentrations of essential oil. Once germination had occurred the ensuing mycelia growth rate becomes a factor of significant importance in determining the degree of virulence achieved by the studied root-rot fungi. gradual decrease in the dry weight were observed in the tested fungi at 40 µl mL^{-1}.

Table 2: Effect of the extracted essential oil on dry mass yield of sclerotia of *R. solani*, *S. rolfsii* and macro-conidia of *F.solani* at 27°C

Essential oil concentration (µL mL^{-1})	Dry weight (mg L^{-1})		
	R. solani	*S. rojfsi*	*F. solani*
0	1020.1	612.1	`357.2
10	980	487.1	321.1
20	221.1	203.1	283.7
30	87.7	69.3	241.4
40	30	0	197.1
LSD			
1%	15.6	16.1	11
2%	9.4	8.2	5.6

R. solani and *S. rolfsii* dry mass yield was assessed after 9 days. Medium supplemented with extracted essential oil at concentrations 10, 20, 30 and 40 µl mL^{-1} in the medium were presented in Table 1 and 2 indicated that the growth of the tested pathogens were significantly decreased compared to control. No growth was recorded at the concentration of 40 µl mL^{-1}. For *S. ro/fsii* pathogen

Table 3: Effect of the extracted essential oil on production of sclerotia of *R. solani*, *S. rolfsii* and macroconidia of *F. solani* at 27°C

Essential oil concentration (µL mL^{-1})	No. of sclerotia/plate		
	R. solani	*S. rolflsii*	*F. solani*
0	63	623	314.2
10	59	562	300.1
20	21-0	214	285.2
30	7	43	250.7
40	3	0	180.1
LSD			
1%	6.2	16.5	18.1
2%	3	10.2	7.5

The production of sclerotia by *R.solani* and *S. rolfsii* were assessed after 9 days

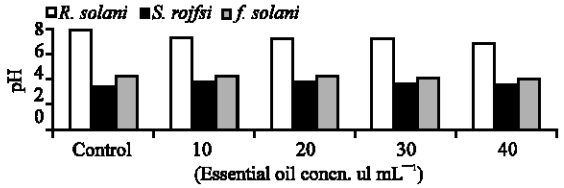

Fig. 1: Effect of essential oil different concn. on the final pH in the pathogens cultures. Initial pH 5.7

Effect of the extracted essential oil on culture pH: The pH of the growth medium shifted towards alkalinity for the *R. solani.* The pH increase in the culture medium during fungal growth may have been caused by differential uptake of cations and anions. Transport of anions such as phosphates may act as the hydroxide exchange system with the medium becoming more basic (Griffin, 1994). Fig 1 shows The rapid decline in the pH of the culture for *S. rolfsii, F. solani* was probably due to the production of organic acids (oxalic acid) through the oxidation of carbon source (Punja and Jenkins, 1984).

Effect of the extracted essential oil on the production of conidia or sclerotia: Table 3 indicated that the number of sclerotia produced/plate by *R. solani* and *S. rolfsii* at

concentrations ranging from 10 to 40 μl mL^{-1} were reduced proportionally to the natural fungicide concentrations. For *R. solani,* no sclerotia were produced with the fungicide at a concentration higher than 40 μl mL^{-1} as compared with the control.

The macro-conidia of *F. solani* germinated in a wide range of the purified natural f ungicide concentration 10-40 μl mL^{-1} decreasing steadily with an increase in the fungicide. Maximum inhibition was recorded at a concentration of 40 μl mL^{-1} while no macro-conidia was observed at a concentration higher than 40 μl mL^{-1}. These results are consistent with those obtained by other investigators who found an antimicrobial activity of some essential oils and plant extracts against several pathogens *in vitro* (Ismail *et al*., 1989; Farag *et al*., 1989; Denns *et al*., 1992; McCutcheon *et al*., 1994; Navarro *et al*., 1996; Ali *et al*., 1999; Mahmoud, 1999; Emam and Eweis, 2005; Mamdouh and Eweis, 2007).

Secondary compounds, considered as final products of plant metabolism or metabolite refuses, have important ecological functions for the plant which synthesize them. One of these functions is to protect the plants against infection by pathogens (Taiz and Zeiger, 1991). Therefore, many essential oils exhibited inhibitory properties in challenge tests against microorganisms (Beuchat, 1994). These oils, however, contained specific component that can inhibit the growth of certain microorganisms (Saito *et al*., 1991; Lawless, 1995).

The direct effects of many pesticides (herbicides or fungicides) on the fungal pathogen S. *rolfsii,* the causal pathogen of sugarbeet root-rot disease in Egypt have been evaluated *in vitro* by many investigators (El-Abyad and Abu-Taleb, 1991; Eweis, 1995; Eweis *et al*., 2006; Mamdouh and Eweis, 2007). Many essential oils, have been found to be potent fungitoxic agents (Singh *et al*., 1980; Reuveni *et al*., 1984; Chauhan and Singh, 1989; Dubey, 1991; Zedan *et al*., 1994; Zambonelli *et al*., 1996). However, the harmful effects on fungi were restricted in: (a) partial or complete inhibition on spore germination, sporulation or mycelia growth and (b) alternation in physiology and biochemistry activities of the fungal cells.

CONCLUSION

All these observations and findings bring further evidence that the extracted essential oil derived from the flowers of *Lantana camara* Linn. have the potential of becoming powerful and safe alternative means of disease control instead of the harmful pesticides like Pyradur.

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