

BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *RADICIS LYCOPERSICI* BY USING AQUEOUS EXTRACTS OF MEDICINAL PLANTS OF WADI RIGH REGION

Research

Lakhdari Wassima*, Institut National De La Recherche Agronomique, Station Sidi Mehdi, Touggourt Algérie.

N° De Téléphone : +213670490404

Dehliz Abderahmane, Institut National De La Recherche Agronomique, Station Sidi Mehdi, Touggourt Algérie.

Guezoul Omar, Département D'agronomie, Faculté S.N.V, Université Kasdi-Merbah, Ouargla, Algérie.

Acheuk Fatima, Département De Biologie, Faculté Des Sciences De La Nature, Laboratoire Valcore, Université Boumerdes, Algérie

Dekkoumi Baderddine, Département D'agronomie, Faculté S.N.V, Université, Gharadia, Algérie.

Benlamouidi Wiam, Institut National De La Recherche Agronomique, Station Sidi Mehdi, Touggourt Algérie.

Département D'agronomie, Faculté S.N.V, Université Kasdi-Merbah, Ouargla, Algérie.

M'lik Randa, Institut National De La Recherche Agronomique, Station Sidi Mehdi, Touggourt Algérie.

Département D'agronomie, Faculté S.N.V, Université Kasdi-Merbah, Ouargla, Algérie.

Hammi Hamida, Institut National De La Recherche Agronomique, Station Sidi Mehdi, Touggourt Algérie.

CORRESPONDENCE AUTHOR

Lakhdari Wassima

Email: Lakhdariwassima@Yahoo.Fr

CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

ABSTRACT

Background: This work deals with the *in vitro* study of the antifungal effect of aqueous extracts of six medicinal plants (*Zygophyllum album*, *Euphorbia guyoniana*, *Atriplex halimus*, *Oudneya africana*, and *Matricaria pubescens*) in Wadi Righ region, at their inhibition of the mycelial growth of a tomato telluric fungus (*Fusarium oxysporum* f. sp. *radicis lycopersici*).

Methods: The study of the antifungal activity of natural extracts was carried out according to the poisoned Food technique defined by Grover and Moore (1962).

Results: The *in vitro* study of the antifungal properties shows that all the aqueous extracts of plant species tested inhibit the mycelial growth of this phytopathogenic agent except that of *Moltkiopsis ciliata*. In fact, the aqueous extract of *Z. album* was found to be a very inhibitory solution because it induced an inhibition rate of 75.29% against the *Fusarium* wilt of tomato after 8 days.

Conclusion: the results indicate that the aqueous extracts studied can be considered as a very promising agent for the plant protection industry.

Keywords: antifungal, aqueous extracts, Forl., medicinal plants.

BACKGROUND:

The majority of yield losses from tomato crops are generally due to phytopathogenic fungi, essentially telluric, which once present in the soil cause a lot of damage [1]. Repeated use of synthetic products often results an en-

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vironment pollution with the appearance of resistant strains and increases the amount of residues on fruit [2]. As a result, the alternative of biological control has proved indispensable today to avoid the frequent appearance of resistant strains during these treatments by using pesticides, limit the environmental impacts due to their repetitive application, valorize the medicinal plants to remedy and minimize the risks of pests and thus maintaining biodiversity.

Our country is endowed with an immense vegetable biodiversity that remains to be discovered, while noting that a large part of this flora is constituted by medicinal species. The latter constitute a valuable heritage for humanity and especially for the majority of the poor communities in the developing countries who depend on those plants for their primary health care and subsistence [3].

We collected six spontaneous plants in the study area. This preliminary study focused mainly on the evaluation of the effect of aqueous extracts of these species, namely their antifungal capacity on the phytopathogenic tomato tulle fungus: *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl.).

The choice of medicinal plants is based on an ethnobotanical study in Wadi Righ region [4, 5, 6]. We chose to test six plants which have very important medicinal virtues in the subject region on human health which make us think that these plants have an antifungal power against phytopathogenic fungi of vegetable crops. *Euphorbia guyoniana* (figure 1) is used against snake bites [6; 7; 8]; *Matricaria pubescens* (figure 2) purgative and laxative, Intensive and stimulating, antiviral and antifungal and used for indigestion also [4; 6; 7; 9] *Oudneya Africana* (figure 4) used in skin care [6; 7; 8] *Atriplex halimus* (figure 5) used as herbal tea for ovarian cysts; in prevention of uterine fibroids in women, anti diabetics, against stomach pain, constipation, diarrhea, cyst, fever, fibroid, hypertension, antiseptic, burns, diabetes, jaundice, anemia, cardiological diseases, cough, rheumatism, obesity, tumor, diuretic, vermifuge, urinary inflammation, sores and ulcers, calming, fortifying gums, sterility, prostate, hypercholesterolemia [5; 6]. Also we used *Zygophyllum album* (figure 6) which is known as a diuretic, local anesthetic, antihistamine [4; 6; 10].



Figure 1: *Euphorbia guyoniana* (Lobbina)



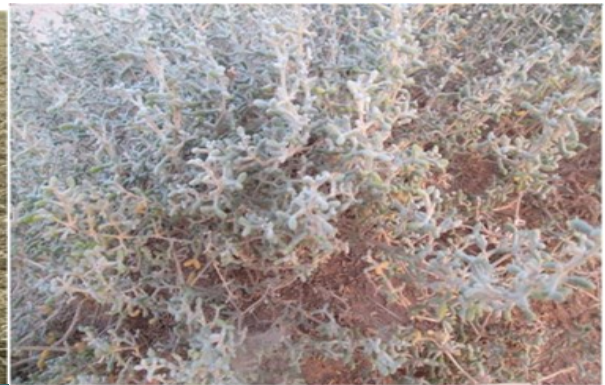
Figure 2: *Matricaria pubescens* (Chohia)



Figure 3: *Moltkiopsis ciliata* (El Helma)



Figure 4: *Oudneya africana* (Hanet Lebiir)

Figure 5: *Atriplex halimus* (Gutef)Figure 6: *Zygophyllum album* (Al Agga)

MATERIALS AND METHODS:

The plant material used consists of the aerial and subterranean part of these spontaneous plants, dried in the shade, protected from moisture and stored in hermetically sealed containers at ambient temperature and protected from light until they are used.

Fungi material

Sampling was carried out in the Barkadjia station, on a greenhouse tomato crop. The organs bearing diseases symptoms (morphological or chlorotic deformations, spots, burns, rotting ...) were collected.

The attacked subjects were rinsed, cut into small pieces and disinfected with sodium hypochlorite (2%) for three minutes (to eliminate the saprophyte flora). The fragments were then rinsed twice with distilled water, dried on a sterile filter paper near the Bunsen spout.

The fragments were seeded aseptically in an agar medium (PDA) in petri dishes at the rate of 4 fragments per dish. Incubation takes place at a temperature of 25 ° C and under a hemerperiodic regime (12 h dark / 12 h light) to further promote sporulation. Aseptically successive transplanting of the non-contaminating culture explants is selected at the peripheral growth zone of the colonies and rearranged in petri dishes containing the PDA medium. The identification of fungi essentially requires morphological cultural characteristics: macroscopic and microscopic aspect.

Preparation of the aqueous extract

The plant material is first crushed. By adopting the protocol of [11], 25 g of powder of each plant were placed in 150 ml of distilled water and then placed in a stirrer for 30 min to finally obtain the filtrate.

Test of antifungal activity of aqueous extracts of selected plants

The study of the antifungal activity of natural extracts was carried out according to the poisoned Food technique defined by [12]. This method consists in spreading 0.2 ml of the extract on 180 ml of culture medium, in our case the PDA. A disk of 5 mm of tested fungus (young culture), was deposited on the culture medium mixed with the aqueous extract, as much for the control (on a PDA medium only), in order to follow the mycelial growth of Forl. The experiment is carried out at the rate of 5 repetitions. The incubation was carried out at a temperature of 26 ° C until the control batches were filled. The manipulations were carried out under aseptic conditions. The results obtained are expressed as a percentage (inhibition rate).

Calculation of inhibition percentages

The inhibition rates were calculated to compare the efficacy of the extracts using the formula of [13]:

$$I (\%) = [(A-B) / A] \times 100$$

I: Inhibition rate of fungus tested;

A: Mean diameter of the estimated mycelial growth of the fungus in the control;

B: Mean diameter of the estimated mycelial growth of the fungus in the presence of the extract.

Determination of mycelial growth speed (VC)

According to [14], the rate of mycelial growth of each concentration is determined by the following formula:

$(D3-D1) / Te2] / Te3] + \dots + [(Dn-Dn-1) / Te2]$
 D: daily diameter of the colony growth zone (mm).
 Te: incubation time (h).

Statistical analysis

The experimental device used in this experiment is total uni-factorial randomization with 5 repetitions. The factor studied is represented by the inhibition rate (%) of the aqueous extracts against the phytopathogenic fungus. The statistical analysis used is ANOVA (analysis of variance) by the SPSS software. For all tests, the level of significance was assessed at the 5% and 1% thresholds. If necessary, the comparison of the averages is made on the basis of the Student test (t-test) of Newmann-Keuls. The aim was to distinguish homogeneous groups according to the values of the means of six variables tested which are represented by the chosen spontaneous plants.

Results

Isolation and identification of phytopathogenic fungus

This species forms white fluffy or cottony colonies (Figure 7, A) and then becomes light pink. The main morphological character of Forl. is the presence of spindle-shaped and fusiform macro conidia (Figure 7, B). The phialides are short and large and formed on the aerial mycelium, micro-conidia are absent.

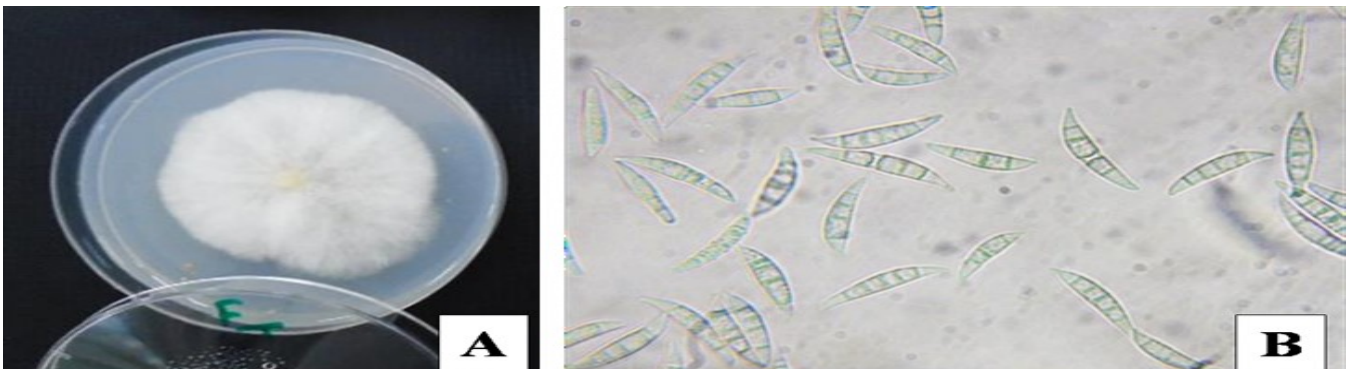


Figure 7: Macroscopic (A) and microscopic (B) appearance of *Fusarium oxysporum* f. sp. *radicis lycopersici*

In vitro efficacy of aqueous extracts of plants tested against Forl.

All plant species tested reduced Forl. mycelial growth, compared with the control. However, the *M. ciliata* extract does not indicate any mycelial inhibition against this fungus (Figure 8). After 48 hours, extracts of *Z. album*, and *E. guyoniana* recorded the highest inhibition levels (over 50%) compared to *A. halimus* (37.21%), *M. pubescens* (33.93 %) And *O. africana* (29.81%). After 192 hours, five plants had an inhibition rate of more than 50%, var *Z. album* (75.29%); *A. halimus* (63.29%); *M. pubescens* (62.12%); *O. africana* (59.21%) and *E. guyoniana* (56%) (figure 9).

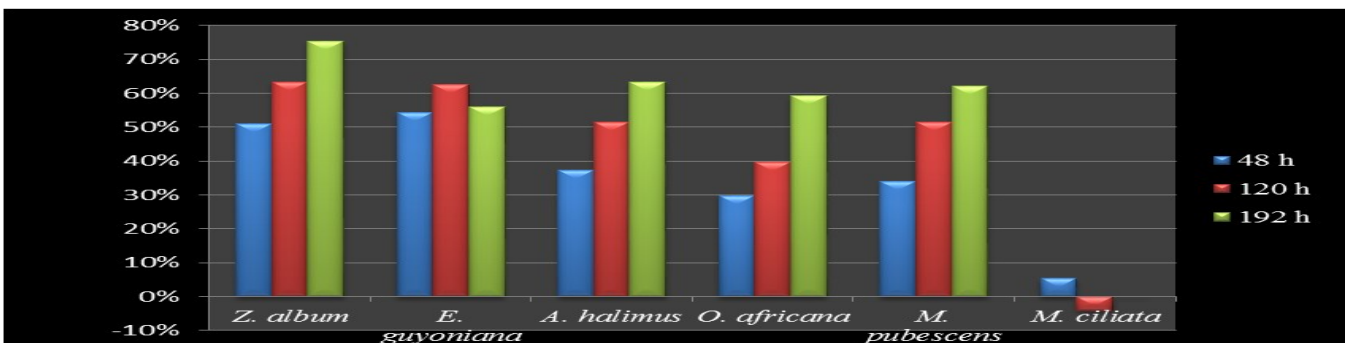


Figure 8: Effect of the aqueous extracts of the plants tested on Forl.

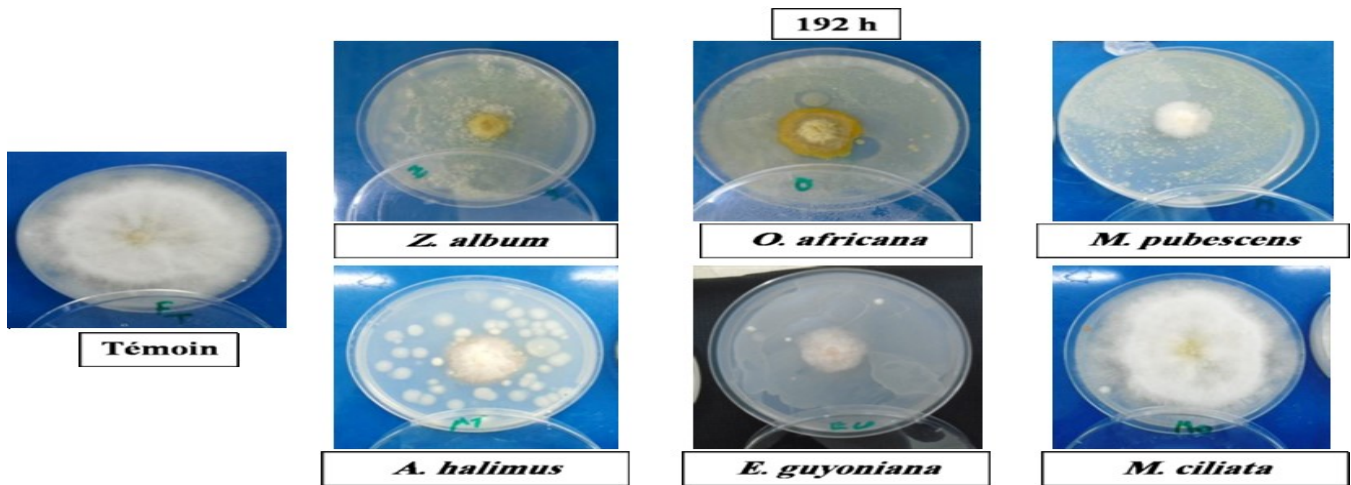


Figure 9: *In vitro* test of the antifungal activity of the aqueous extracts of plants tested on Forl.

Mycelial growth rate of fungus tested

The species Forl. recorded a very high mycelial growth rate in the presence of the aqueous extract of *Moltkiopsis*

	<i>Zygophyllu m album</i>	<i>Oudneya africana</i>	<i>Matricaria pubescens</i>	<i>Euphorbia guyoniana</i>	<i>Moltkiopsi s ciliata</i>	<i>Atriplex halimus</i>
mycelial growth rate of Forl.	0.40	0.61	0.57	0.40	0.93	0.44

Table: *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl) mycelial growth rate under the effect of aqueous extracts of selected plants.

Effect of aqueous extracts tested on spores morphology

We observed the effect of extracts on spores and their formation under a microscope. Forl. spores were deformed (Figure 10) with formation of chlamydo spores.

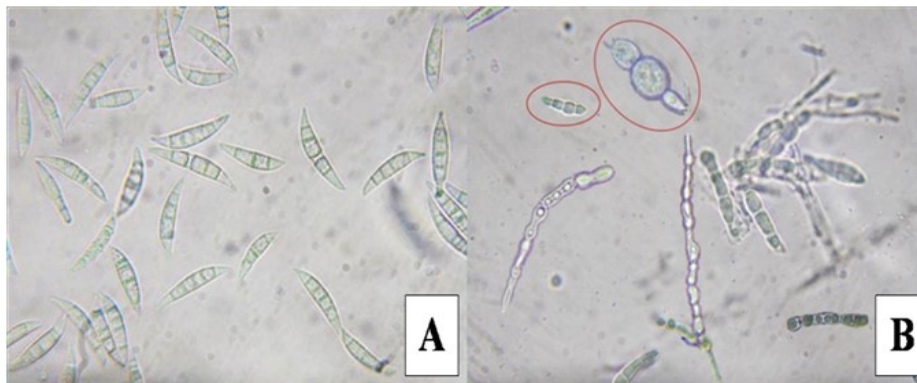


Figure 10: Microscopic appearance of Forl (A: control; B: deformation of spores with formation of chlamydo spores).

DISCUSSION

The active substances of a medicinal plant are components naturally present in this plant, they confer its thera-

peutic activity due to the presence of chemical compounds (saponin, flavonoids, alkaloids, ...). These metabolites attract attention both for their biological and pharmacological properties, which needs their industrial exploitation. The case of saponins whose anti-inflammatory, antibacterial, antifungal, antiparasitic, antiviral, haemolytic and cytotoxic activities have been reported in several studies [15].

Moreover, there are no studies at the regional level that sensitize us on the effect of these spontaneous plants on this tomato fungus.

The potential of *Z. album* may be explained by its richness in saponosides and glucosides [14; 16; 17]. This zygophyllaceae is known for its pesticidal effect because in a work on *Fusarium* sp. of the date palm, [18] obtained an inhibition of the mycelial growth of this microorganism by more than 50%.

Like *Z. album*, *E. guyoniana* also exerted an antifungal effect on this fungus with an inhibition rate of 56%. *E. guyoniana* also showed an insecticidal effect because its aqueous extract induced mortality of all individuals in a population of *Schistocerca gregaria* after 14 days [19]. The pesticidal effect of this euphorbiaceae may be due to its richness in secondary compounds. In fact, euphorbiaceae contain a large number of chemical compounds such as flavonoids [20] and saponins [21] that enable them to protect themselves against bio-aggressors.

This study also showed that *A. halimus* inhibited the development of Forl. Recording an inhibition rate of 63.29%. Variable inhibition values (between 28% and 91%) have been proposed by [22] using the extract of this plant against *Fusarium oxysporum* and *Fusarium solani*. The phytochemical screening of the aqueous extract of this plant reveals the presence of saponins, flavonoids and tannins [23]. These secondary metabolites which have a wide range of biological activity are probably at the origin of its phytotherapeutic virtues.

As regards *O. africana*, his extract acted on Forl. with an inhibition rate of 59.21%. This action can be explained by the phytochemical composition of this brassicaceae because it is very rich in saponins, flavonoids, and tannins [24; 25; 27]. Although, [26] proved that seeds of *O. africana* treat microbial infections by using it as proteinaceous extract against of *L. monocytogenes*, *E. coli*, *B. subtilis*, *E. hirae*, *P. aeruginosa*, *S. aureus* and *C. albicans*.

As for *M. pubescens*, his extract inhibited the evolution of Forl. with an inhibition rate of 62.12%. The antifungal activity of this can be explained by its richness in alkaloids, saponins, terpenes, flavonoids, tannins, steroids and cardioids [9]. Essential oils of this plant species can exert a remarkable antifungal activity against fungi such as *Aspergillus niger* [27].

[28] noted that the ethanolic extract of *M. ciliata* also has an antibacterial effect on *Candida albicans*. Indeed, this borraginacea is rich in alkannines and shikonins such as β -acetoxisovalerylalkannin; B-dimethylacrylshikonin and benzoylshikonin [29; 30] in addition to a significant number of phenolic molecules, flavonoids and saponins [28; 31].

Our study also showed that the extracts of the plants tested acted on the Forl. by different mechanisms. A change of color from white to orange yellow and formation of chlamydo-spores were noted in this fungus. Exposed to the preparations of *Z. album* and *O. Africana*. These observations were also reported by [32] in *A. Niger* treated with *Cymhopogonnordus* essential oil. According to these same authors, the change in color may correlate with the loss of mycotoxin production.

According to the statistical analyzes we note that all the extracts of the plants tested have a significant effect on the inhibition of mycelial growth of the fungus under study, while presenting a F of the order of 21.086.

CONCLUSION

The use of synthetic products (chemical control by pesticides) harmful to humans and the environment would be of great value in the search for suitable bio-solutions to the problems posed by fungal diseases.

Since there is no study that mentions the place of aqueous extracts of medicinal spontaneous plants specific to the region in the biological control of the Forl., the main objective of the work carried out was to evaluate the antifungal efficacy of six (06) aqueous extracts of the spontaneous plants (*Z. album*, *E. guyoniana*, *A. halimus*, *O. Africana*, *M. pubescens* and *M. ciliata*) against the phytopathogenic agent which attacks tomato (Forl.) isolated in greenhouse from infected organs in the Barkadjia perimeter.

The study of the antifungal properties *in vitro* shows that all the plant species tested inhibit the mycelial growth of this fungus: with the exception of *M. ciliata* which has no effect. Our results indicate that the aqueous extracts studied can be considered as a very promising agent for the plant protection industry.

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Contact Us:

SIFT DESK

Deerpark Dr, #75,
Fullerton, CA, 92831

United States.

E-mail:

helpdesk@siftdesk.org

Visit us on the web at:

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