

IN VITRO EFFICACY OF SOME PLANT EXTRACTS AGAINST DAMPING OFF DISEASE OF TOMATOES

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Abstract

Plant diseases control represent a major challenge that farmers are facing in the management of cropping systems. Rhizoctonia solani is a soilborne fungal pathogen causing tomato (Solanum lycopersicum) root-rot and damping off, and generating compromised quality of crops and reducing yields.

Ethanol extracts of five medicinal plants, marigold (Tagetes patula), horsetail (Equisetum arvense), elderberry (Sambucus nigra), burdock (Arctium lappa), liquorice (Glycyrrhiza glabra) were investigated to determine their antifungal potential against Rhizoctonia. Solani. In vitro testing demonstrated a high growth inhibition of the extracts. Four of the extracts totally inhibited the mycelial growth of R. solani compared to the untreated control, at 10% concentration in growth media. For all plant extracts considered, the growth inhibition increased with the concentration of each extract.

The obtained results support an interesting direction of research, meaning the use of plant extracts in controlling diseases attacking economically important crops for integrated pest management programmes.

Key words: antifungal effect, ethanol plant extracts, Rhizoctonia solani, damping off tomatoes

1. INTRODUCTION

Taking into consideration the demands of a growing population, food production must rise by 70% by 2050 to feed nine billion people (FAO statistics). Food and beverages industry and commerce are dependent on agricultural sector as their main supplier of raw materials. The challenge is to provide more output with limited resources available.

Plant diseases control represent a major issue that farmers are facing in the management of cropping systems. The fungal diseases represent one of the major cause of decreased yields of agricultural crops all over the world (Makovitzki et al. 2007). At this moment, the prevention and control management of plant pathogenic fungi is achieved mainly by the use of synthetic fungicides.

However, the massive and sometimes inappropriate use of the synthetic fungicides in agricultural practices resulted in severe negative effects on multiple levels. On the one hand, the plant pathogens have developed resistance to the fungicidal treatments in use (Secor and Rivera, 2012; Rossall, 2012), and on the other hand, the contamination of the environment - soil (Komárek et al. 2010; Wightwick, et al. 2010), water (Wightwick et al. 2011) and air (Brent and Hollomon, 2007) and of the final products due to fungicide treatment was reported. Therefore, new control formulations for plant diseases represent a real need in the nowadays context of sustainable development in agriculture and ecology area.

Plants produce multiple secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids (Butu et al. 2014a; Kennedy and Wightman, 2011) being in the same time important sources of biologically active molecules possessing antibacterial, antifungal (Negi, 2012) and antioxidant properties (Butu et al. 2014b)

In recent years, plants extracts obtained by various methods have raised scientific research interest for their antimicrobial potential (van Vuuren, 2008; Rakholiya and Chanda 2012; Ncube et al. 2012; Mamoci et al. 2011). Moreover, an increasing number of studies are developed for investigation of the antimicrobial effects of medicinal plants for plant disease control. (Satish et al. 2007; Sharmin and Shamsi 2013; Rodino et. al 2013; Manasi et al. 2014)

R. solani, a soilborne fungus, is one of the most common phytopathogens that attack tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), cucumber (*Cucumis sativus*), peas (*Pisum sativum*) and many other crops, causing disease such as root rot and damping off (Ohkura et al., 2009; Agrios, 2005)

Current chemical treatments with traditional fungicides and integrated disease management methods used for control of *R. solani* diseases seem not to be completely effective, and therefore the diseases it causes remains a persistent issue (Huang et al. 2012) for the farmers to deal with. No matter the cropping system - green house or open field - each season are reported outbreaks of *R. solani* diseases.

In this respect, the objective of the present paper was to evaluate the capability of the selected medicinal plant extracts, namely, marigold (*Tagetes patula*), horsetail (*Equisetum arvense*), elderberry (*Sambucus nigra*), burdock (*Arctium lappa*), and respectively, liquorice (*Glycyrrhiza glabra*), to inhibit the mycelial growth of *R. solani*.

2. MATERIALS AND METHODS

2.1. Plant material

The medicinal plants used in this study were selected from the locally available spontaneous flora, and used as presented in Table 1. The plant parts were chosen according to traditional medicinal use of the refereed plants.

Table 1. Plant material used for study

	<i>Popular name</i>	<i>Scientific name</i>	Plant part used
1.	Marigold	<i>Tagetes patula</i>	Flowers
2.	Liquorice	<i>Glycyrrhiza glabra</i>	Roots
3.	Burdock	<i>Arctium lappa</i>	Leaves
4.	Horsetail	<i>Equisetum arvense</i>	Whole Plant
5.	Elderberry	<i>Sambucus nigra</i>	Fruits

The sample plants were collected from different agricultural fields located in the Southern part of the country. The plant material was washed with distilled water, dried at room temperature, in dark and then grinded to a fine powder in order to be used for the extraction.

2.2. Preparation of plant extract

The alcoholic extracts used in the antifungal assay were obtained by maceration. This is a cheap and simple preparation technique which allows the further transfer of the technology to the field. A quantity of 10g of dried and fine powdered plant material was added to 100 ml of 70 % ethanol. The mixture was left in sealed glass recipients for 96 hours, at room temperature, in darkness, with occasionally stirring. The obtained extract was filtrated through filter paper (Whatman no.1) under vacuum. The extracts obtained were kept at 4°C in sealed recipients until further use.

2.3. Fungal inoculum

The fungal culture belongs to the collection of the Faculty of Biotechnologies from the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The tests were carried out using a 7 day old culture.

2.4. Antifungal activity testing

The effect of the different concentrations of plant extracts against the mycelial growth of *R solani* was tested by poisoned food technique.

An appropriate quantity of each extract was incorporated in sterilized potato dextrose agar (PDA) medium to reach desired concentrations for each treatment. Three concentrations of ethanolic extracts were used in the assessment, respectively, 10%, 4% and 2%. The positive control was represented by untreated PDA plates. The plates were left to solidify in the laminar flow hood and then mycelial discs of 6 mm diameter, taken from the margins of an actively growing culture of the fungal phytopathogen were placed in the center of the Petri dishes.

Three replicates were used for each treatment. The Petri dishes were incubated in dark at 25 ± 2 °C until the control colony reached the margins of the Petri dish. The measurement of the diameter of the mycelial growth of the fungus were recorded on a daily basis, beginning with 24 hours after inoculation.

The percentage of the growth inhibition of the mycelium (I) due to treatment with extracts was calculated using the following formula:

$$I (\%) = (1 - d_t/d_c) * 100 (\%),$$

where, d_c is the average fungal colony diameter measured in control plate, with no treatment, and d_t is the average fungal colony diameter measured in treated plates (Ogbebor et al., 2008)

2.5. Statistical analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) using Microsoft Excel 2010. The treatment means were compared by Tukey's post-test at P (0.05) level of significance.

3. RESULTS AND DISCUSSIONS

R. solani *in vitro* growth proved to be sensitive to treatment with the selected plant extracts. The fungal colony started to develop on the control dish 24 h after inoculation and grew rapidly reaching the margins of the Petri dish in 5 days. Four of the extracts totally inhibited the mycelial growth of *R. solani* compared to the untreated control, at 10% concentration in growth media. In this case, the extract obtained from *A. lappa* had only a fungistatic effect, with a growth inhibition of 85% against the untreated control.

The highest antifungal activity at a concentration of 4% in the growth media, was observed for the *T. patula* extract, with 82% growth inhibition, followed by *S. nigra* -77%, *G. glabra* 75%, *E. arvense* 62% and respectively, *A. lappa* 59% (Figure 1). When compared to the control, all of the extracts had a statistically significant ($P < 0.05$) antifungal activity against the tested fungal inoculum.

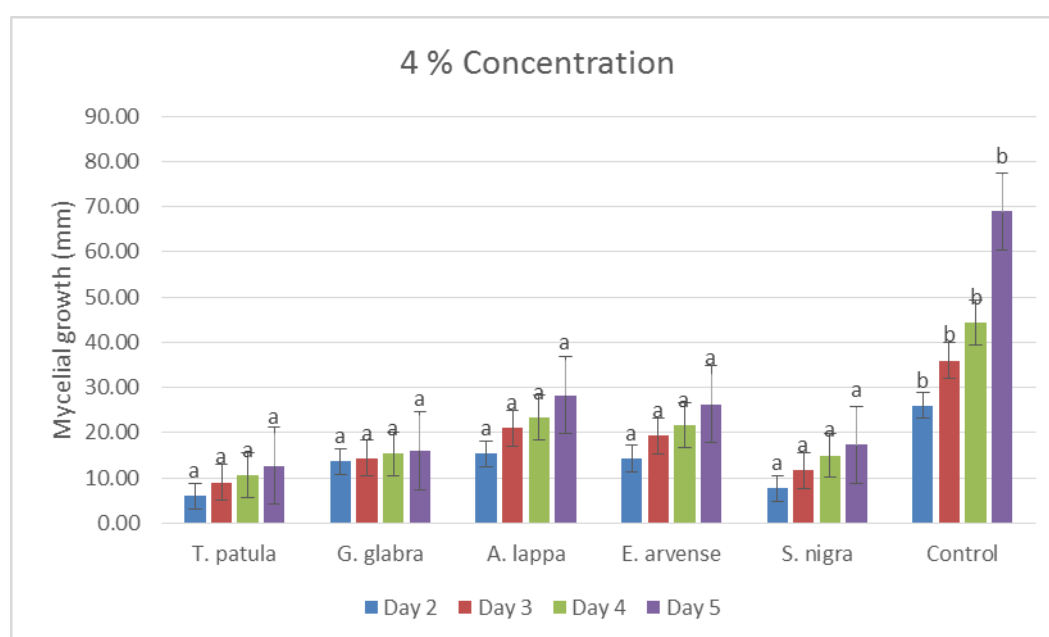


Figure 1. Diameter growth of *R. Solani* colony on PDA after treatment with plant extracts at 4% concentration. Bars with the same letters are not significantly different, according to Tukey test ($P < 0.05$).

For the 2% concentration the distribution of the antifungal activity dynamics followed the same trend as before (Figure 2). In the same time, it could be observed that the growth inhibition increased with the concentration of each extract in the growth media. The treatment with *T. patula* extract resulted in 73% growth inhibition, followed by *S. nigra* 72% and *G. glabra* 72%, *E. arvense* 46% and respectively, *A. lappa* 38%. The mean

diameter growth on the plates containing hidroalcoholic extracts obtained from marigold, elderberry and licorice were statistically significant when compared to the untreated control ($P < 0.05$).

From these results it can be concluded that the extract of marigold, licorice and elderberry possesses strong *in vitro* antifungal activity against *R. solani* and can be subjected to further investigation of their potential for *in vivo* efficacy study.

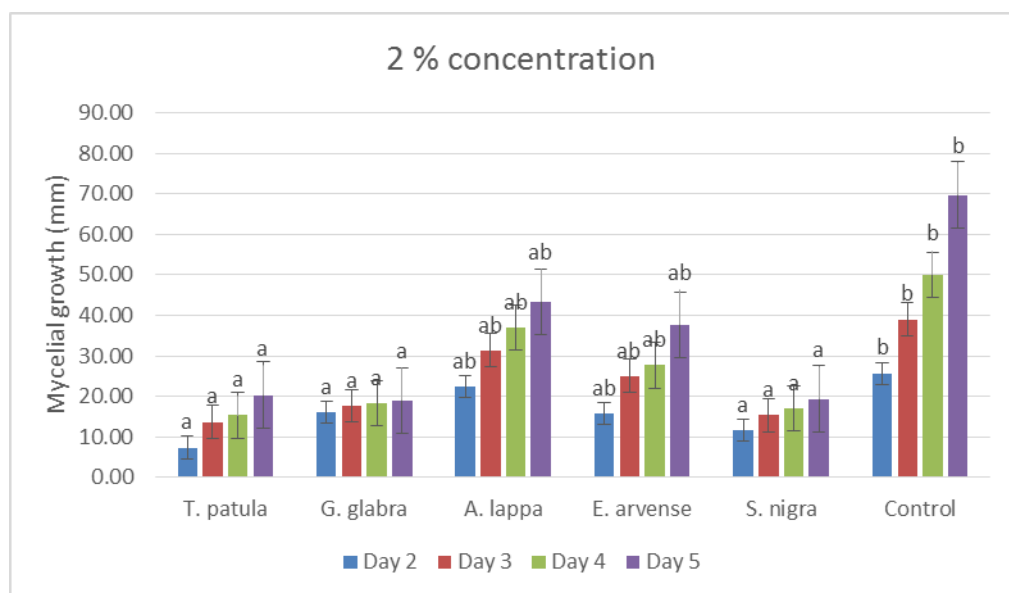


Figure 2. Diameter growth of *R. Solani* colony on PDA after treatment with plant extracts at 2% concentration. Bars with the same letters are not significantly different, according to Tukey post-hoc test ($P < 0.05$).

4. CONCLUSIONS

Due to economic, environmental and technical issues and to the more restrictive legal regulations in force, phytopathogens control is a difficult task to accomplish, and therefore complementary natural formulations could be a solution. The results obtained in this paper support an interesting direction of research, meaning the use of plant extracts in controlling diseases attacking economically important crops for integrated pest management programmes.

The studied medicinal plants extracts might be used as potential source of natural compounds based fungicides for organic and traditional farming, being possible to be used as an alternative or at least complementary and synergistic derivatives to synthetic chemicals.

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