PharmacologyOnLine

Archives • 2021 • vol.2 • 485-491

ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OIL OF MORROCAN MYRTLE (*MYRTUS COMMUNIS L.*): APPLICATION IN AGRICULTURE

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Abstract

Anthracnose, caused by *Ascochyta sp. (Pass.) Lab.,* is the most important disease of chickpea (*Cicer arietinum L.*) which occurs mainly in the regions of the Mediterranean, the Near and Middle East and the North. West India. It is a handicap to the development of winter sowing and therefore is a brake on improving yields. For this, our study was carried out in order to deepen our knowledge of the nature of the relations of the couple *Cicer arietinum - Ascochyta rabiei* and to specify the heredity of resistance of chickpeas to this pathogen.

The extraction of essential oil from the aerial part of *Myrtus communis L.*, harvested from the region of khenifra (Middle Atlas central in Morocco) was carried out by hydrodistillation, the yield was 0.87%, the chemical composition of the extracted ETs was determined by the use of gas chromatography (GC) and gas chromatography / mass spectrometry (GC / MS) coupling. The results obtained revealed the existence of the following major components: Limonene (22.34%), Linalool (17.11%), α-pinene (12.22%), 1,8 cineole (10.54%), geranyl acetate (7.91%) , Myrcene (7.21%) and linalyl acetate (6.31%). The antifungal activity of the essential oil of Moroccan *Myrtus communis L.* at different concentrations is evaluated *in vitro* against four phytopathogenic fungi, *Ascochyta rabiei (Pass.) Labr.*, *Fusarium oxysporum f. sp. Ciceris*, *Botrytis cinerea*, and *Fusarium solani f.sp. pisi*.. The results show the growth inhibiting power with a remarkable difference for each concentration and according to each strain. on the other hand, the fungicidal power of the essential oil of *Myrtus communis L.* was marked for all the strains studied.

Keywords: *Myrtus communis L., Essential oil, chemical composition, fungal activity.*

Introduction

Food legumes occupy an important place in the human diet for many developing countries [1-2]. These, rich in proteins, make it possible to a certain extent to correct the deficiencies in animal proteins of a population whose diet is exclusively based on cereals. Among legumes, the chickpea (*Cicer arietinum L.*) which occupies the third position in the world after beans and peas as a cultivated food legume, it is considered an essential source of protein in particular, in the South and West Asia and North Africa. In addition to its role in food, chickpea has a great agronomic interest in enriching the soil with nitrogen which can exceed 70 kg / ha and therefore its ability to maintain soil fertility for a long time [3-4]. In Morocco, chickpea cultivation comes second after the bean with an area of 77,000 ha in 1989-90 and 43,600 ha in 2011-2012 and 59,000 ha during the 2014-2015 agricultural campaign, i.e. a decline of 23% [5]. The chickpea sector in Morocco remains traditional and faces several agronomic and climatic constraints which only reduce the area sown and production [6]. The national average yield of 6.8qx / ha remains low and lower than the global average yield of 7qx / ha and the average yield of some countries such as that of Italy 12qx / ha [7]. Spring plantings which expose the crop to drought during the critical flowering period, coinciding with certain diseases and pests, are the main causes of low yields. In fact, chickpea cultivation, like all other field crop species, is generally exposed to various unfavorable environmental stresses which limit production.

Materials and Methods

Plant collection

Myrtus communis L., is a plant of the Myrtaceae family, this aromatic and medicinal plant belonging to the eighth largest family of flowering plants, which has more than 140 genera and approximately 5,600 species. In this work this species was collected in the regions of Khenifra (Middle Atlas central of Morocco). Then this species was verified by a specialized botanist team at the Forest Research Center of Khenifra (Morocco).

Extraction and analysis of essential oil

The operation of extracting essential oil was used by the hydrodistillation method in a Clevenger type apparatus. Distillation was carried out by boiling for an hour and a half 200 g of plant material with one liter of distilled water. The essential oil obtained is dried over anhydrous sodium sulfate (Na_2SO_4). The essential oil was stored at four degrees Celsius in the refrigerator for later use [8].

The yield "Y" of the essential oil is the ratio of the weight w of the essential oil extracted to the weight W of the plant material. It is expressed in ml of distillate per 100 g of dry matter (w / w%):

$$Y(\%) = \frac{w}{W} \times 100$$

The essential oil of *Myrtus communis L.* is analyzed by gas chromatography and gas chromatography coupled with mass spectrometry (CPG and CPG / MS).

For the sample analysis, 20 µL of essential oil was used in 980 µL of dichloromethane, which was then injected into the gas chromatograph of the Agilent Technologies 7890A type coupled to a detector of the Agilent Technologies 5975C Selective type. mass. The separation of the compounds from the mixture was carried out using an apolar capillary column (5% methylsiloxane phenyl, length 60 m and 0.25 mm internal diameter, the film thickness is 0.25 µm). The injector temperature was programmed to 250 ° C and the injection was maintained in split mode (20: 1). The oven temperature program was as follows: initial temperature 50 ° C, later it was increased to 2.5 ° C / min up to 180 ° C, then 10 ° C / min up to 200 ° C , and finally at 20 ° C / min up to 240 ° C. The run time was 60 minutes. The carrier gas is helium with a constant flow rate of 1 mL / min. The different constituents of essential oils were identified by comparing each peak with those from the mass spectra library databases by: National Institute of Standards and Technology (NIST 17).

Mushroom collection

Four fungal strains were used in this study: *Ascochyta rabiei (Pass.) Labr.*, *Fusarium oxysporum f. sp. Ciceris*, *Botrytis cinerea* and *Fusarium solani f.sp. pisi.*, harvest on the spot with the help of an agronomist in EST-Khenifra (Morocco).

Antifungal activity

Ascochyta rabiei (Pass.) Labr., *Fusarium oxysporum f. sp. Ciceris*, *Botrytis cinere*, and *Fusarium solani f.sp. pisi* were screwed using the food poisoning technique [9]. The essential oil fractions are dissolved in 0.5 ml of 5% (v / v). Tween-80 was added to various sterilized Petri dishes (9 x 1.5 cm) which contained 9.5 ml of PDA medium in order to provide the required concentration of 0.001 up to 1 mg / ml. The witnesses without essential oil were inoculated by following the same process.

A disc of mycelium (diameter of 5 mm) of pathogenic fungi, taken from the periphery of the culture for seven days previously, was aseptically inoculated in the center of the Petri dishes containing the treatments and the control. Petri dishes were incubated at 22 ± 2 ° C for seven days. Three repetitions were performed for each treatment. The diameter of the fungal colonies of the treatment lots and the diameter of the positive control were measured. The percentage inhibition PI of the mycelium was calculated by the mean value of the colony diameters by the following formula [10]:

$$PI(\%) = (\frac{d_t - d_T}{d_t}) \times 100$$

d_T: the average diameter of the treated fungal colonies.

d_t: the average diameter of the fungal colonies in the control.

The food poisoning technique was used to determine the MIC of the essential oils necessary for inhibiting the growth of the mycelium of the fungi tested [11]. Different concentrations of the oil from 0.001 to 1 mg / ml and the control without essential oils were prepared by separately dissolving its necessary amount in 0.5 ml, 5% (v / v) of tween-80 and then by dissolving it. mixing with 9.5 ml of medium PDA. The inoculated Petri dish was incubated for seven days at 22 ± 2 ° C and the lowest concentrations without growth observable with a binocular magnifying glass were defined as the minimum inhibitory concentration (MIC). Three repetitions were performed for each concentration.

Result and discussion

Essential oil analysis

The average essential oil yield of *Myrtus communis L.* was calculated by the dry plant matter of the aerial part of the plant. The *Myrtus communis L.* species sample yielded a rate of approximately 0.46%.

The results obtained for the yields agree with those of the iteration, in Morocco the results according to Satrani et al. (2006) [12] it varies between 0.3 to 0.4% and it is calculated from the dry plant matter it is significantly higher than that of Tunisian and Algerian Myrtle [13-14]. So, we can conclude that the factors influence the yield are numerous, the harvest season, the origin of the plant the parts of the plant used, the extraction processes.

The analysis of the results of the chemical composition carried out by gas chromatography (GC-MS) of essential oil of *Myrtus communis L.* studied is mentioned in Table 1. Analysis of the results given in this table showed all of the following results: Limonene (22.34%), Linalool (17.11%), α-pinene (12.22%), 1.8 cineole (10.54%), geranyl acetate (7.91%), Myrcene (7.21%) and linalyl acetate (6.31%) were obtained as major chemotypes, and which represent a percentage of (83.64%) in the essential oil of *Myrtus communis L.* The plant contains many biologically active compounds. The oil has been widely studied, its composition is quite variable depending on the geographical region of production, the harvest season and the duration of distillation [15-16]. However, in most regions, terpenoids compounds (1,8-cineole, α-pinene, myrenyl acetate, limonene, linalool, α-terpinolene) are the main constituents present in the essential oil obtained from the leaves [17-19]. The results obtained by Yadegarinia et al. (2006) [20] show that the essential oil composition of *Myrtus communis L.* Iranian has thirty-two compounds including a-pinene (29.1%), limonene (21.5%), 1,8-cineole (17.9%) and linalool (10.4%) were the major compounds, but the results presented by Akin et al. (2010) [21] show that the main components in oils of *Myrtus communis L.* from northern Cyprus were eucalyptol (50.13%),

Linalool (12.65%)), α-Terpineol (7.57%) , Limonene (4.26%).

Antifungal activity

The antifungal activity *in vitro* of the essential oil of Moroccan *Myrtus communis L.* at different concentrations is evaluated against four phytopathogenic fungi, *Ascochyta rabiei (Pass.) Labr.*, *Fusarium oxysporum f. sp. Ciceris*, *Botrytis cinerea*, and *Fusarium solani f.sp. pisi.*, expressed as the percent inhibition of growth (PI%). The results of Table 3 show the growth inhibiting power with a remarkable difference for each concentration and according to each strain. The most precise antifungal properties of *Myrtus communis L.* essential oil were determined by determining the minimum inhibitory concentrations (MIC) as well as the nature of the fungitoxicity (Table 3). *Myrtus communis L.* has a low MIC of 0.01 mg/ml for *Ascochyta rabiei (Pass.) Labr.*, *Fusarium solani f.sp. pisi* and *Botrytis cinerea*, versus *Fusarium oxysporum f. sp. Ciceris* which was 0.05 mg/ml.

Conclusion

Through this study the antifungal activity increases as the concentration of essential oil increases this is induced to regress the rate of mycelial growth. This study once again allows the development of the use of essential oils in other industrial fields such as bio fungicides, and at the same time, it confirms their use as a preservative in the field of the food industry, where it It appears that the essential oil of *Myrtus communis L.* could be valued more particularly in the fight against many fungal species responsible for the various phytopathogenic forms. The in vitro efficacy could be explained by the richness of this plant in aromatic compounds. All of these results obtained in vitro only constitute a first step in the search for substances from a natural, biologically active source. These preliminary results can be supplemented by other more in-depth studies: testing other fungal strains and exploiting the antifungal properties as well as the fungistatic / fungicidal activity test. And other in vivo and in situ tests.

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Table 1. Chemical composition of *Myrtus communis L.* essential oil.

Peak	RT	Component	(%)
1	10.51	α-thujène	0.31
2	12.37	α-pinène	12.22
3	13.64	β- pinène	0.01
4	14.24	Myrcène	7.21
5	15.55	α-phéllandrène	0.01
6	16.82	p-cymène	0.19
7	17.01	Limonène	22.34
8	18.73	1,8 cinéole	10.54
9	19.27	(E)- β ocumène	0.15
10	20.64	α-terpinolène	0.01
11	21.61	Linalool	17.11
12	22.71	*allo*-ocimène	0.12
13	23.55	terpinène-4-ol	0.18
14	24.67	α-terpinéol	1.57
15	25.37	Estragole	1.87
16	26.59	trans carvéol	0.01
17	27.99	Nérol	0.23
18	28.37	cis-carvéol	0.01
19	29.46	Carvone	0.01
20	30.52	linalyl acétate	6.31
21	31.00	Géranial	0.25
22	32.61	bormyl acétate	0.01
23	34.37	methyl géranate	0.21
24	35.71	α-terpényl acétate	0.62
25	36.68	néryl acétate	1.02
26	37.46	géranyl acétate	7.91
27	38.79	methyl eugénol	1.36
28	39.21	E-caryophyllène	0.75
29	40.31	γ-élemène	0.01
30	42.83	α humulène	0.74
31	45.15	(E-E), α-famésène	0.01
32	46.16	δ-cadinène	0.55
33	47.53	oxyde de caryophyllène	5.93
34	50.98	Caryophylladienol II	0.21
Total			**99.90**

Table 2. Antifungal activity of essential oil of *Myrtus communis L.*

Essential oil	Dose (mg/ml)	Percentage Inhibition (PI %)			
		A. rabiei	***F. oxysporum***	***B. cinerea***	***F.solani***
Myrtus communis L.	**1.000**	100±0.0	100±0.0	100±0.0	100±0.0
	0.800	100±0.0	100±0.0	100±0.0	100±0.0
	0.600	100±0.0	73.01±0.4	100±0.0	75.82±0.8
	0.400	88.95±0.5	51.55±1.2	76.09±0.6	55.34±0.3
	0.200	65.42±.0.8	41.34±0.8	62.54±0.7	44.05±1.5
	0.100	49.52±0.9	19.45±0.9	42.37±0.5	21.16±2.3
	0.050	18.11±0.6	3.43±1.1	15.25±1.5	12.33±1.6
	0.010	3.64±1.0	NA	2.38±1.3	2.51±0.4
	0.001	NA	NA	NA	NA

Table 3. Nature of the fungitoxicity of essential oil *of Myrtus communis L.* and MIC (mg/ml).

Essential oil	Fungal strain							
	A. rabiei		***F. oxysporum***		***F. solani***		***B. cinerea***	
	MIC (mg/ml)	**NF**	**MIC (mg/ml)**	**NF**	**MIC (mg/ml)**	**NF**	**MIC (mg/ml)**	**NF**
Myrtus communis L.	0.01	-	0.05	-	0.01	-	0.01	-

- , Fungicidal; +, fungistatic