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Evaluation of the antifungal effect of the aqueous extract of *Drimia numidica* (Jord. & Fourr. J.C.) from Algerian Northeast to *Fusarium solani*

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Abstract

At the present time, the world is moving towards sustainable agriculture that supports the production of healthy food and the preservation of the environment. This requires the use of new, effective and biodegradable pesticides. In this context, a study was carried out in order to evaluate in vitro the antifungal activity of the aqueous extract from the bulb of an Algerian Northeastern asparagus *Drimia numidica* (Jord. & Fourr.) J.C. against fungus *Fusarium solani*, causal agent of tomato blight. Following preliminary tests, two concentrations of 30 g/l (T2D1) and 50 g/l (T2D2) were chosen. The effect of these two concentrations was examined on the mycelial growth, growth rate and sporulation of the fungus, compared to a negative control (T0), untreated (Malt-Agar medium) and a positive control (Malt-Agar supplemented with a fungicide, T1). Analysis of the variance (ANOVA) reveals very highly significant differences (P = 0.000) between the averages of the four treatments for the different parameters. Concerning the two doses of the aqueous extract, the concentration 50 g/l (T2D2) was more depressed on mycelial growth and sporulation with inhibition rates of 66.15% and 86.91%, respectively, relative to the fungicide which gives 77.58% and 97.19%. These results indicate that the aqueous extract of the bulb of *Drimia numidica* (Jord. & Fourr.) J.C. possesses an antifungal activity comparable to that of the fungicide. This is probably due to the richness of this organ in bioactive compounds that can be exploited in the biological control of phytopathogenic fungi.

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Introduction

The tomato *Lycopersicon esculentum* Mill. Of Solanaceae family is one of the most cultivated vegetables in the world. The cultivation of tomatoes occupies a prominent place in the Algerian agricultural economy. The Wilayates of Annaba, El Tarf, Guelma and Skikda in Northeastern of Algeria represent the main producing region of industrial tomatoes. It covers an area of 12173 ha with a production of 3822731 q (Snoussi, 2010). This crop is threatened by several insects and fungal diseases such as, downy mildew, powdery mildew and fusariosis (Anonymous, 2010). The latter is caused by phytopathogenic fungi of the genus *Fusarium*. This group is responsible for seedling slits, root and collar rot, and vascular disease in several crop plant species. At present, the use of chemical fungicides remains the only means used to control these pathogens.

However, global agricultural research is increasingly concerned with preserving the environment and biodiversity and is mobilized to replace synthetic molecules with other molecules of animal and vegetable origin. Historically, plant products with phytosanitary action have a much longer history than most other pesticides. Indeed, plant extracts played an important role early in old agricultural activities (Regnault-Roger *et al.*, 2008).

Therefore, the objective of this study is to evaluate the antifungal activity of the aqueous extract of the medicinal plant *Drimia numidica* (Jord. & Fourr.) J.C. on the growth of the fungus *Fusarium solani* isolated from the tomato.

Materials and methods

The plant species used for the preparation of the aqueous extract are the sea scallop (*Drimia numidica* (Jord. & Fourr.) JC) (Dobignard and Chatelain, 2010), it belongs to the Asparagaceae family (Spichiger *et al.*, 2016). It was collected from the Wilaya of El-Tarf (Northeastern of Algeria). The pathogen was isolated from the roots and the collar of a tomato plant with symptoms of *Fusarium* wilt from an industrial tomato field in the Wilaya of El-Tarf.

Isolation of the fungus

The isolation was carried out in the mycology laboratory of the INPV (National Institute for the Protection of Plants of El-Tarf). Fragments of about 1 cm were cut from the collar and tomato roots. These fragments were first washed and then disinfected with bleach diluted to 10%. After several rinses with sterile distilled water, the fragments were dried with sterile Whatman paper and then placed in five Petri dishes of 90 cm diameter containing Mat-Agar medium. These dishes were incubated in an oven at 22° C. for 48 hours.

Identification and purification of the fungus

A sample of the mycelium removed from the two-day primary culture was stained with blue methylene and then observed under an optical microscope. The identification of *Fusarium solani* was performed using the morphological characters described by Leslie and Summerell (2006). For purification, samples of the same culture, about 2 mm² are cut and transplanted into Petri dishes which are incubated under the same conditions.

Preparation of the aqueous extract

The aqueous extract is prepared from the bulb of *Drimia numidica* (Jord. &Fourr.) JC and obtained by maceration of 30 g (D1) and 50 g (D2) of bulb fragments in one liter of distilled water for 48 Hours in the dark. After filtering, the two concentrations are stored at 4 ° C.

Preparation of culture media

Four types of culture medium were prepared with five replicates for each: The first Malt-Agar medium is composed of a mixture of 5 g of Agar-Agar with 2.5 g of Malt and 250 ml of distilled water. This medium is autoclaved for 20 minutes at 120° C. It is considered as the control treatment (T0).

The second medium is prepared by mixing 225 ml of a Mat-Agar medium, autoclaved and cooled at 45° C., with 25 ml of a suspension of the Thirame fungicide at a concentration of 3g/l. It is considered the treatment (T1).

The third medium is obtained by adding 25 ml of the D1 concentration of the aqueous extract of 225 ml of the Malt-Agar medium under super cooling at 45° C. This is the treatment (T2D1).

The fourth medium is prepared by the addition of 25 ml of the D2 concentration of the aqueous extract of 225 ml of the super cooled Malt-Agar medium at 45° C. It constitutes the treatment (T2D2).

Transplanting of the fungus on the different media

In each Petri dish filled with one of the prepared media, a sample of 2 mm², taken from the purified colonies, is deposited in the center of the dish. Five cans were used for each treatment. The dishes were incubated in an oven for seven days at 22° C.

Tests of antifungal activity

Mycelial growth: Mycelial growth was daily assessed by measuring two perpendicular diameters of each colony for 7 days.

Growth rate:The growth rate is calculated on the 7th day, according to the following formula:

$$Vc = \frac{D2 - D1}{T2} + \frac{D3 - D2}{T3} + \cdots + \frac{Dn - Dn - 1}{Tn}\ cm/d$$

VC: growth rate
D1: diameter of first day colony
T1: first day
Cm: centimeter
d: day

Sporulation: On the seventh day, the colonies of the fungus of each Petri dish of the various treatments were rinsed with 10 ml of sterile distilled water. After stirring, the recovered solution was used to count the number of spores using a Malassez cell.

Evaluation of the percentage inhibition: The evaluation of the inhibition by the fungicide and the two concentrations of the aqueous extract is estimated by calculating the percent inhibition of mycelial growth, growth rate, and the number of spores according to the formula of Wang *et al.* (2005).

$$I\% = \frac{(D0 - Dc)}{D0} X100$$

I%: percent inhibition of mycelial growth, growth rate or sporulation.
D_0: mycelial growth, growth rate or sporulation of the control.
D_c: mycelial growth, growth rate or sporulation for fungicide or aqueous extracts.

Statistical analysis

The different characteristics of the fungal culture are described by calculating the mean (m), the standard deviation (s) and the minimum (X min) and maximum (X max) values for each treatment. The STUDENT t-test (Dagnelie, 2009) is used to compare the two doses of the *Drimia numidica* aqueous extract (T2D1 and T2D2) for each day (Minitab Inc, 2016) between diamond growth averages. Analysis of the variance (ANOVA) of the general linear model (GLM) of the Minitab software for statistical analysis of data (Minitab Inc, 2016) is used to compare the averages among the four treatments for each variable studied (Dagnelie, 2009).

The TUKEY test (Dagnelie, 2009) determined homogeneous treatment groups by fungal culture variable (Minitab Inc, 2016).The DUNNETT test (Dagnelie, 2009) was used to compare the means of the control treatment (T0) with each of the averages of the other treatments (T1, T2D1 and T2D2) for each variable of the fungal culture (Minitab Inc, 2016).

Results

Mycelial growth

The results of the following Table (1) show that the effect of the aqueous extract on the mycelial growth of *Fusarium solani* is different according to the concentration.

In fact, the diameter of the fungus colonies, in the medium containing the 50 g/l (T2D2) dose on the 7th day of incubation, is very close to that recorded in the medium containing the Thirame fungicide (T1) with mean values of 1,54 and 1,020 cm and inhibition rates (Table 06) of 66.15% and 77.58% respectively

compared to the control (T0) which displays an average value of the order of 1.886 cm. However, this inhibition is less important in the medium containing the dose 30 g/l (T2D1) whose mean diameter and inhibition rate are 2.610 cm and 42.63%.

These results also show that the inhibitory effect of the two concentrations of the aqueous extract, which is manifested by the decrease in the diameters of the colonies of the fungus, are different according to the day of incubation. Indeed, the inhibition was very low on the first two days, but on the third day, it became more important and followed an evolutionary trend up to the 7th day compared to the chemical fungicide, which showed a significant inhibitory effect from the first day of incubation.

Table 1. The values of the basic statistical parameters calculated from the colonies of the fungus. The number of samples (n), the mean (m), the standard deviation (s), the minimum values (X_{min}) and the maximum values (X_{max}).

Variable	Days	Treatment	n	m	s	X_{min}	X_{max}
Diameter	1	T0	5	0,710	0,089	0,600	0,800
		T1	5	0,340	0,022	0,300	0,350
		T2D1	5	0,620	0,075	0,500	0,700
		T2D2	5	0,720	0,067	0,650	0,800
	2	T0	5	1,570	0,175	1,400	1,850
		T1	5	0,640	0,054	0,600	0,700
		T2D1	5	1,250	0,224	1,000	1,600
		T2D2	5	1,040	0,163	0,800	1,250
	3	T0	5	2,380	0,225	2,100	2,700
		T1	5	0,720	0,057	0,650	0,800
		T2D1	5	1,650	0,226	1,450	2,000
		T2D2	5	1,310	0,096	1,200	1,450
	4	T0	5	3,030	0,451	2,500	3,550
		T1	5	0,824	0,131	0,650	1,000
		T2D1	5	2,230	0,268	1,950	2,550
		T2D2	5	1,620	0,125	1,500	1,800
	5	T0	5	3,720	0,678	2,850	4,400
		T1	5	0,920	0,156	0,750	1,150
		T2D1	5	2,500	0,200	2,250	2,750
		T2D2	5	1,720	0,178	1,500	1,900
	6	T0	5	4,310	0,851	3,000	4,950
		T1	5	0,960	0,151	0,750	1,150
		T2D1	5	2,800	0,269	2,550	3,150
		T2D2	5	1,800	0,141	1,700	2,000
	7	T0	5	4,550	0,799	3,200	5,250
		T1	5	1,020	0,097	0,900	1,150
		T2D1	5	2,610	0,416	2,000	3,000
		T2D2	5	1,540	0,181	1,400	1,850
	7	T0	5	1,886	0,229	1,550	2,110
		T1	5	0,618	0,050	0,550	0,680
		T2D1	5	1,292	0,212	1,030	1,570
		T2D2	5	1,206	0,273	0,960	1,660
Number of spores	7	T0	5	21,40	4,770	16,000	26,000
		T1	5	0,600	0,894	0,000	2,000
		T2D1	5	14,00	1,581	12,000	16,000
		T2D2	5	2,800	1,789	1,000	5,000

The TUKEY test (Table 4) classifies the treatments To and T1 each as a separate group. But for the two treatments T2D1 and T2D2 (30 and 50 g/l) this test classifies them, for the first two days, in a single homogeneous group. However, from the 3rd to the 6thday each treatment is stored in a separate group. For the 7th day, it is observed that the treatments (T1 and T2D2) possessing the most inhibitory effect meet and form a single homogeneous group.

Table 2. Results of the STUDENT t test of the comparison, between concentrations, of the averages for each day. Observed value of the variable t of STUDENT (t_{obs}), probability of highlighting significant differences (P), there are no significant differences between the two means (NS), there are significant differences between the two averages, Highly significant (**), very highly significant (***).

Variable	Days	Means per day		Statistical parameters	
		T2D1	T2D2	t_{obs}	P
Diameter	1	0,620	0,720	2,21	0,058 NS
	2	1,250	1,040	1,69	0,129 NS
	3	1,650	1,310	3,09	0,015 *
	4	2,230	1,620	4,60	0,002 **
	5	2,500	1,720	6,50	0,000 ***
	6	2,800	1,800	7,35	0,000 ***
	7	2,610	1,540	5,27	0,001 ***

Table 3. Results of the analysis of the variance (ANOVA) to a criterion for all the studied variables: The number of degrees of freedom (ddl), the sum of the squares of the deviations (SCE), the mean square (CM), the observed value of the Fisher F variable (F_{obs}) and the probability (P).

Variable	Days	Source of variation	ddl	SCE	CM	F_{obs}	P
Diameter	1	Treatment	3	0,472	0,157	33,59	0,000 ***
	2	Treatment	3	2,280	0,760	27,52	0,000 ***
	3	Treatment	3	7,202	2,400	83,87	0,000 ***
	4	Treatment	3	13,096	4,365	56,62	0,000 ***
	5	Treatment	3	21,341	7,113	51,18	0,000 ***
	6	Treatment	3	31,117	10,372	49,41	0,000 ***
	7	Treatment	3	36,535	12,178	57,02	0,000 ***
Growth rate	7	Treatment	3	4,038	1,346	30,84	0,000 ***
Number of spores	7	Treatment	3	1429,00	476,33	65,03	0,000 ***

Growth rate

Tables (1 and 6) show that the growth rate in the medium containing the fungicide (T1) is the lowest (0.618 cm/d) with an inhibition rate of 67.23%. However, the two concentrations of the aqueous extract (T2D1 and T2D2) show a lower decrease in the order of 1.292 and 1.206 cm/d with inhibition rates of 31.49%and36.5% compared to the control that recorded the fastest growth rate in the order of 1.886 cm/d. The variance analysis (Table 3) gives very highly significant differences (P = 0.000) between the treatments.

The TUKEY test (Table 4) classifies the different treatments into three distinct groups. A homogeneous group composed of the T2D2 and T2D1 treatments

(the 50g/l dose and the 30g/l dose of the aqueous extract); while the other two treatments (T0 and T1) each form a separate group.

Sporulation

The results in Table 1 represent the number of spores collected from the fungal colonies for the different treatments.

Table 4. Homogeneous treatment groups by variable according to the TUKEY method: The same alphabetical letter indicates that the treatments produce, on average, homogeneous results.

Variable	Days	Homogeneous treatment groups and their means			
		T1	T2D2	T2D1	T0
Diameter	D1	0,3[b]	0,6[a]	0,70[a]	0,70[a]
	D2	0,6[c]	1,0[b]	1,3[b]	1,6[a]
	D3	0,7[d]	1,3 [c]	1,6 [b]	2,4 [a]
	D4	0,8[d]	1,6[c]	2,2[b]	3,0[a]
	D5	0,9[d]	1,7[c]	2,5[b]	3,7[a]
	D6	1,0[d]	1,8[c]	2,8[b]	4,3[a]
	D7	1,0[c]	1,5[c]	2,6[b]	4,6[a]
Growth rate	D7	0,6[c]	1,2[b]	1,3[b]	1,9[a]
Number of spores	D7	0,6[c]	2,8[c]	14,0[b]	21,4[a]

It is observed that the treatment T0 has the highest average number of spores, which is of the order of 21x10^5 spores/ml of solution. Conversely, T1 treatment (Thirame fungicide) gives the lowest mean number with 0.6x10^5 spores/ml, corresponding to an inhibition percentage of 97.19% (Table 6).

For the two doses of the aqueous extract, a more pronounced inhibitory effect of the 50 g/l (T2D2) dose was observed with 2.8x10^5 spores/ml and aninhibition percentage of 86.91% with respect to the 30 g/l dose T2D1) which exhibited an average efficiency with an average number of 14x10^5 spores/ml solution and aninhibition percentage of only 34.57%.

Table 5. Results of DUNNETT test of comparison of the control treatment with each of the other treatments.

Variable	Days	Averages and treatments identical to the control				Number of treatments
		T1	T2D2	T2D1	T0	
Diameter	1	0,3	0,60	0,70	0,71	02
	2	0,6	1,0	1,3	1,6	00
	3	0,7	1,3	1,6	2,4	00
	4	0,8	1,6	2,2	3,0	00
	5	0,9	1,7	2,5	3,7	00
	6	1,0	1,8	2,8	4,3	00
	7	1,0	1,5	2,6	4,6	00
Growth rate	7	0,5	1,2	1,3	1,9	00
Number of spores	7	0,6	2,8	14,0	21,4	00

The variance analysis (Table 3) reveals very highly significant differences (P = 0.000) between the averages of the four treatments. The TUKEY test (Table 4) gives three distinct treatment groups.

Treatment T0 constitutes the first group. The T2D1 treatment alone forms the second group. The two treatments T2D2 and T1 form a third homogeneous group.

Discussion

In this study, we evaluated *in vitro* the effect of the aqueous extract of the bulb, the medicinal plant *Drimia numidica* (Jord. & Fourr.) JC of the Northeast Algerian, on a soil fungus *Fusarium solani*, causal agent of *Fusarium* wilt of tomato. This plant was chosen on the basis of its use in traditional Algerian medicine (Hammiche *et al.*, 2013).

Mycelial growth

The inhibitory power of the aqueous extract on the mycelial growth of *Fusarium solani* was demonstrated by the significant reduction in the diameter of fungal colonies compared to the untreated control (Table 2).

This reduction is related to the concentration of the extract and the incubation time. Similar studies have reported that the use of different concentrations of aqueous extract of *Datura stramonium* (Sharma *et al.*, 2014), *Crotalaria retusa* (Dabé *et al.*, 2017) and *Artemisia absinthium* (Nikan and Dehghanpour, 2015) provoke the inhibition, to varying degrees, of *Fusarium solani.*

Table 6. Inhibition percentage (%).

Variable	Days	Inhibition percentage (%)		
		T1	T2D1	T2D2
Diameter	1	52,11	12,67	1,40
	2	59,23	20,38	33,75
	3	69,74	30,67	44,95
	4	72,80	26,40	46,53
	5	75,26	38,79	53,76
	6	77,72	35,03	58,23
	7	77,58	42,63	66,15
Growth rate	7	67,23	31,49	36,05
Number of spores	7	97,19	34,57	86,91

Growth rate

The results of the table (3) confirm a significant decrease in the growth rate of the colonies. These are less evolved in the media treated with the two concentrations of the *Drimia numidica* extract compared to the negative control. It is observed, on the one hand, that there is a slight difference between the inhibition levels of the two concentrations (30g/l and 50g/l) and on the other hand that the inhibition of the growth rate is not as effective as the Thirame fungicide.

Sporulation

Fusarium solani sporulation was significantly affected, to varying degrees, by both concentrations. Indeed, the number of spores decreased with increasing concentration; reason for which the concentration 50 g/l displays a similar inhibitory effect to the fungicide (Table 4). This is consistent with the results obtained by Onyeani *et al.* (2012).

These authors reported a significant reduction in sporulation of *Aspergillus niger* and *Rhizopus stolonifer* under the effect of the extracts of *Acalypha ciliata*, *Aloe vera*, *Azadirachta indica* and *Vernonia amygdalina*. Similarly, high concentrations of garlic extract reduced the number of spores of *Fusarium oxysporum* f. sp. *lycopersici* (Agbenin and Marley, 2006).

Thus, in view of the results achieved at the end of this work, it appears that the concentration 50 g/l of the extract of the bulb of *Drimia numidica* is as effective as the chemical molecule (Thirame) against the mycelial growth and the sporulation of *Fusarium solani*. Nevertheless, the expression of this concentration is less when it comes to the rate of growth.

The efficiency of the extract of the bulb is probably due to the richness of this organ in bioactive compounds. According to Hammiche *et al.* (2013), it contains more than 50 heteroside compounds, the most important being scillarene A and B, which possess antibacterial activities (Bozorgi *et al.*, 2017) which are toxic (Spichiger *et al.*, 2016).

Conclusion

The results obtained showed that the aqueous extract of this plant exhibited an antifungal effect similar to that of the chemical fungicide mainly when it was used at the highest concentration (50 g/l).

This efficacy is probably due to the presence of bioactive compounds in the bulb. In addition, the increase in antifungal activity with incubation time may be due to a gradual accumulation of antifungal properties in the culture medium that can be used to develop a potential and biodegradable fungicide.

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