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ANTIFUNGAL ACTIVITY OF FOUR PLANTS AGAINST ALTERNARIA ALTERNATA

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Abstract

Natural fungicides are gaining increased attention because of their environmentally friendly properties, being easily accessible and relatively cost effective, in the perspective of sustainable methods of plant disease control The aim of the present work was the investigation of the in vitro antifungal activity of the ethanolic and aqueous extracts obtained from four locally available traditional medicinal plants from Romania, collected from different regions of Southern part of the country. The four plants taken into consideration were absinth (Artemisia absinthium), rosemary (Rosmarinus officinalis), jimson weed (Datura stramonium) and cocklebur (Xanthium strumarium), and their antifungal properties were tested against the plant pathogenic fungus Alternaria alternata. All extracts obtained from the selected plants presented antifungal potential, demonstrated by the inhibition of the mycelial growth. Generally, the ethanolic extracts showed a higher antifungal activity than the aqueous extracts, for all the tested variants. The results of this study confirm that the ethanolic and aqueous extracts of the selected plants can be used as an alternative in control of the tested phytopathogenic fungus.

Keywords: *Alternaria alternata, antifungal activity, plant extracts.*

INTRODUCTION

Fungal plant diseases represent an important cause of increased annual crop losses. More than 70% of all major crop diseases are caused by fungi (Agrios, 2005).

Alternaria alternata causes leaf spots and blight on a large variety of agricultural and horticultural crops such as: tomato *(Lycopersicon esculentum)*, potato (*Solanum tuberosum*), carrot (Daucus carota), cauliflower (*Brassica oleracea var. botrytis)*, broccoli (*Brassica oleracea -Botrytis Group*), cabbage (*Brassica oleracea var. capitata*), peppers (*Capsicum annuum.*), beans (*Pisum sativum)*, apple (*Malus domestica*), peach Prunus persica) and citrus species. Moreover, *A. alternata* can also attack a several weeds and ornamental plants.

There is also little doubt that sensitivity to *Alternaria* is an important factor in the induction of allergic rhinitis and asthma on immunodepressed patients, especially in children (Kuna et al., 2011)

Generally, the control of plant diseases and pests is well established with synthetic fungicides and other agricultural practices such as crop rotation inter-cropping and sanitation (Pretty, 2008). However, in the recent years the farmers all over the world have reported an efficacy decrease of the treatments with traditionally used fungicides to control early blight and other plant diseases (Fairchild et al., 2013). Furthermore, the inappropriate use of fungicides, such as applying increased and more frequent dosage units (Genet et al., 2006) has resulted on the one hand in the occurrence of fungal resistance (Brent and Hollomon, 2006; Mcgrath, 2001; Haouala, 2008) and on the other hand in hazardous effects in human and animal health and on the environment resulting in ecological imbalances (Pramila and Dubey, 2004).

Products based on natural components used in controlling and combating phytopathogenic agents have gained increased attention lately, in search of eco-friendly methods that can be used either for mass production or for some the organic and bio farming niche production.

Moreover, the natural fungicides are easily accessible and relatively cost effective, in the perspective of sustainable methods of plant

The research in this area started initially in a more likely confirmation direction of the antimicrobial potential, with medicinal plants used empirically in folk's medicine to treat various diseases. Subsequently, the scope of work expanded to applications of the use of products obtained from these plants in the phytopathogens area.

Various reports on medicinal plants extracts have shown inhibitory effects against phytopathogenic fungi *in vitro* (Rodino et al. 2013a; Haouala, 2008; Rodino et al. 2013b; Singh et al. 1998; Yasmin et al., 2008; Harlapur et al., 2007; Prashith et al 2010)

Currently, there are also conducted studies on some species of plants such as weeds (Srivastava and Singh, 2011) or trees (Mahlo et al., 2010) with less known medicinal value, that could be used to control plant diseases and pests.

In view of this, in the present paper was investigated the antifungal activity of extracts obtained from locally available plants, namely absinth or wormwood (*Artemisia absinthium*), rosemary (*Rosmarinus officinalis*), jimson weed or thornapple (*Datura stramonium*) and cocklebur (*Xanthium strumarium*) against the fungus *Alternaria alternata.*

MATERIALS AND METHODS

Plant material collection

The plant materials and plant parts used in this study were as follows:

1- absinth (*A. absinthium*) aerial part,
2 - rosemary (*R. officinalis*) aerial part,
3 (a) - jimson weed (*D. stramonium*) leaves.
3 (b) - jimson weed (*D. stramonium*) fruits.
4 - cocklebur (*X. strumarium)* fruits.

The plants were collected from different agricultural lands in Southern part of the country. The healthy vegetal material was shade dried and minced to a fine powder in order to be used for the extraction.

Preparation of plant extract

The *alcoholic extracts* used in the antifungal assay were obtained by maceration. For this process was used a quantity of 5g of dried, fine powdered plant to 50 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 *aqueous extracts* were prepared by infusion using the same ratio of 1:10 (w:v) of powdered dried plant material to sterile distilled water. The solution thus obtained was kept at 4°C in sealed recipients and used within the next eight hours.

Fungal culture

The fungal culture belongs to the collection of the Faculty of Biotechnologies from the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The present experiments were carried out using a 7 day old culture.

Antifungal activity testing

The effect of the plant extracts taken into study against the mycelial growth of *A. alternata* was tested by poisoned food technique.

An appropriate quantity of each extract was incorporated in sterilized PDA medium to reach desired concentrations for each plant solution treatment. Three concentrations of ethanolic extracts at 10%, 5% and 2.5% were used in the assessment. Regarding the aqueous extracts a concentration of 10% was evaluated.

Mycelial discs of 6 mm diameter, taken from the margins of an actively growing culture of the fungal pathogen were aseptically placed in the centre of the 70 mm plates containing solidified poisoned PDA. The experiment was run in triplicate. The Petri-plates were incubated at 25 ± 2 °C. The measurement of the mycelial growth dynamics of the fungus were recorded on a daily basis, beginning with 24 hours after inoculation. The whole experiment was carried out for 9 days until the control colony reached the margins of the Petri plate.

The control was considered the plate containing the organic solvent (sterile double distilled water or ethanol) incorporated and the control with non-poisoned PDA.

The percentage of the inhibition of the mycelial growth (I) due to different treatments was calculated on the formula:

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

Where,

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

RESULTS AND DISCUSSIONS

All the extracts obtained from the selected plants presented antifungal potential against the tested fungus, demonstrated by the inhibition of the fungal mycelial growth. In general, the ethanolic plant extracts showed more intense antifungal activity than the aqueous extracts for all the tested variants.

The results revealed that the antifungal activity of the extracts was dose dependent, being negatively influenced by decreasing the concentration of the extracts in the growth media.

When using the concentrations of 10% ethanolic plant extract in PDA medium resulted in a fungistatic effect demonstrated by the 100% inhibition of mycelial growth in all variants studied.

Regarding the growth inhibition dynamics of the variant using 5% extract concentration in growth medium it could be observed a very slow development of the fungus compared to the not treated control.

The beginning of growth was delayed with 3 days in the case of rosemary and four days for cocklebur, and after that, a slow growth was seen. The final measurements revealed that the extract obtained from *X. strumarium* had the highest inhibitory activity, equal to a percentage of 86.7%, (mycelium growth was 4 mm), while the one obtained from *D. stramonium* fruits presented the lowest inhibition activity against *A. alternata,* mycelium growth reaching 15 mm on the 9th day since inoculation. At that moment the on the control plate the tested fungus reached the margins.

The daily measurements of the radial growth are given in *Figure 1*.

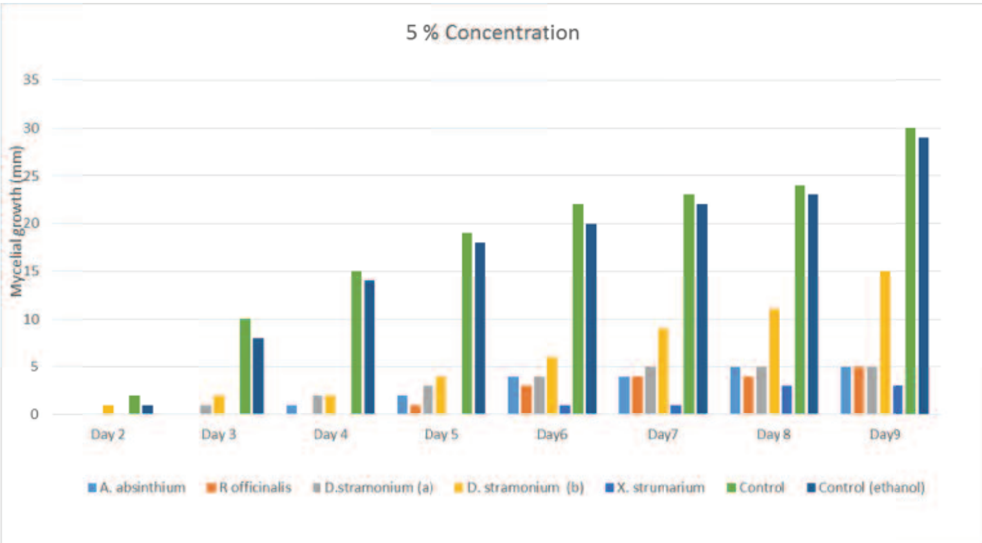

Figure 1. The mycelial growth of A. alternata under the treatment with selected ethanolic plant extracts

In the next phase of experiments, when incorporating the appropriate ethanolic plant extracts quantity to reach 2.5% concentration in the inoculated growth media, the antimicrobial activity decreased in intensity, the fungus developing more rapidly than in previous set of variants, its dynamics being closer to the one of the control.

The *X. strumarium* extract reduced the radial growth of the fungus to 15 mm, while the jimson weed fruits extract treatment resulted in a mycelium growth of 24 mm, this value being close to the development of the control colony with no treatment included, as can be observed in Figure 2.

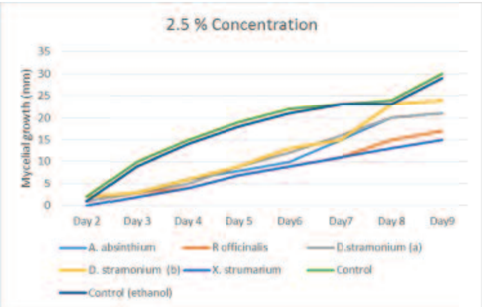

Figure 2. The mycelial growth of A. alternata under the treatment with selected ethanolic plant extracts

The absinth and jimson weed leaves extracts respectively, showed similar results, of 30% inhibition of growth (Table 1), calculated after the formula mentioned in the previous section.

The ethanolic extract of *X. strumarium* was found to be most effective for the control of the test fungus.

All of the aqueous plant extracts showed a lower effect than the ethanolic ones. Their activity at 10% concentration against the fungal colony growth only reached a maximum inhibition percentage of 60% for the *D. stramonium* (a) extract. The lowest results were obtained for the fruit extract of the same plant, approximately 16.7%. The daily measurements effectuated on radial growth are graphically reflected in Figure 3.

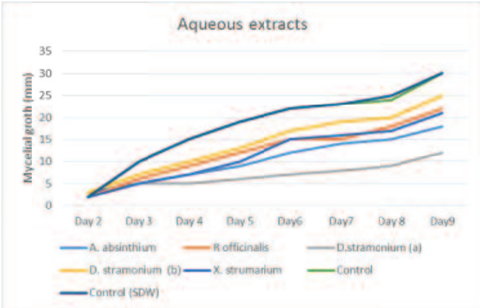

Figure 3. The mycelial growth of A. alternata under the treatment with selected aqueous plant extracts

Therefore, among the aqueous extracts, the one obtained from *D. stramonium* was found to possess the most effective fungitoxic potential for the control of *A. alternata.*

Table 1. The effect of the plant extracts of the inhibition of *A. alternata* growth

Plant extracted	**Percentage of inhibition % of the mycelial growth**		
	5% Ethanolic Extract	**2.5% Ethanolic Extract**	**10% Aqueous Extract**
A. absinthium	83.33	30.00	40.00
R. officinalis	83.33	43.33	26.67
D. stramonium (a)	83.33	30.00	60.00
D. stramonium (b)	50.00	20.00	16.67
X. strumarium	86.67	50.00	30.00

The vegetal material taken into study in the present paper, two medicinal plants and two weed species, is easily available belonging to spontaneous flora of our country. Simple methods of extraction were chosen, in order to make them to be easily exploited in the practical *in vivo* control of plant diseases.

Choosing as raw material the weed species for plant derived fungicides can lead both to an ecofriendly method of disease control and deliver a solution for weed management of agricultural crops, creating economic uses for these unwanted species (Srivastava et al., 2011).

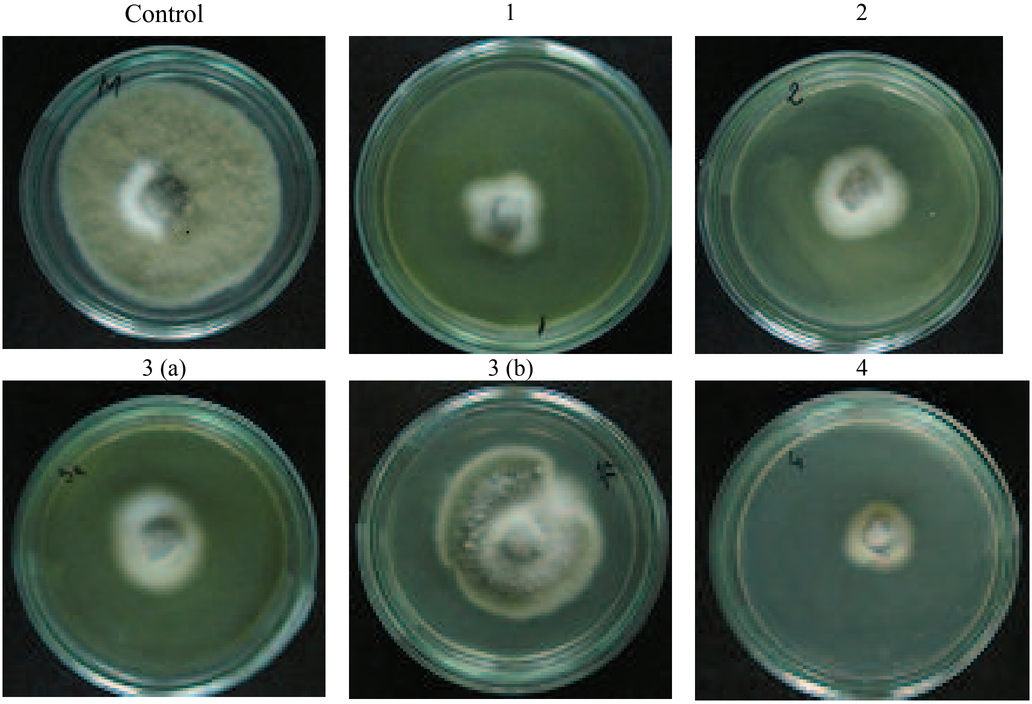

Figure 4. Antifungal activity of the selected plant extracts against *A alternata*. 1. *A absinthium,* 2. *R. oficinallis*, 3. (a). *D. stramonium* leaves, 3. (b). *D. stramonium fruits*, 4. *X. strumarium*,

CONCLUSIONS

Given the fact that there are a limited number of studies presenting evidence of the antimicrobial activity of the medicinal plants against phytopathogen fungi, investigation on this direction still needs to be done.

Further studies should also be done on phytochemical characterization of the extracts obtained.

The results obtained support and confirm the ethnopharmacological uses of the tested plants and provide preliminary information that can be used for further evaluation.

This study shows optimistic results regarding the potential of plant species as sources of plant based products with activity against plant pathogenic fungi.

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