Ethnopharmacological Study and Antifungal Activity of Three Plants (Asteraceae Family) From

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ABSTRACT

Anvillea radiata, Asteriscus graveolens and Dittrichia viscosa are plants belonging to Asteraceae family; they are commonly used in traditional medicine in south west of Morocco, for the treatment of several diseases. The aim of this work was to find an alternative to chemical fungicides currently used in the control of pathogenic fungi. In this study, we screened three Moroccan medicinal and aromatic plants extracts for their anti-fungal activity against Fusarium oxysporum f. sp. albedinis (Foa) (causing vascular wilt of date palm). The aqueous extract of A. radiata and dichloromethanic extract of D. viscosa have produced the greatest inhibitory effect on mycelial growth (more than 50%).

The spore germination was strongly inhibited using aqueous and chloroformic extracts of A. radiata. Thus, it can be concluded that the use of A. radiata, D. viscosa and A. graveolens extracts could be considered as an antifungal available to develop novel types to control several plant pathogenic fungi.

Keywords: Antifungal activity, *A. radiata, A. graveolens, D. viscosa, Fusarium oxysporum f. sp. albedinis, extract*

INTRODUCTION

Tafilalet region is a part of south-eastern Morocco which has historically played an important role as the crossroads for desert caravans between South and North Africa. It is largely called the door to the desert. It is considered among the regions of Morocco in which phytotherapy knowledge is very developed. Its climate is arid inducing various flora with concentrated active substances (Eddouks, et al., 2002).

Palm trees (*Phoenix dactylifera* L.) constitute the ecological and socio-economic womb of the Saharan populations. They offer a suitable microclimate for other crops, and they protect them from the wind. Palm trees represent a basic food source for the people and animals of the Sahara and make a significant economic contribution to the country. This harmony represents the Saharan ecosystem (Ouinten, 1996).

The most destructive fungal disease of date palm is undeniably the wilt caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa) commonly known as 'Bayoud' (Sedra, 2013). The impact of this disease is very serious in North Africa, especially in Morocco where it has killed more than 10 million palm trees during the last 100 years (Saaidi, 1992).

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The search of more new antifungal natural plant products could be more useful against plant pathogens, especially Foa. The aim of this work is to make a pre-ethnobotanical survey and reports the antifungal efficacy of three plants belonging to the Asteraceae family: *Dittrichia viscosa* (L.), *Anvillea radiata* (Coss & Dur) and *Asteriscus graveolens* (Forssk.) less, against Foa tested for spore germination inhibition, radial growth inhibition of *Foa*.

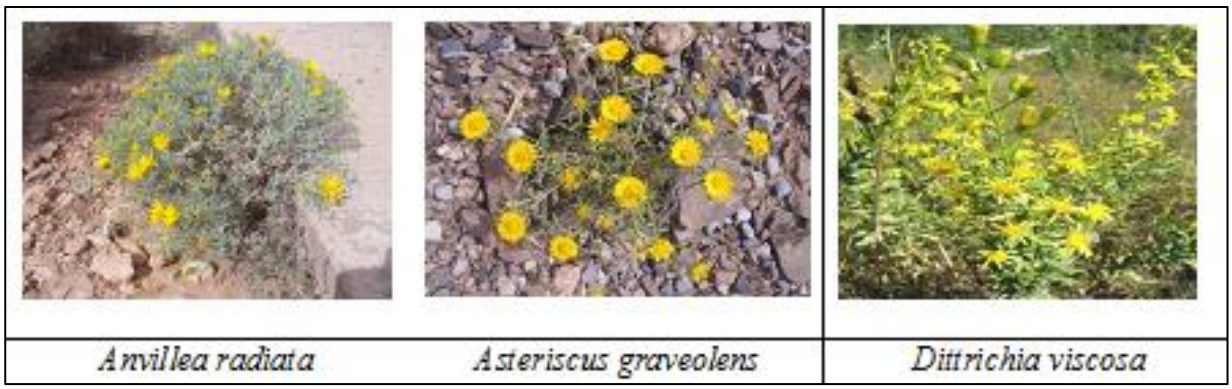
MATERIALS AND METHODS

The ethno pharmacological investigation

This ethno pharmacological investigation was performed in different areas of Tafilalet region, including the following villages: Errachidia, Goulmima, Rissani and Tinjdad, during two months and including two hundred persons. The questionnaire includes questions on plant parts used, Therapeutic use, medicinal preparation methods. Gross checked by interviewing old people aged between 50 and 70 years old, who had traditional a knowledge about plants (Ziane, et al., 2013).

Plant Materials

At flowering stage, fresh aerial parts of *Anvillea radiata* and *Asteriscus graveolens* were collected in April 2013 from Tafilalet (Southeast of Morocco) and from Mohammadia (northwest of Morocco) for *Dittrichia viscosa*.



Preparation of Plant Extracts

Preparation of crude extract by Soxhlet method

Aerial parts of three medicinal plants were collected and air-dried by keeping them in shade for 2 weeks. The dried plants were grinded to fine powder with electronic grinder. Sixty-gram aerial parts of *A. radiata* and *D. viscosa* powder was extracted by Soxhlet method. Using two solvents (300 ml each) in ascending order of polarity (chloroform and methanol) for *A. radiata* and dichloromethane for *D. viscosa*.

In the end of soxhlation, the solvents was evaporated at lower temperature under reduced pressure in rotary flash evaporator to get the crude extract. The extracts were stored in dark vials at 4°C for future uses.

Aqueous extraction

In this method, 20 gram of the crushed plants aerial part (*Asteriscus graveolens* and *Anvillea radiata*) were dipped separately in 250 ml of the distilled water for 48 h at room temperature in a conical flask and were homogenized using a magnetic stirrer-type IKAMAG RCT. The extracts were filtered and the filtrates were evaporated. The aqueous extracts were stored in dark vials at 4°C for future uses.

Fungal Strain

The fungal strain used in this study is *Fusarium oxysporum* f. sp. *albedinis*, (Foa) the causal agent of Bayoud disease that affects the date palm trees (*Phoenix dactylifera* L). The strain used in this study was received from Biotechnological Plant Laboratory, Faculty of Sciences and Techniques, Errachidia, Morocco.

Antifungal efficacy

Poisoned food technique

The sterilized (passing through 0.20 µm pore-size Millipore Nalgene filters) plant extracts were added to PDA (at 45°C) to give a final concentration 7.4, 3.84, and 1.9 mg ml^{-1} for each extract and the resulting media were poured in petri dishes (9 cm in diameter). Then, inoculum discs (5 mm in diameter) from seven days growing cultures of Foa were placed in the center of petri plates containing PDA and extracts, Pathogen grown on PDA without plant powders was used as control. Each treatment was tested on three plates as replications. The plates were incubated at 25±2^{o}C. On the 2, 4, 6 and 8th day (when the fungus overgrow on control plates), radial growth of Foa was recorded for each plate by calculating the average of two perpendicular diameters. Growth inhibition of the treatment against the control was measured by mycelial growth inhibition and calculated as per formula of Pandey et al.,1982 (Pandey, et al., 1982).

$$\text{Percentage of mycelial growth inhibition (IP)} = \left(1 - \left(\frac{Dc}{Dt}\right)\right) \times 100$$

Where: D_c: Average diameter (in mm) of mycelial in control; D_t: Average diameter (in mm) of mycelial in treatment.

Spore germination assay

Spore germination assay was performed as described by Badoc & al. (BADOC, 2001): Spores were collected by adding 5 mL of sterile water containing 0.1% (v/v) tween 80 (for better spore separation) to each Petri dish and rubbing the surface with a sterile L-shaped spreader, then, filtrated through two layers of sterile cheesecloth to eliminate mycelia fragments. 0.1 ml of a spore suspension (approximately 10^6 spores ml^{-1}) of Foa, (counting using a Malassez cell) was spread on the Petri dishes containing PDA medium incorporated with three concentrations (7.4, 3.84 and 1.9 mg ml^{-1}) of various extract (separately). The Petri dishes were incubated at 25 °C for 24 h. The counting of spores germinated or ungerminated was determined under a microscope on a total of 200 spores and the number of spores germinated was scored to calculate the percentage of the spore germination (Surender, et al., 1987). A spore is considered germinated if the germ tube length is greater than its diameter (Amiri and Bompeix, 2011). All tests were performed in triplicates.

RESULTS AND DISCUSSION

Therapeutic use of three plants

A. radiata, *A. graveolens* and *D. viscosa* are widely three of the plants utilized in traditional medicine in the Tafilalet for the treatment of several diseases. The ethno botanical information on these plants are indicated in Table (1) showing some of medicinal uses of these plants in different regions. The results of the survey are included in Table 1 with a comparison of our results and those of other investigations.

The noted convergence between the results of our investigation and the results of other surveys (especially the Algerian territory) is probably due to the nomads movement between the two countries, transmitting their knowledge during their travels local populations (Maiza, et al., 1996).

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Table 1. Some of medicinal uses of *A. radiata, A. graveolens* and *D. viscosa* according to ethno_ botanical studies in different regions

Plants	Preparation used	Part uses	Region of study	Therapeutic use	Our Survey (Tafilalet)	Corresponding references
Asteriscus graveolens (Forsck.) Less	Dec	Wp	Tata South of Morocco	Fertility and problem menstrual disorder	**Gum diseases, Stomach pain, Fever, Toothache**.	(Bellakhdar, et al., 1987)
	Inf, Ch	Wp, L		**Gum diseases and Toothache**		
	Po	L		Headaches and migraine		
	Dec	Ap	Southeastern Morocco (Tafilalet)	Diabetes, Obesity and **Stomach pain**		(El Rhaffari and Zaid, 2002)
	Dec	R		**Toothache**		
	Inf	L, St	Tassili n'Ajjer, central Sahara	**Fever** and Pathologies of the respiratory system		(Benchelah, et al., 2004)
	Po	L, St		Intoxication by venoms of scorpions and insects		
	Ju	L		Sinusitis		
	Dec	Ap	Om Shehan, North Sinai Egypt	Blennorrhagia, **Diabetes**, Diarrhea, Headache and **Cold**		(El-Seedi, et al., 2012)
Anvillea radiata (Coss & Dur)	Dec	Ap	North Western Sahara Algeria	**Diabetes**, Indigestion, Chest pain, Cooling pulmonary and Aches	**Gastro enteritis, Spasms, colic, Diabetes Arthritis, Cold and Influenza.**	(Maiza, et al., 1996),
	Mac, Inf	L, St	Ouargla Algeria	Cooling Pulmonary and Indigestion		(Ould El Hadj, et al., 2003)
	Dec	L, St	South of Algeria	**Diabetes**, Indigestion, Cooling Pulmonary, **Cold** and Upset stomach		(Djellouli, et al., 2013)
	Dec	Pa / R	Southeastern Morocco (Tafilalet)	**Gastro enteritis**, **Spasms**, **Colic**, Hepatitis, **Arthritis**, **Cold and Influenza**		(El Rhaffari and Zaid, 2002)
	Inf	L		**Diabetes** and Upset stomach		
	Dec	R/Pa		Leucorrhoea, Rheumatism		

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	Inf	Pa	South of Morocco	**Gastro-enteritis**, **Spasms**, and **Colic- Arthritis** and Rheumatoid		(Bellakhdar, 1997)
	Dec, Po	L	Tan Tan South of Morocco	**Diabetes**		(Douira, et al., 2013).
	Po	L		Kidney problems		(Ghourri, et al., 2014)
	I	Wp	South East of Algeria. F	**Diabetes** and indigestion		(Fatiha and Arrezki, 2013)
Dittrichia viscosa (L.) Greuter	Inf	Ap	Rissani oasis (SE, Marocco)	**Nervous irritation, Calming and Hair loss.**	**Nervous irritation, Calming, diabetes, Hair loss in cosmetic.**	(El Mansouri, et al., 2011)
	Po	Ap	Southeastern Morocco (Tafilalet)	Cutaneous leishmaniasis		(El Rhaffari, et al., 2002)
	Dec	L,S	south-east of Morocco	**Diabetes**, Hypertension and Renal diseases		(Eddouks, et al., 2002)
	Dec	L	south-east of Morocco	**Diabetes**		(Zeggwagh, et al., 2006)
	Inf	R	Oriental Morocco	Hypertension		(Ziyyat, et al., 1997)
	Inf	L	Morocco	Skin diseases, Treats cutaneous abcesses, Wound healing, Tuberculosis and Bronchial infections		(Bellakhdar, 1997)
	-	L	Palestinian	Skin diseases		(Ali-Shtayeh, et al., 2000)
	Dec	Wp	Israeli	Infertility and Rheumatic pains		(Palevitch, et al., 1986)
	T	L	Samaria in Israel	**Diabetes**		(Yaniv, et al., 1987)
	Inf	Ap	Atlas, Rif and Sahara of Morocco	Cold		(Mouhajir, et al., 2001)

Ap: aerial part; L: leaves; Wp: whole plant; St, stem; R: root, S: seeds; Po: poultice; Ju: Juice; T: tisane; Dec: decoction; po: powder; Inf: infusion; Mac: maceration; Ch: Chew; Fr: fresh.

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Antifungal activity

Different extracts of medicinal plants have shown inhibitory effects against phytopathogenic fungi in vitro. In this study, the biological tests showed that all extracts exhibited appreciable inhibitory effect against Foa in all tests (Fig.1).

Our results showed that the aqueous extract of *A. radiata* has presented an inhibitory effect both on mycelial growth and spore germination neatly superior than those of other extracts. Indeed, the effect of the extracts at the same concentration is more marked on the inhibition of sporulation than the mycelial growth.

The results obtained from the spore germination test of each extracts are shown in Table 2. There was a significant inhibition of fungal spore germination by different concentrations of all extracts. The lower germination percentage (20.16%) was observed at 7.4 mg ml^{-1} for aqueous extract of *A. radiata*, followed by that of *D. viscosa* with a percentage about 33.66%. while the largest percentage germination was observed for the aqueous extract of *A. graveolens* (48.50%).

Regarding the mycelia development, the percentage of fungal growth inhibition (IP) of extracts on Foa was ranging from 4.32 to 56.59% (Table 3). At the concentration equal to 7.4 mg ml^{-1}, the methanolic extract of *A. radaiata* has a low activities against Foa (16.59%), while the chloroformic extract of *A. radiata* and the aqueous extract of *A. graveolens* have a moderate activities (42.33% and 33.59% respectively). The aqueous extract of *A. radiata* proved too active against Foa (IP = 56.59%). Similar results were obtained for dichloromethanic extract of *D. viscosa* (IP = 65.71%).

Table 2. Effect of different extracts on spore germination of Foa

Plant	Extract	Concentration (mg ml^{-1})			Control	Percentage of spore Germination (%)
		1.9	3.84	7.4		
A. radiata	aqueous	69.00±4.00	42.66±2.44	20.16±1.77	90.16±6.44	
	methanolic	84.33±2.44	52.00±1.33	39.83±2.22	90.16±6.44	
	chloroformic	72.16±2.88	44.16±1.11	29.50±2.00	90.16±6.44	
A. graveolens	aqueous	87.16±2.88	62.66±2.44	48.50±2.00	90.16±6.44	
D. viscosa	dichloromethanic	81.50±2.00	48.66±1.11	33.66±1.77	90.16±6.44	

Table 3. The percentage inhibition of mycelial growth after 8 days

Plant	Extract	Concentration (mg ml^{-1})			Percentage inhibition of mycelial growth (%)
		1.9	3.84	7.4	
A. radiata	aqueous	17.32±0.45	23.07±0.71	56.59±1.06	
	methanolic	04.32±0.45	09.07±0.71	16.59±1.06	
	chloroformic	21.19±0.91	34.00±1.33	42.33±1.55	
A. graveolens	aqueous	06.32±0.45	18.07±0.71	33.59±1.06	
D. viscosa	dichloromethanic	29.00±0.66	37.87±1.91	55.53±1.68	
DMSO		2.10±0.66			

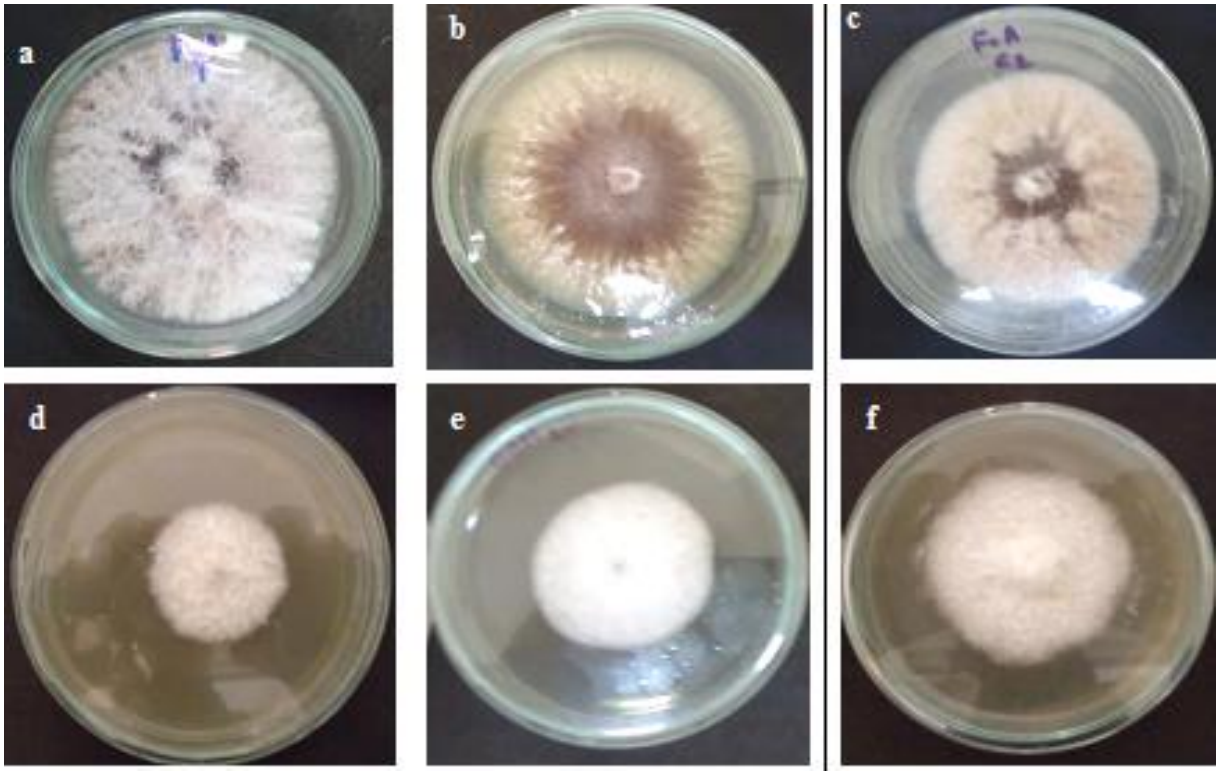

Figure 1: Effect of different plant extracts on the inhibition percentage of mycelial growth after 8 days. a: control*;* b: methanolic extract of *A. radiata;* c: aqueous extract of *A. graveolens*; d: aqueous extract of *A. radiata;* e: dichloromethanic extract of *D. viscosa;* f: chloroformic extract of *A. radiata*

Similar studies have been carried out by different researchers on antifungal activity of plant extracts. Recent studies indicate that the petroleum ether extract from *D. viscosa*, *A. graveolens* and *A. radiata* and chloroformic extract from *A. radiata* have revealed the highest antifungal activity against *Penicillium italicum* (Askarne, et al., 2013).The powders of *A. radiata* completely inhibited mycelial growth of *Penicillium italicum*, at a concentration of 10% (w/v). Furthermore, the powders of *A. graveolens*, and *D. viscosa* were also effective against *P. italicum* with the inhibition of mycelial growth greater than 75% (Askarne, et al., 2012). Flavonoid extracts derived from the flower and leaves of *A. radiata* showed the strongest inhibitory effects on spore germination and on soil population density of Foa (Lakhdar, et al., 2013). The powder of *D. viscosa* is also effective against *Geotrichum candidum* with a percent of inhibition of mycelial growth higher than 80% (Talibi, et al., 2012).

Study of kinetic of mould growth (Fig. 2, 3, 4, 5 and 6) assessed by radial mycelial growth (mm), measured along different time intervals, (2^{nd}, 4^{th}, 6^{th} and 8^{th} days) has shown a fungistatic effect of various extracts at different concentration. The aqueous extract of *A. radiata* at 7.4 mg ml^{-1} provided significant decrease in the mycelial growth when compared with the control assay.

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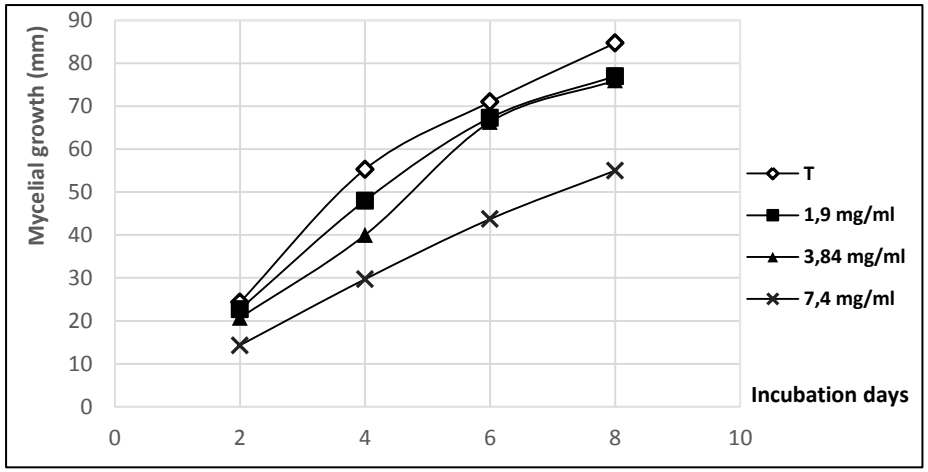


Figure 2. Effect of aqueous extract of *A. graveolens* on the radial mycelial growth kinetic of Foa

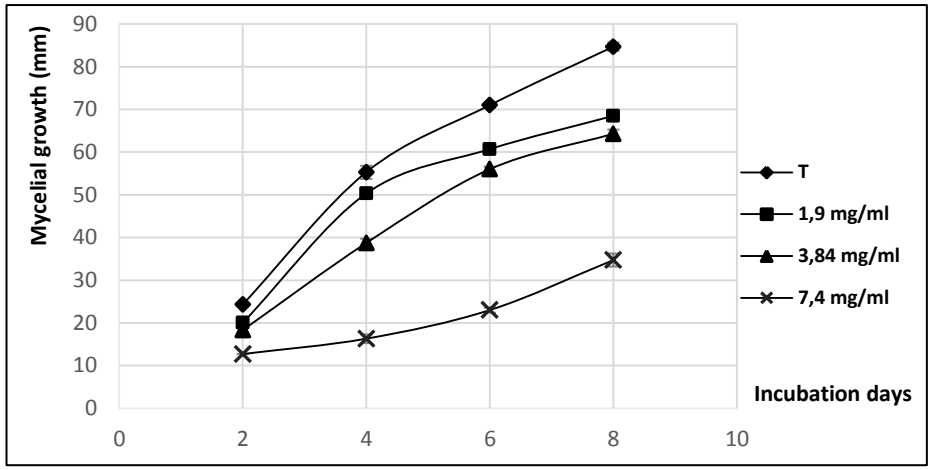


Figure 3. Effect of aqueous extract of *A. radiata* on the radial mycelial growth kinetic of Foa

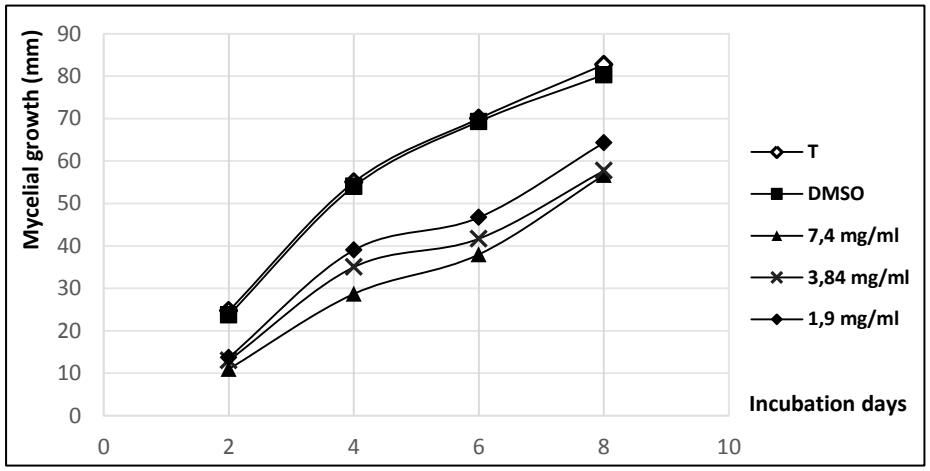
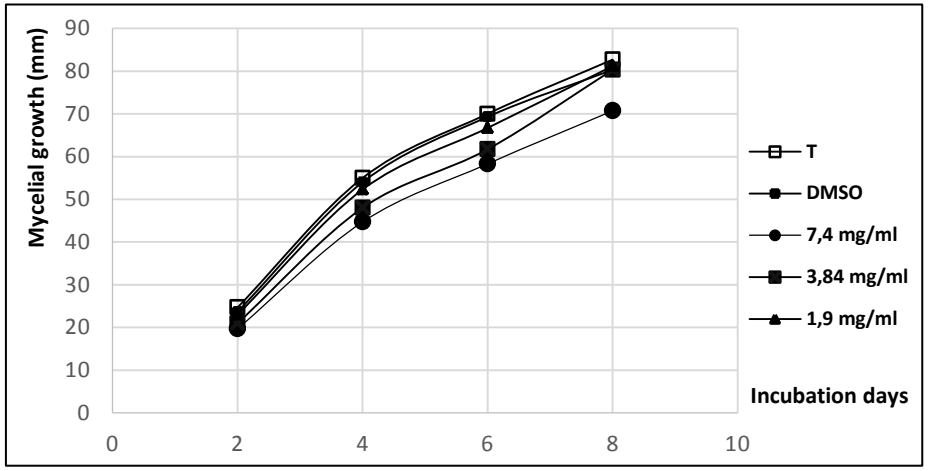


Figure 4. Effect of chloroformic extract of *A. radiata* on the radial mycelial growth kinetic of Foa



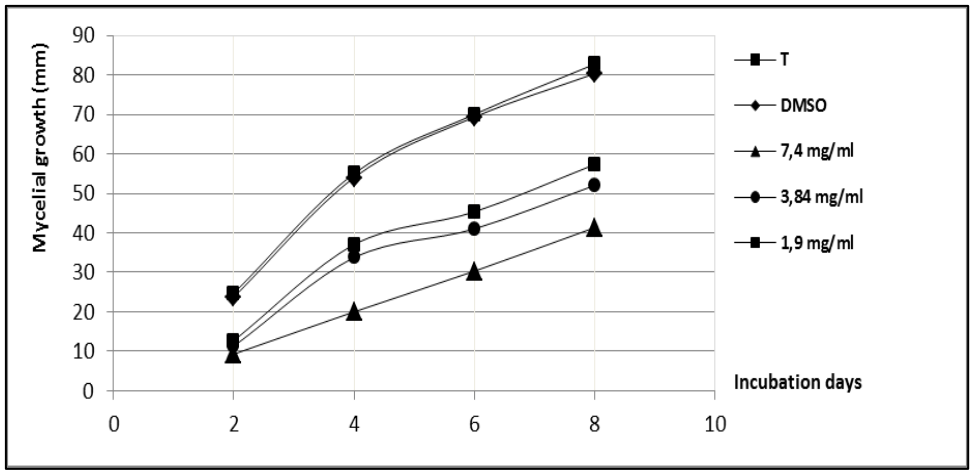


Figure 6. Effect of dichloromethanic extract of *D. viscosa* on the radial mycelial growth kinetic of Foa

CONCLUSION

The ethno pharmacological survey conducted in Tafilalet (South-Eastern of Morocco) shows that the three plant species (*Anvillea Radiata*, *Asteriscus graveolens and Dittrichia viscosa*) are widely used in traditional medicine in this area, in various forms of medicinal preparations; they are used to treat various diseases.

Among the extracts tested, aqueous extract of *A. radiata* and dichloromethanic extract of *D. viscosa* showed promising antifungal activity against Foa, and they can therefore be used as a natural potential alternative to synthetic fungicides for the phytopathogenic fungi.

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