Mycopath (2019) 17(1): 39-43 **Research Article**

In vitro evaluation of the antifungal potential of *Zizyphus lotus* L. against toxigenic molds of hydroponic barley

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Abstract

This study aimed to focus on the evaluation of the antifungal activity of extract prepared from *Zizyphus lotus* leaves harvested from the mountains of the Medea region in the north of Algeria. The extracts were prepared by maceration of leaves in sterile distilled water, methanol, chloroform and ethyl acetate. *In vitro* antifungal activity of the extracts was evaluated at concentrations of 5, 2.5, 1.25 and 0.625 mg mL^{-1} by the direct contact method on the mycelial growth of toxigenic molds of hydroponic barley *namely Fusarium aveaceanum*, *Fusarium culmorum*, *Alternaria* sp. and *Aspergillus niger*. Chloroform and ethyl acetate extracts exhibited pronounced antifungal potential. The inhibition potential varied proportionally with the concentrations. The fungal growth inhibition potentials varied significantly from 5.9–97%. Thus, *Z. lotus* ethyl acetate extract could be used in the food industry for better phytoprotection of barley hydroponic culture and as a natural conservative of its grains in places of storage.

Keywords: Antifungal activity, *Hordeum vulgare*, Molds, Polyphenolic extracts, *Zizyphus lotus.*

Introduction

The barley (*Hordeum vulgare* L.) has always occupied an important place among other cereals (hard and soft wheat) in Algeria. Until a certain period (1900), it was at the head of cultures and intended for human self-consumption. Its role in animal nutrition has always been and remains fundamental (Rahal-Bouziane, 2015). To face the new challenges (climate change, global economic crisis, rising food prices), our country severely affected by desertification and the scarcity of water, will be confronted more than ever with difficulties to ensure food security. Because of this crisis, various hydroponics techniques were adopted around the world (Texier, 2014). The barley hydroponic cultivation has several advantages such as controlling nutrition, conserving water, better yields and better qualities of barley and reducing of pesticides use for a better health and faster growth. Unfortunately, it is more exposed to fungal contamination responsible of deterioration of organoleptic quality, the risk of toxicities caused by mycotoxins and the reduction in the nutritional quality of barley. Some species of fungi are also less susceptible to antibiotics and develop multiple resistances (Pibiri, 2006), hence there is the need to search for an effective natural fungicide. Benaissa (2011) proposed medicinal plants as a real and effective source against this problem. Their major originalities lie in their ability to produce very diverse natural substances and represent an important source of molecules used by humans in the fields of pharmacology or food industry (Macheix *et al.*, 2005).

In recent years, bioactive plant constituents have been characterized for their antifungal activities and are now used as effective fungicides (Naqvi *et al*., 2019; Akhtar *et al*., 2020; Javaid *et al.*, 2020), but there is a great need for more botanical products to be used as fungicides. In addition, very little work has focused on the antifungal activity of *Zizyphus lotus*, particularly in the food industry. It has been mentioned that all parts of this plant are rich in polyphenols such as flavonoids, phenolic acids, and other natural compounds. The variability of their biomolecule composition depends on the environment, soil type, climate, or the age of the plant. Their biological activities have been studied in relation to their active compounds including, flavonoids, several saponins, and alkaloids. It was reported that the alkaloids of *Z. lotus* have significant antifungal and antibacterial properties (Le Crou'eour *et al.*, 2002). In Morocco, fruit extracts of this plant obtained by maceration with different organic solvents of increasing polarity (ether, dichloromethane and methanol) have confirmed an interesting antifungal activity on various fungal species namely *Fusarium culmorum*, *Aspergillus ochraceus*, *Penicillium italicum*. Inhibition rates

ranged from 31 to 85%. Also, a strong correlation was observed between the concentrations of these components in fruit extracts and their antimicrobial activity (Rsaissi *et al*., 2013). In this context, this study aimed to investigate antifungal potential of foliar extracts of *Z. lotus* against toxigenic molds of hydroponic feed barley.

Materials and Methods

Biological materials

Leafy twigs of *Z. lotus* were harvested from jujube shrubs in June 2018, in the mountain of Medea region, located in the north of Algeria. The collected leaves were dried in the open air and protected from light to be used for the extraction. The fungal material consisted of purified cultures of four fungal isolates namely *F. aveaceanum*, *F. culmorum*, *Alternaria* sp. and *A. niger*. These purified cultures were procured from the Mycotheque of Dr. Saida Messgo-Moumene in the Laboratory of Research on Medicinal and Aromatic Plants of the University of Blida 1, Algeria. They were isolated from hydroponics samples from feed barley of the local cultivar called "Saïda" and have been the subject of numerous research studies (Messgo-Moumene *et al.*, 2018).

Preparation of *Z. lotus* leaf extracts

This study was based on the extraction of phenolic compounds, from the leaves of *Z. lotus* by the maceration method, using sterile distilled water and different organic solvents namely methanol, chloroform and ethyl acetate (Venturini *et al*., 2010). The methodology of total polyphenols extraction was that proposed by Mohammedi and Atik (2011) with some modifications. For this purpose, 30 g of dry leaves were extracted by maceration under magnetic stirring for 24 h with successively each of the aforementioned solvent systems with the proportions of 70/20 v/v (Goni and Serrano, 2005). The macerates obtained were filtered using Wattman paper and the filtrates were evaporated to dryness under reduced pressure using a rotary evaporator. The dry extracts obtained were then stored in previously sterilized bottle, covered with aluminum paper, protected from light, then placed in the refrigerator at 4 °C until used.

Antifungal assays

The dry extracts previously stored were collected to prepare aqueous solutions using sterile distilled water at the concentrations of 0.625, 1.25, 2.5, and 5.0 mg mL^{-1}. The *in vitro* antifungal activity was studied using different concentrations of the extracts against the various fungal isolates by the direct contact method on potato dextrose agar medium (PDA) to evaluate mycelial growth inhibition rates (Paranagama *et al*., 2003).

A volume of 10 mL of each prepared sample was placed separately in Petri dishes of 90 mm diameter, followed by adding 15 mL of PDA sterile medium that was still in a melted state. The Petri dishes were shaken manually for homogenization of the extract with the culture medium. The control fungal cultures were prepared with only PDA. The preparation of all the Petri dishes was done in aseptic conditions. After solidification of the medium, the mycelial discs of 5 mm in diameter of each isolate of *F. aveaceanum*, *F. culmorum*, *Alternaria* sp. and *A. niger* were transplanted separately using a Pasteur pipette by depositing the mycelial disc in the centre of the Petri dishes. All the prepared dishes were sealed with parafilm before incubation. Five repetitions were considered for each treatment. The PDA medium without extract served as a control for each isolate (Mishra and Dubey, 1994). The incubation was carried for 7 days at a temperature of 25 °C (Messgo-Moumene, 2018).

Mycelial growth of tested isolates and those of the positive controls was determined by measuring two perpendicular diameters of the colonies formed, passing through the centre of the deposited mycelial disc. Mycelial growth inhibition rates were calculated for each plant extract prepared, for each concentration selected and for each fungal isolate tested, according to the formula described by Mishra and Dubey (1994):

$$I\ (\%) = (DT-Dt) / DT \times 100$$

Where, I: % inhibition of mycelial growth of each fungal isolate tested; DT: mycelial growth (mm) of each control fungal isolate tested; Dt: mycelial growth (mm) of each fungal isolate developed on medium in the presence of extracts of different concentrations. The development of fungal cultures having shown an interesting mycelial growth inhibition under the effect of the various prepared extracts was continued under the same incubation conditions for three weeks in order to confirm their fungicidal or fungistatic power translated respectively by the stability in inhibition or the resumption of their mycelial growth (Mahanta *et al.*, 2007).

Statistical analysis

In order to verify a possible effectiveness of *Z. lotus* against each of the fungal isolates tested and to compare their *in vitro* antifungal potency on mycelial growth, statistical analyses were carried out on the inhibition rates of mycelial growth using the software SYSTAT version 12, by determining the variance using the ANOVA test and the GLM (Generalized Linear Models) model. The differences were considered significant for $P \leq 0.05$ (Philipeau, 1989). Tukey's test was also carried out to classify the inhibition rates into homogeneous groups according to the studied parameters, using the SPSS software. The results were expressed as mean ± standard deviation. P values <0.01 were considered statistically significant (Athamena *et al.*, 2010).

Results and Discussion

The polyphenolic extracts prepared from the leaves of *Z. lotus* showed antifungal activity against the various tested fungal isolates (Table 1). Mycelial growth inhibition rates showed very highly significant effect according to the nature of the tested extracts, their concentrations and according to the studied fungal species. However, the greatest inhibition in the fungal growth was shown by the ethyl acetate extract (45–96%) followed by chloroform extract (28–85%). On the other hand, methanolic and aqueous extracts showed weak inhibitory activities (Table 1).

According to the Tukey's test, *F. aveaceanum* was highly significantly inhibited by methanol (97 ± 0.1%) followed by ethyl acetate, (96.3% ± 0.24), aqueous (92.9% ± 0.1) and chloroform (85.9% ± 0.05) extracts. Likewise, aqueous and chloroform showed 90% reduction in the growth of *Alternaria* sp., while ethyl acetate and methanol extracts displayed 86-87% inhibition. In case of *F. culmorum*, methanol (85 ± 1.33%) followed by ethyl acetate (77.5 ± 0.3%), chloroform (76 ± 0.6%), aqueous (62.5 ± 1%); while for *A. niger*, ethyl acetate (45.6 ± 0.2%) followed by chloroform (28.8 ± 0.2%) and methanol (6.42 ± 0.3%) extract exhibited marked antifungal activity (Table 1). Inhibition in growth appeared to be moderate for all of the fungal isolates at the MIC of 0.625 mg mL^{-1}, except for *A. niger*. The ethyl acetate extract was the most efficient against all the tested isolates (Table 1). Referring to the inhibition rate recorded by extracts of *Z. lotus*, several studies corroborate with the current findings. Mushatq *et al*. (2012) found 48% inhibition in mycelial growth of *Alternaria alternate* by chloroform extract of *Lantana camara*. In addition, moderate inhibition *i.e.* 40% was recorded by some plant extracts against *A. niger* such as ethanolic garlic extract (37%) and *Azadirachta indica* (38%) aqueous extract, respectively proved by Qadir *et al.* (2017) and Keta *et al.* (2019). These results coincided with those revealed by the ethyl acetate extract from our plant but seemed more interesting than those presented by the other extracts. Indeed, differences in results of bio-effectiveness may be due to the amount and quality of bioactive metabolites contained in the extract, which depends on the different plant species, used parts of the plant, the extraction methodology, concentrations tested, of the bioactivity assessment technique, as well as the sensitivity or resistance of the different microorganisms isolates.

Moreover, the difficulty of developing an antifungal molecule is linked firstly to the ultra-structure of the fungal cell which has three gates: the chitinous cell wall, membrane ergosterol and eukaryotic nucleus (Chami, 2005) and on the other hand, the antifungal molecules themselves which can generate resistance (Prasad and Kapoor, 2004). Some authors have described the mode of action of plant extracts by changing the morphology of the tested fungus. Romagnoli *et al.* (2005) explained the inhibitory effect of essential oils on the mycelial growth of *Candida albicans*, by observations with a scanning and transmission electron microscope, the results showed an increase in permeability followed by a rupture of the membrane plasma resulting in leakage of cytoplasmic content and, therefore, death of the yeast (Cox *et al*., 2000). Bouchra *et al*. (2003) revealed the toxic effect of plant extracts components on the functionality and structure of the cell membrane. Fungal morphological changes resulted in mycelial lysis, the destruction of the mycelial content as well as of preventing conidial germination and growth. Sharma and Tripathi (2006) have in turn translated the lysis and vesiculation of the mycelium by the activity of the chemical compounds of essential oils and polyphenolic extracts on the hyphae, leading to the release of cytoplasmic inclusions, the loss of rigidity and the integrity of the cell wall, leading to its fragmentation and the death of the fungus.

Conclusions

In conclusion, the significant growth inhibition of all fungal isolates tested was granted to *Z. lotus* ethyl acetate extract, which was very effective in its inhibitory potential for its use in the food industry for better phytoprotection of barley hydroponic culture and as a natural conservative of its grains in places of storage.

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Table 1: Mycelial growth inhibition of fungal pathogen isolates according to the nature and concentrations of *Zizyphus lotus*polyphenolic leaves extracts.

Nature of Plant extract	Concentration of plant extracts (mg mL^{-1})	Inhibition in growth (%)			
		Fusarium avenaceum	***Fusarium culmorum***	***Alternaria alternata***	***Aspegillus niger***
Aqueous	5	92.9 ±0.1a	62.5±1a	90.3±0.1a	0
	2.5	70±0.23a	50±1.3b	86.5±0.6b	0
	1.25	51±0.08b	37.4±0.3c	77.5±0.3c	0
	0.625	47±0.1b	30±1.3d	40±0.33d	0
Methanol	5	97±0.1a	85±1.33a	86±0.1a	6.42±0.3a
	2.5	76±0.6b	70±3.3b	77.5b	5.9±0.27a
	1.25	72.2±1.2c	51±0.6c	71b	0
	0.625	61.1±1.26d	45±3.3d	60±0.3c	0
Chloroform	5	85.9±0.05a	76±0.6a	90.3±0.1a	28.8±0.2a
	2.5	85.1±0.06b	71.2b	77.5±0.3b	23.47b
	1.25	74.09±0.07c	64±0.1c	67.7±0.5c	18±0.03c
	0.625	66.4±0.26 bc	62.5c	60±1.3d	12±0.2d
Ethyl acetate	5	96.3±0.24a	77.5±0.3a	87.5±0.3a	45.6±0.2a
	2.5	88.7±0.15b	67.6b	85.6±0.2b	43.5±0.3b
	1.25	70.3±0.1c	56±0.3c	74.5±0.3c	42±0.04c
	0.625	58.5±0.3d	42.5±0.3d	58.6±0.2d	39±0.06d

In a column, means followed by the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

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