Anales de Biología 40: 115-120, 2018
DOI: http: //dx.doi.org/10.6018/analesbio.40.13 ARTICLE

In vitro effect of crude extracts from eight Algerian steppe plants on mycelial growth and sporulation of *Ascochyta pisi* Lib.

Ammar Tiaiba[1,3], Malika Derbali[2] & Boubekeur Seddik Bendahmane[3,4]

1 Department of Agricultural Sciences, Faculty of Sciences, University of M'Sila, BP 166 Echbilia, 28000 M'Sila, Algeria.
2 Department of Natural and Life Sciences, Faculty of Sciences, University of M'Sila, BP 166 Echbilia, 28000 M'Sila, Algeria.
3 Department of Agronomy, University of Mostaganem, Mostaganem, Algeria.
4 Plant Protection Laboratory, University of Mostaganem, Mostaganem, Algeria.

Correspondence
A. Tiaiba
E-mail: ammar.tiaiba@univ-msila.dz
Received: 27 June 2018
Accepted: 21 September 2018
Published on-line: 30 November 2018

Resumen

Efecto in vitro *sobre el crecimiento micelial y la esporulación de* Ascochyta pisi *Lib. de extractos crudos de ocho plantas esteparias de Argelia*

Se ha estudiado el efecto de extractos crudos de ocho plantas, que crecen en la estepa argelina, contra *Ascochyta pisi* Lib., uno de los miembros del complejo Ascochyta que causa la antracnosis del guisante, con el objetivo de sustituirlos por fungicidas químicos con reconocida nocividad para el medio ambiente y la salud pública. Con este propósito, se realizó una prueba de control biológico *in vitro* del crecimiento y la esporulación de *A. pisi*, agregando extractos de plantas crudas o sus sub-fracciones a su medio de cultivo. Al final de este ensayo, encontramos que los extractos de *Artemisia alba* Turra, *Lycium arabicum* Schweinf. Ex Boiss. y *Peganum harmala* Linn. registraron las mejores tasas de inhibición del crecimiento de patógenos, mientras que *Artemisia campestris* L. es más efectiva contra su esporulación.

Palabras clave: Antracnosis, Guisante, Inhibición de crecimiento, Esporulación, Plantas esteparias, Extracto crudo

Abstract

This work consists of studying the effect of crude extracts of eight plants growing in the Algerian steppe against *Ascochyta pisi* Lib., one of the members of the Ascochyta complex causing the Ascochyta blight of pea, with the aim of substituting them for chemical fungicides recognized for their harmfulness to the environment and public health. For this purpose, an *in-vitro* biocontrol test of the growth and sporulation of *A. pisi* was achieved by adding to its cultural medium a crude plant extracts or their sub-fractions. At the end of this trial, we found that the extracts of *Artemisia alba* Turra, *Lycium arabicum* Schweinf. Ex Boiss. and *Peganum harmala* Linn. recorded the best growth inhibition rates of pathogenic whereas, *Artemisia campestris* L. is more effective against its sporulation.

Key words: Ascochyta blight, Pea, Growth inhibition, Sporulation, Steppe plants, Crude extract.

Introduction

Ascochyta blight is the most important and most devastating disease effecting pea (*Pisum sativum* L.). It is caused by a group of fungi belonging to the genus *Ascochyta* Lib., known as the Ascochyta complex and including *Ascochyta pisi* Lib., *Mycosphaerella pinodes* (anamorphic syn. *Ascochyta pinodes* L. K. Jones) Boerema) and *Didymella pinodella* (L.K. Jones) Q. Chen & L. Cai (anam. syn. *Ascochyta pinodella* L. K. Jones) (=*Phoma medicaginis* var. *pinodella* (L.K. Jones) (Schoeny *et al.* 2007, Tivoli & Bannisa 2007). In recent years, in Australian literature has cited *Phoma koolunga* Davidson, Hartley, Priest, Krysinska-Kaczmarek, Herdina, McKay & Scott as a pathogen causing, alone or in association with the other three species, Ascochyta blight on pea (Davidson *et al.* 2009, Tran & You 2014, Ahmed *et al.* 2015, Barilli *et al.* 2016). In Algeria, and despite the presence of the three species of *Ascochyta* causing Ascochyta blight, *A. pisi* predominates in addition to the other two species (Tadja *et al.* 2009,). The disease affects peas all over the world, including Algeria, where it causes important losses. The lack of effective control measures and the severity of attacks can sometimes lead to the total destruction of crops (Fondevilla *et al.* 2007). The most commonly used control methods focus mainly on the use of chemical pesticides (Onfroy *et al.* 2007), except seed treatments, these products are not very effective in addition to their harmful effects on the environment and the health of the consumer.

Fungal diseases in plants can be controlled by using fungicides. Use of fungicides may have adverse effects including toxicity to humans and organisms in the environment. Alternatively, biological control using natural substances may be used against plant pathogens (Mahlo *et al.* 2010). The search for alternative solutions to the usual chemical control methods is more than necessary. Multiple studies on biopesticides show that some plant-derived products can be a cure for conventional plant protection problems by being effective in controlling plant pathogens (El Guilli *et al.* 2009).

We have conducted this work which aims to study the effect of some crude extracts of plants from the Algerian steppe on the growth and sporulation of *A. pisi*, one of the three pathogens of Ascochyta blight of pea and we also want to a contribution of valorization of plants which grow naturally in the steppe in the research and the development of new molecules of natural origin allowing us to reduce the use of the chemical products, whose problems with which there are bound are well established.

Material and methods

Plant Material

The plant material constituting the source of plant extracts is a set of eight plants collected in the natural El-Mergueb reserve located in the Hodna Steppe (Central Algeria). These eight species belonging to different families and selected according to their importance in pharmacopoeia and popular medicine as well as their abundance which has been visually estimated. These plant species are: *Artemisia alba* Turra., *Artemisia campestris* L., *Cleome arabica* L., *Lycium arabicum* Schweinf. Ex Boiss., *Peganum harmala* Linn., *Thapsia garganica* L., *Thymelaea hirsuta* (L.) Endl., *Thymelaea microphylla* Coss & Dur.

The aerial parts of the steppe plants were collected in the spring of 2015 and kept in the shade to minimize photo-oxidation (Mahlo *et al.* 2010). According to Ogbebor *et al.* (2005), the plant material has undergone drying. Drying of the plants was done at room temperature (about 20-23 °C) for two weeks after being examined and the old and infected leaves were removed. Drying is stopped when the plants are sufficiently brittle. Once dried, the plant material is then ground into a very fine powder using a laboratory plant grinder. The vegetable powder obtained is stored in paper bags in the dark until extraction.

Fungal material

The fungal material is the fungus *Ascochyta pisi*, causal agent of ascochyta blight of a pea. It is isolated from pea plant showing the symptoms of the disease. The cultures used in the bioassay come from pure and single-spore cultures to ensure maximum genetic uniformity (Bowen *et al.* 1997). CDA medium (Chickpea seed meal Dextrose Agar) was selected as a basic culture medium.

Plant extracts and sub-fractions

The crude plant extracts were obtained in a Soxhlet type assembly (Pretorius *et al.* 2002) and whose solvent is absolute ethanol. Part of the

crude extracts (crude extracts will show high levels of inhibition of mycelial growth) are then fractionated separately by successive washing with HCl and NaOH solutions, Dichloromethane, Methanol and Hexane in according to a protocol described by William *et al.*, (2006). In our test, the fractionation only concerned with *L. arabicum* and *A. alba*. Once the different sub-fractions have been obtained, the solvents are then removed from the solutions of the sub-fractions by evaporation under reduced pressure using a rotary evaporator (ROTAVAPOR R-210 BUCHI). The dry extract resulting from evaporation is then dissolved in ethanol at a known concentration and stored at -18 °C. to use. The sub-fractions prepared for testing are detailed in Table 1.

Antifungal activity

The bioassay consists of measuring the mycelial growth and sporulation of *A. pisi* on the CDA medium supplemented separately with plant extracts at 1.5, 3 and 6%. Mycelial growth and sporulation are also evaluated on the same medium supplemented with ethanol at a dose of 3% (solvent control) and on the same medium free of plant extract and ethanol (negative control). Mycelial discs of diameter 5mm taken are then deposited in the centre of each of Petri dish containing the medium with or without added plant extract. The incubation is carried out in the room temperature at 21 °C ± 0.1.

When the growth of the fungus in the Petri dishes of control treatment reaches the edges, the incubation is stopped and the mycelial growth and the number of spores per cm^2 with the MALASSEZ Hemocytometer, are then measured in order to evaluate the effect of the extracts on sporulation and calculate the growth inhibition (GI) rate of the different extracts according to the formula shown below (Dalili *et al.* 2015):

$$GI\,\% = \frac{A-B}{A} x\ 100$$

A: The average mycelial growth in the control treatments.
B: The average mycelial growth in treatments with different plant extracts.

In the same way with the trial with crude plant extracts, another test is carried out by adding the sub-fractions of plant extracts of *L. arabicum* and *A. alba* following the same protocol and with the same measurements carried out previously.

Sub-fraction extraction solvent	Name of sub-fraction
HCl and NaOH Solutions	F2
Dichloromethane	F4
Methanol	F5
Hexane	F6

Tabla 1. Descripción de las sub-fracciones utilizadas en el bioensayo.

Table 1. Description of the sub-fractions used in the bioassay.

Data Analysis

All the results obtained have been statistically analyzed using SAS software (SAS System 9.4, SAS Institute Inc.). ANOVA tests are performed at the 0.05 and 0.01 levels.

Results and discussion

The results clearly show that at the end of the incubation, the mycelial growth of *A. pisi* was totally inhibited compared to the negative control and the solvent control at 6% of all the plant extracts tested. Except for 6% of plant extract, the inhibition rate of growth is clearly proportional to the dose of extract where the growth is more inhibited at 3% than at 1.5% of the extract in the medium. However, at 3% of plant extract in the medium, the highest levels of inhibition were recorded with *L. arabicum*, *A. alba* and *P. harmala* extracts, note that in this case, the inhibition of mycelial growth is around 98%. On the other hand and at the same dose, the extracts from *A. campestris* and *T. microphylla* were less effective than the extracts cited above and recorded average rates which oscillate around 85% (Fig. 1).

Concerning sporulation, it is to be noted that when *A. pisi* is cultivated on a medium containing 6% of plant extract of any plant, does not sporulate contrary to the control and the cultures on medium containing 3% of ethanol. The lowest sporulation levels were recorded with extract at 3%, particularly with the crude extract of *A. campestris* when the medium contains 1.5% of plant extract, the fungus could sporulate at levels sometimes exceeding the controls (Fig. 2).

The result of the evaluation of the inhibition rates of the various crude plant extracts, it appeared to us that the level of 3% of crude extract in the medium of the fungus constitutes the lowest effective dose where, *L. arabicum* and A. alba were relatively effective and their crude extracts were then fractionated into four sub-fractions mentioned.

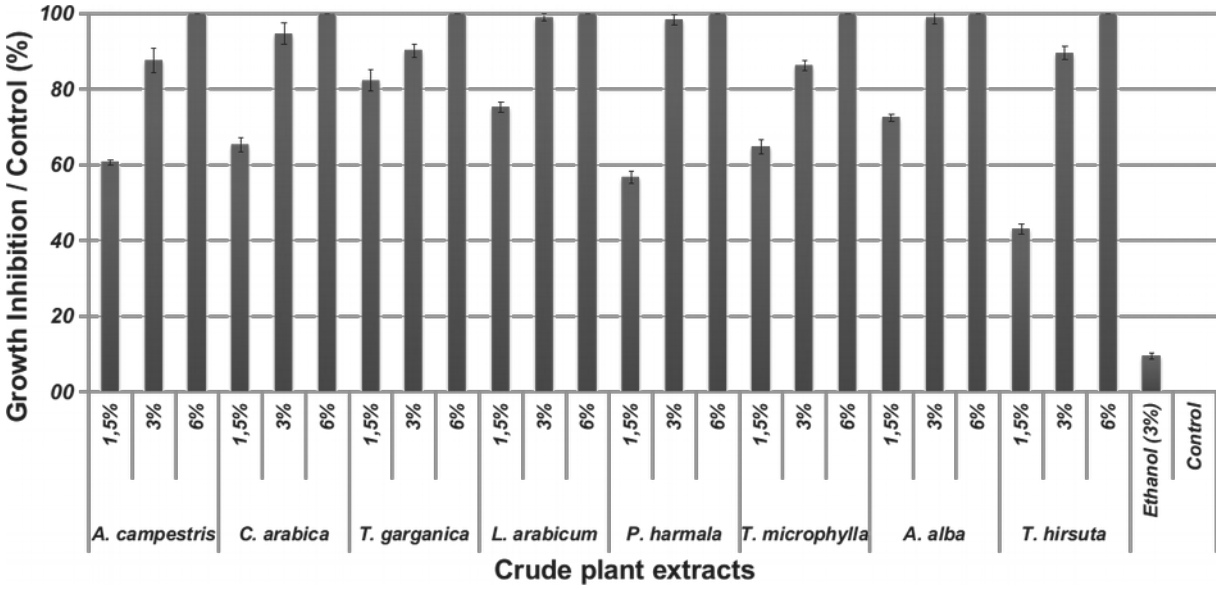

Figura 1. Efecto de los extractos de plantas crudas en el crecimiento micelial de *Ascochyta pisi*.
Figure 1. Effect of crude plant extracts on mycelial growth of *Ascochyta pisi*.

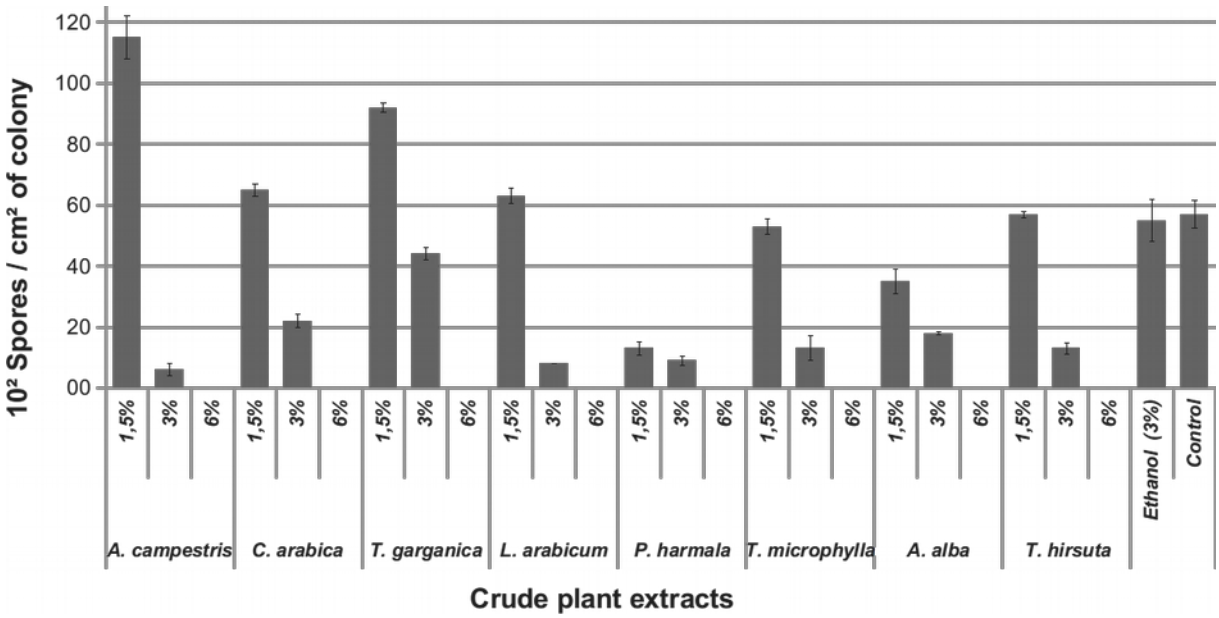

Figura 2. Efecto de extractos de plantas crudas en la esporulación de *Ascochyta pisi*.
Figure 2. Effect of crude plant extracts on sporulation of *Ascochyta pisi*.

The result of the evaluation of the inhibition rates of the various crude plant extracts, it appeared to us that the level of 3% of crude extract in the culture medium of the fungus constitutes the lowest effective dose where the extracts of *L. arabicum* and *A. alba* were relatively effective compared to other extracts and their crude extracts were then fractionated into four sub-fractions that we called F2, F4, F5, F6. In comparison, the effect of the sub- fractions of each of the two extracts of plant species, it seems that all the sub-fractions of *A. alba* significantly inhibit ($P< 0.01$) the growth of *A. pisi* compared to the sub -fractions of *L. arabicum*. The statistical treatment of the results relating to the effect of the different sub-fractions shows a significant action of the type of extract ($P<0.01$) and such results are almost similar to those reported by Tegegne *et al.* (2008) in South Africa. Unlike the effect of the type of the sub-fraction where the differences found were not statistically significant. Numerically, F4 of *L. arabicum* recorded the best rate of inhibition com-

pared to other sub- fractions of the same plant species; nevertheless this rate remains low compared to the rates recorded with all the sub-fractions from *A. alba* (Fig. 3).

The present bioassay, has allowed us to confirm the possibility of valuing some plants of the Algerian steppe known for its phytogenetic biodiversity. The valuation of these naturally growing plants, which are known for their richness in secondary metabolites such as alkaloids, phenolic compounds and essential oils, can be an important source of bioactive natural substances, antifungals especially, potentially able to substitute for conventional phytopharmaceutical products for the purpose of sustainable agriculture that protects the environment and public health.

The eight tested plants showed to be able to inhibit *in vitro* mycelial growth and sporulation at very appreciable levels. The bioassay with the sub-fractions of *A. alba* and *L. arabicum* further supports their effectiveness in biocontroling the growth of *A. pisi* causative agents of Ascochyta blight. All the observations that we have been able to record through this study open up promising prospects for in plant or *in vivo* assays in addition to other trials including other species of *Ascochyta* (*P. medicaginis* var. *pinodella* and *M. pinodes*) which are also involved in Ascochyta blight. Moreover, it is more than necessary to deepen and push the research towards the precise identification of the chemical substances responsible for antifungal activity.

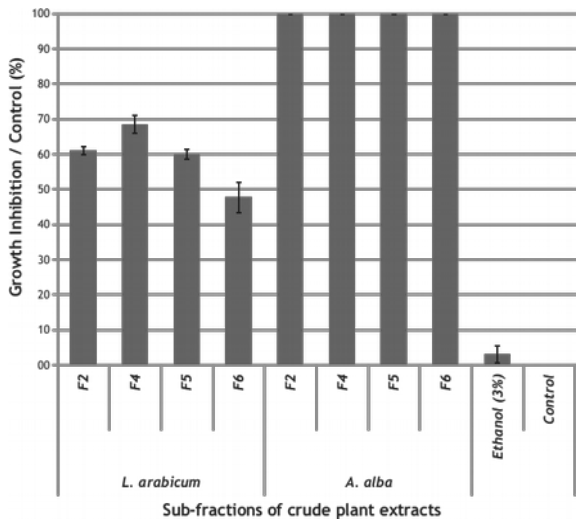

Figura 3. Efecto de la subfracciones de extractos de plantas crudas sobre el crecimiento micelial de *Ascochyta pisi*.

Figure 3. Effect of sub-fractions of crude plant extracts on mycelial growth of *Ascochyta pisi*.

References

Ahmed H, Chang KF, Hwang SF, Fu H, Zhou Q, Strelkov S, Conner R & Gossen B. 2015. Morphological characterization of fungi associated with the ascochyta blight complex and pathogenic Variability of Mycosphaerella pinodes on field pea in central Alberta. The Crop Journal 3: 10-18.

Barilli E, Cobos MJ & Rubiales D. 2016. Clarification on Host Range of Didymella pinodes the Causal Agent of Pea Ascochyta Blight. Frontiers in Plant Science 7:592 [16].

Bowen J K, Lewis BG & Matthews P. 1997. Discovery of the teleomorph of Phoma medicaginis var. pinodella in culture. Mycological Research 101(1): 80-84.

Dalili A, Bakhtiari S, Barari H & Aldaghi M. 2015. Effect of some fungicides against the growth inhibition of Sclerotinia sclerotiorum mycelial compatibility groups. Journal of Plant Protection Research 55 (4): 354-361.

Davidson JA, Hartley D, Priest M, Krysinska-Kaczmarek M, Herdina-McKay A & Scott ES. 2009. A new species of Phoma causes ascochyta blight symptoms on field peas (Pisum sativum) in South Australia. Mycologia 101: 120-128.

El Guilli M, Achbani E, Fahad K & Jijakli H. 2009. Biopesticides: Alternatives à la lutte chimique. Symposium International «Agriculture durable en région méditerranéenne», 14-16 Mai 2009, Rabat Maroc.

Fondevilla S, Cubero JL & Rubiales D. 2007. Inheritance of resistance to Mycosphaerella pinodes in two wild accessions of Pisum. European Journal of Plant Pathology 119: 53–58.

Jones WP & Kinghorn AD. 2006. Extraction of Plant Secondary Metabolites. From: Methods in Biotechnology, Natural Products Isolation. Totowa: Humana Press Inc.

Mahlo SM, McGaw LJ & Eloff JN. 2010. Antifungal activity of leaf extracts from South African trees against plant pathogens. Crop Protection 29: 1529-1533.

Ogbebor N & Adekunle AT. 2005. Inhibition of conidial germination and mycelial growth of Corynespora cassiicola (Berk & Curt) of rubber (Hevea brasiliensis muell. Arg.) using extracts of some plants. African Journal of Biotechnology 4(9): 996-1000.

Onfroy C, Baranger A & Tivoli B. 2007. Biotic factors affecting the expression of partial resistance in pea to ascochyta blight in a detached stipule assay. European Journal of Plant Pathology 119: 13-27.

Pretorius JC, Craven P & Van der Watt E. 2002. In vivo control of Mycosphaerella pinodes on pea leaves by a crude bulb extract of Eucomis autumnalis. Annals of Applied Biology 141: 125-131.

Schoeny A, Jumel S, Rouault F, Le May C & Tivoli B. 2007. Assessment of airborne primary inoculum availability and modelling of disease onset of ascochyta blight in field peas. European Journal of Plant Pathology 119: 87-97

Tadja A, Youcef Benkada M, Rickauer M, Bendahmane BS & Benkhelifa M. 2009. Characterization of Ascochyta as Pathological Species of Pea (Pisum sativum L.) at the North-West of Algeria. Journal of Agronomy 8(3): 100-106.

Tivoli B & Banniza S. 2007. Comparison of the epidemiology of ascochyta blights on grain legumes. European Journal of Plant Pathology 119: 59-76.

Tegegne G, Pretorius JC & Swart WJ. 2008. Antifungal properties of Agapanthus africanus L. extracts against plant pathogens. Crop Protection 27: 1052-1060.

Tran HS, You MP, Lanoiselet V, Khan T N & Barbetti M J. 2014. First Report of Phoma glomerata Associated with the Ascochyta Blight Complex on Field Pea (Pisum sativum) in Australia. Plant disease 98 (3): 427-427.