Advances in Biology & Earth Sciences
Vol.7, No.2, 2022, pp.104-115

IN VITRO ANTIFUNGAL EFFECT OF *COTULA CINEREA* EXTRACTS AGAINST *FUSARIUM OXYSPORUM* F. SP. *ALBEDINIS* AND SOIL POPULATION ASSAY

Archaoui Cherifa[1], Gafa Nacira[1], Hasni Haouaria[1], Mebarki Lakhdar[1,2*], Boulenouar Ali[1,2], Benlarbi Larbi[1,2]

[1]Faculty of Natural Sciences and Life; University of Bechar, Bechar, Algeria
[2]Laboratory of Valorization of Vegetal Resource and Food Security in Semi Arid Areas, South West of Algeria, University of Bechar, Bechar, Algeria

Abstract. This study was conducted to evaluate the antifungal activities of the cell-wall polysaccharide extracts of *Cotula cinerea* against *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of vascular wilt of date palm and their effect on the density of this pathogen population in the soil. The results obtained show that these extracts have presented an inhibitory effect both on the germination and sporulation neatly superior to that on the mycelial growth. For germination, the best inhibitory effect was exercised under the effect of foliar hemicelluloses, with a rate up to 84.45% at 1 mg/ml. Concerning the mycelial growth, cellulose-based media have developed a weakly dense mycelium compared to PDAa. By contrast a stimulatory effect was recorded in the presence of highly methylated pectins (HMP) at all concentrations. Sporulation was strongly inhibited on cellulose-based media, whose inhibition rate exceeded 97%. The hemicelluloses and HMP proved to be less active than the cellulose. *Foa* infested soils were treated with cellulose, hemicellulose and HMP separately. After 21 days, the 5% and 10% cellulose doses, in addition to 1% HMP had an average inhibitory effect, while the other treatments had no inhibitory effect.

Keywords: *Cotula cinerea, effectiveness, Fusarium oxysporum f. sp. Albedinis, polysaccharide extracts, soil.*

Corresponding Author: *Mebarki Lakhdar, Faculty of Natural Sciences and Life, Tahri Mohamed University, BP417, Bechar. Algeria, Phone: +213 663890531, e-mail : mebarki.lakhdar@univ-bechar.dz*

Received*: 12 June 2022;* ***Accepted****: 7 August 2022;* ***Published****: 9 August 2022.*

1. Introduction

The date palm (*Phoenix dactylifera* L.) is one of the fruit species whose culture has existed since the earliest antiquity (Munier, 1973). “It is a stem of great interest not only for its high productivity and the quality of its fruit which are very looked for, but also due to its abilities to adapt the Saharan regions, where it allows to create a meso-climate inside the desert oasis that is favorable to the culture of several arboreal species, grain, forage and vegetable, which are associated with date palm whenever water is available” (Saaidi, 1990).

However, this culture has continued to deteriorate in the Maghreb after being attacked by a lethal vascular wilt called "Bayoud disease" and caused by *Foa*. This vascular fusarium, affects especially the best producing varieties of dates (Djerbi, 1982; Bounaga & Djerbi, 1990)

Indeed, various control methods have been developed in the control strategy of this disease. Faced with the many socio-economic and environmental issues related to

chemical control, control methods based on varietal resistance have been explored (Krad *et al*., 2009). By contrast, the introduction and the multiplication of resistant cultivars are often frequently encountered with two types of constraints; the poor adaptation to local soil and climatic conditions, in addition to the mediocre quality of the dates that does not have commercial value desired by the producers (Robert and Benkhalifa, 1991). In Morocco, palm trees that were thought to be resistant proved that they are sensitive after 8 to 10 years of their plowing (Bounaga & Djerbi, 1990).

In this context, alternative methods of controlling the disease have been studied with emphasis on novel compounds derived from plant sources (Garibaldi *et al*., 1990; Alabouvette, 1999). Indeed, many plants and plant products have been reported to be effective antimicrobials against food and grain storage fungi, foliar pathogens, soilborne pathogens and in control of vascular wilt of several cultures (Bowers & Locke, 2000; Krad *et al*., 2009; Soro *et al*., 2010; Singha *et al*., 2011; Hadian *et al*., 2011; Doumbouya *et al*., 2012; Mebarki, 2020). Plant products are good alternatives to chemical pesticides, as they are biodegradable. Indeed, *Cotula cinerea* is a medicinal plant of Asteraceae family widely used in traditional medicine to combat several diseases (Maiza *et al*., 1993; Mebarki, 2018), which presents appreciable antimicrobial activities (Markouk *et al*., 1999).

The objective of the present research is to evaluate the effect of the cell-wall polysaccharide extracts of *Cotula cinerea* on the *in vitro* development of *Foa* and assess their ability to reduce the population of this pathogen in the soil in order to select the products that can be used in controling Bayoud disease.

2. Materials and methods

Fungal sampling

The fungal strain used in this study was isolated according to the protocol of Djerbi (1990) from rachis of an infected date palm (showing the typical symptoms of fusarium wilt) in infested palm grove in the region of Bechar, Algeria. The isolated strain is coded by KE21 and deposited in the bank of Laboratory of Valorization of Vegetal Resource and Food Security in Semi Arid Areas, South West of Algeria, University of Bechar.

Plant material

Cotula cinerea specimens were harvested at the flowering stage from the area of Bechar. The harvested parts (leaves and flowers) were air-dried in shade, ground and stored separately in closed containers at room temperature and protected from light until their use. The extracts used in fighting this fungal strain were the cell-wall polysaccharides (cellulose, hemicellulose and HMP).

Preparation of extracts

Accorging to Harche *et al*. (1991), the extraction of the polysaccharides from the cell-wall requires a preliminary operation consisting in the preparation of the parietal residue. Plant powder (30 g) were placed in an Erlenmeyer flask containing a mixture of methanol and chloroform (1:1, v/v) and had been stirred under a fume hood (for 14 h) in order to remove the lipid soluble, tannins and other cytoplasmic constituents. The operation was repeated twice. After filtration, the residue was placed in 95% ethanol for 2 h with stirring to remove traces of chloroform and then was incubated in boiling 95%

ethanol for 2 h to get better elimination of chloroform traces. The residue was then dried in an oven at 60°C for 48 h (Redgwell & Selvendran, 1986).

Extraction of cellulose and hemicelluloses

5 g of parietal residue were put in an Erlenmeyer flask containing 100 ml of 4% NaOH and were stirred for 14 h. After filtration, the residue was rinsed with distilled water and then with acetone, then the residue was dried in an oven (60°C/14 h) before being weighed. The resulting part represents the cellulosic fraction.

The two filtrates obtained were neutralized by pure acetic acid and then precipitated in ethanol (1:3, v/v) for 14 h. After centrifugation (3600 *g*/30 min) at room temperature for, the pellet was washed with distilled water and then with acetone. Next, it was dried in an oven (60°C/14 h) and finally weighed. This part represents the hemicellulosic fraction (Chanda *et al.*, 1950).

Extraction of HMP

5 g of parietal residue were put in an Erlenmeyer flask containing 100 ml of distilled water, and stirred for 14 h at room temperature, and were put to boiling under reflux twice for two hours. After filtration, the filtrate was concentrated in a rotary evaporator. Then it was precipitated in cold acetone (1:2, v/v) for 14 h. After centrifugation (3600 *g*/30 min) at room temperature, the pellet was rinsed with distilled water and with alcohol and then the residue was dried in an oven at 50°C and was weighed. This part represents the HMP (Thibault, 1980).

Antifungal tests

Hemicelluloses and HMP were diluted in NaCl solution (5 mM) and sterile distilled water respectively, then were added to the PDAa medium. A range of concentrations (0.25; 0.5; 1; 2; 4 mg/ml of culture medium) of the extract were used.

Cellulose was intended to reconstruct cellulose-agar medium ($NaNO_3$ 2 g; K_2HPO_4 1 g; $MgSO_4,7H_2O$ 1 g; KCl 0.5 g; $ZnSO_4,7H_2O$ 0.005 g; $CuSO_4,5H_2O$ 0.001 g; $MnCl_2$ 0.001 g; Cellulose 2.5 ; Agar 16 g; Distilled water 1000 ml; pH 5), on which development of *Foa* was studied (Lekchiri *et al*., 2006).

Effect of the extracts on spore germination

To test plant extracts for inhibition of spore germination, 0.1 ml of a spore suspension (10^5 spores/ml) of *Foa*, prepared in sterile distilled water, was spread on the Petri dishes containing PDAa media incorporated by hemicellulose and HMP extracts (separately) at different concentrations and on the cellulose-based media. Germination was recorded under microscope on a total of 200 spores after incubation at 25°C for 24 h. A spore is considered germinated if the germ tube length is greater than its diameter (Maouni *et al*., 2001).

Effect of the extracts on fungal growth

The method of Hassikou *et al.* (2002) was used to determine the effect of plant extracts and the on fungal mycelia growth. Plates containing PDAa media with hemicelluloses and HMP extracts at different concentrations were inoculated with a mycelial disc (6 mm diameter) obtained from a youth *Foa* colony. The Plates with cellulose-based medium were inoculated in the same way. The plates were incubated at

25°C for 7 days. The diameter of the *Foa* colony was obtained by calculating the average of two perpendicular diameters (Hassikou *et al.*, 2002).

Effect of the extracts on sporulation

To test plant extracts for inhibition of sporulation, all colonies used to asses mycelial growth were incubated until the 10th days at 25°C. For this, four washers measuring 5 mm in diameter were taken along the diameter of the same colony and were collected in a tube containing 1 ml of distilled water. After crush washers and agitation to the vortex for 30 s, the spores were counted using a Malassez cell with three counts by suspension (Maouni *et al*., 2001).

Each experiment was repeated three times and control tests were performed under the same conditions in absence of extracts. Toxicity against *Foa* was calculated as percent inhibition:

Inhibition (%) = (C - T). 1OO / C.

Where C and T represent *Foa* germination (radial growth or sporulation) in control and treated plates, respectively.

Effect of the extracts on Foa population density in the soil

Foa was incorporated into soil (previously sifted and sterilized) at an inoculum density of 10^6 spores/g of soil. Aliquots (10 g) of the infested soil were placed in a sterile test tube and treated by 1, 5 and 10% (w/w) of cellulose, hemicellulose and HMP in separated experiments. Population density of *Foa* was determined using dilution plate techniques at 0 (before soil treatment), 1, 3, 7, 14, and 21 days after soil treatment (Bowers and Locke, 2000). 0.5 g of soil (sample unit) from each tube (replication) was placed in 5 ml of distilled water. 0.1 ml from the appropriate dilution (depending on the treatment) was pipetted onto the surface of Petri plates containing PDAa media. Plates were incubated at 25°C for 24 to 48 h. Colony forming units (CFU) were counted in Petri dishes and then reported per gram of soil (Simoussa *et al.*, 2010). Control tests (infested soil with no medicinal plant treatment) were performed under the same conditions.

Data analysis

The statistical evaluation of results for the study on the products effects on the mycelial growth, sporulation inhibition percentage and on the soil population reduction percentage was performed to determine statistically significant differences ($p<0.05$) by using Fischer test. For all experiments three replicates were realized per treatment and concentration.

3. Results

The drying of *Cotula cinerea* showed that it is rich in water with a rate of about 68%, which is a considerable proportion.

The extraction results have indicated that the cell-wall fraction found in this species is more important. The estimated weight of the different categories of cell-wall polysaccharides (Table 1) showed that cellulose is the most abundant fraction in both organs of *Cotula cinerea* compared to the hemicelluloses and HMP.

Table 1. Yield of cell-wall polysaccharide extracts

	Parietal residue	Cellulose	Hemicellulose	HMP
Leaves	68.33%	17.66%	10.73%	6.80%
Flowers	60%	13.86%	11.23%	10.50%

Effect of extracts on spore germination

The evaluation of the effectiveness of the extracts tested was based on the calculated percentages of spore germination inhibition (Figure 1).

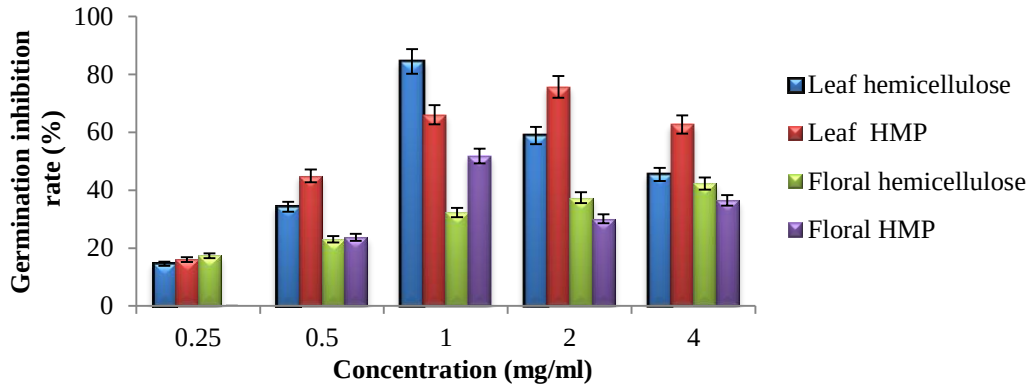

Fig 1. Effect of hemicellulose and HMP extracts on the germination of *Foa*

The results obtained show that the hemicellulose and HMP of both organs have exerted an inhibitory effect on spore germination. The percentage of this inhibition increases with dose increasing for the floral hemicellulose, where the best rate was at an average of 42.25% at a concentration of 4 mg/ml. By contrast for leaf hemicellulose and HMP of both organs, the dose increase is not necessarily accompanied by better efficiency. Leaf hemicellulose and HMP flowers had a lower level of inhibition at 4 mg/ml than at 1 mg/ml. A strong inhibition of germination was registered under the effect of the foliar hemicellulose to 1 mg/ml, with a rate of about 84.45%. HMP leaves also presented a remarkable inhibition with a percentage of 66% at 2 mg/ml. HMP flowers were totally ineffective at 0.25 mg/ml, but presented a maximum inhibition at an average rate of 51.78% to 1 mg/ml.

On cellulose-based media, spore germination was compared with that on PDAa medium with no addings. The results show that the leaf cellulose-based agar is more effective on germination (23.66% inhibition) than floral cellulose-based agar which showed no effect.

Effect of extracts on mycelial growth

Mycelial growth in the presence of cell-wall polysaccharide extracts was assessed after incubation at a temperature of 25°C corresponding to the optimum growth of Foa (Hibar *et* al., 2002). The measurement of the mean diameter of each colony was performed after 7 days of incubation (Table 2).

These results show that the addition of hemicellulose at high doses in PDAa medium had weakly prevented the mycelial growth. By contrast, at low doses Foa develops at the same rate as in PDAa with no addings. No significant difference was observed between the hemicellulose of both organs.

Table 2. Effect of hemicellulose and HMP extracts on mycelial growth of *Foa*

			Concentration (mg/ml)					
Plant organ	extract	control	0.25	0.5	1	2	4	
leaves	hemicellulose	5.31	5.41	4.73	5.6	4.28	4.93	Colony diameter (cm)
	HMP	6.05	6.08	6.2	6.38	6.11	7.06	
flowers	hemicellulose	5.7	4.95	5.72	5.62	3.97	4.52	
	HMP	5.9	6.4	5.91	6.19	6.16	6.21	

In the presence of HMP, *Foa* does not behave in the same manner as in the presence of hemicellulose. Indeed, diameters of all *Fusarium* colonies in the presence of HMP, whatever the concentration, were greater than those of control. This allowed saying that these extracts had a stimulating effect on the mycelial growth of this plant pathogen.

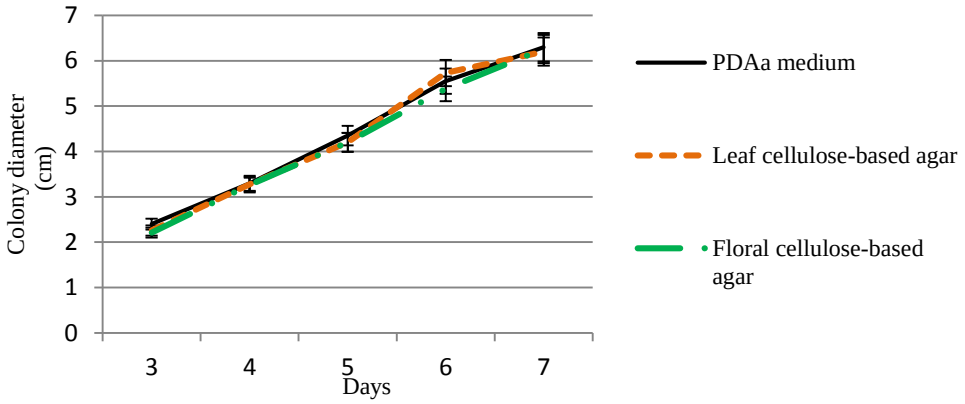

Fig 2. Growth curves of *Foa* on cellulose-based agar and on PDAa medium

The development of the mycelium on cellulose-based agar was followed in terms of time in parallel with the PDAa media (Figure 2). The pace of the growth curve showed that the mycelial hyphae elongation of Foa on cellulose-based agar occurs in a similar way as on PDAa without addings. The diameters of the colonies were between 6.2 and 6.3 cm. The significant difference is that the cellulose-based media have developed a very weakly dense mycelium compared to the PDAa with no addings.

Effect of extracts on sporulation

The evaluation of the effectiveness of the extracts tested was based on the calculated percentages of sporulation inhibition.

The results obtained indicate that the foliar hemicellulose exercised an inhibition around 45% for the concentrations between 0.25 and 1 mg/ml. This inhibition decreased at a dose of 2 mg/ml, then increased at 4 mg/ml until reaching 33.08%. At 0.25 mg/ml the floral hemicelluloses have presented the maximum inhibition with 23.72%, and then the inhibitory action vanished at 0.5 mg/ml. By increasing the concentration to 1 mg/ml, the extract tends to induce a stimulation of sporulation with a percentage of 27.9%. This stimulatory effect was decreased at 2 mg/ml and then, the inhibitory effect of hemicellulose returned at 4 mg/ml with a low rate at an average of 9.3% (Figure 3).

These results show that HMP of both organs had presented an antisporulant effect with a slight difference. The effectiveness of leaf HMP extracts was generally low to medium. At low concentration, these extracts have exercised an inhibition with a rate 22.87%. The increase in concentration was accompanied by a greater effectiveness until reaching 44.47% at 1 mg/ml. Then, this rate remained stable. By contrast, the effect of the floral HMP on sporulation was moderately effective at 0.25 mg/ml. The increase in the concentration to 1 mg/ml resulted in a decrease in inhibition, followed by a maximum antisporulante action at an average of 61.1% at 2 mg/ml and then at an average of 31.83% to 4 mg/ml.

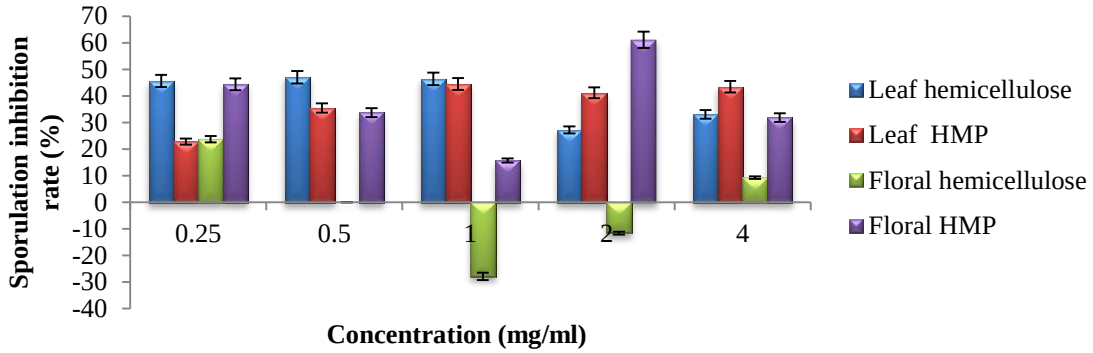

Fig 3. Effect of hemicellulose and HMP extracts on sporulation of *Foa*

On cellulose-based media, no significant difference was observed between the extracts of both organs. These media have shown a significant inhibitory efficacy on sporulation. The inhibition percentage of the sporulation calculated in relation to the PDAa medium with no addings has reached 97.92% and 98.25% on the cellulose-based agar of leaves and flowers respectively.

Effect of the extracts on Foa population density in the soil

CFU were transformed to log 10 CFU per gram of soil (Table 3). These results show that *Fusarium* population was slightly reduced in the first week after treatment of 1% by the foliar polysaccharides. 21 days after treatment, these extracts seemed with little activity. The same results were obtained under the action of the extracts of flowers, with a remarkable reduction exercised under the effect of HMP. For the soil treated with 5 %, a remarkable drop in the density of the pathogen was registered under the effect of leave extracts compared to 1%. The reduction rate after 21 days varied between 16.13% and 33.44% under the effect of HMP and the cellulose respectively. Polysaccharides flowers have resulted only in a slight reduction after 21 days, compared to untreated soil, with a rate of around 5%. Soil treated with 10% of leaf extracts showed no significant difference compared to 5%. Meanwhile a moderate reduction in the density of the Foa in the soil was exercised by the floral cellulose. In contrast, flower HMP have allowed for an increase of the population after 21 days compared to control.

Table 3. Soil population densities of *Foa* over time as affected by soil treatment with cell-wall polysaccharide extracts of *C. cinerea*

Plant organ	Rate treatment	Days after treatment					
		1	3	7	14	21	
Leaves	1%						LOG10 (CFU/g soil)
	cellulose	6.03	6.11	5.78	5.49	5.79	
	hemicellulose	6.24	5.9	6.35	6.4	5.69	
	HMP	5.47	6.44	5.56	5.07	4.69	
	5%						
	Cellulose	6.16	4.6	4	4.47	4	
	hemicellulose	5.73	5.59	6.18	5.87	4.77	
	HMP	7.3	5,94	5.43	5.38	5.04	
	10%						
	Cellulose	4.3	4	4	5.4	4	
	hemicellulose	5.79	5.62	4	6.03	5.25	
	HMP	6.7	4.9	6.84	5.73	5.59	
Flowers	1%						
	cellulose	6.3	5.96	5.726	5.72	5.98	
	hemicellulose	5.97	6.04	6.45	5.92	5.74	
	HMP	5.3	6.46	5.03	4.84	4	
	5%						
	cellulose	6.16	5.92	5.87	5.51	5.59	
	hemicellulose	4.47	5.7	6.27	5.93	5.68	
	HMP	4	6.41	5.96	5.56	5.81	
	10%						
	cellulose	6.05	5.87	5	4.3	4.84	
	hemicellulose	6.31	6.02	6.2	6.36	5.56	
	HMP	6	5.9	6.28	6.94	6.27	
	Control	5.74	5.91	6.46	6.09	6.01	

4. Discussion

Bayoud of date palm (*Phoenix dactylifera* L.), caused by the fungus *Foa*, is the most important disease of this crop. It constitutes a veritable plague in the date growing areas of parts of North Africa and a threat to those countries still unharmed by it. The diversity of the situations in these countries imposes a diversity of control strategies against this disease. In this context, biological agents and plant products have been shown to exert potential antifungal activity against plant pathogenic fungi.

Therefore, the main goal of the present study was to evaluate the antifungal activities of cell-wall polysaccharide extracts derived from the floral parts and leaves of *Cotula cinerea*, against *Foa*.

About the spore germination, and according to Simoussa *et al.* (2010), The *Foa* does not react the same way toward medicinal plants, as toward their extracts and also toward their concentrations in the culture medium. In effect, spore germination of *Foa* was strongly inhibited by essential oils of *Anacyclus valentinus; Artemisia herba alba; Inula viscosa ; Thymus vulgaris* to that by the essential oils of *Eucalyptus sp; Rosmarinus officinalis L; Laurus nobilis; Mentha piperita; Salvia officinalis; Tetraclinis articulata.* Moreover, the essential oils of these plant species were more active on the germination of conidia of this same pathogen compared with their aqueous extracts.

The results of effect of cell-wall polysaccharide extracts of *Cotula cinerea* on mycelial growth corroborate those obtained by Lekchiri *et al.* (2006) and Mebarki *et al.* (2013). From these results, it is appropriate to say that these cellulosic extracts play a role of inductor of cellulase at *Foa*, although *C. cinerea* does not represent the specific host of this fungus, and that these extracts may be used as the sole source of carbon and energy for the growth of *Foa*, as Amraoui *et al.* (2004) suggested. According to Roussos and Raimbault (1982) and Mebarki (2016) this strain is already known in the literature as a producer of cellulase. According to previous works, several phytopathogenic microorganisms secrete active toxins and hydrolytic enzymes against tissue structures of the host plant. These are mainly cellulases and pectinases. *In vitro*, these hydrolases were able to degrade cellulose compounds, and pectic (Baracat *et al.*, 1989; Ortega, 1990; Enokibara & Kitamoto, 1992). Deese and Stahmann (1962) had suggested that cellulases and pectinases of *Fusarium orysporum* f. sp. *lycopersici* are responsible for the degradation of pectin and cellulose; essential compounds of the plant cell-wall into soluble fragments. Moreover, the cellulase activity which was demonstrated by *Foa* can contribute to understanding the biochemical mechanisms of the pathogenesis of this fungus. This Latter in contact with the cell-wall constituents of the tissues of date palm induced cellulases which will a release of reducing sugars, especially glucose which is necessary to the growth of the fungus. In this context, histological studies and enzymatic digestions carried by Belarbi et al. (1984) showed that tissue alterations in the infected date palm may involve hydrolases mainly cellulases, pectinases and even the proteases since these degradations affect the polysaccharides, pectins and proteins of date palm tissues.

Concerning sporulation, research in this context has shown that the sporulation of *Fusarium oxysporum* f.sp. *lycopersici* was completely inhibited by the *Ocimum gratissimum* oil (Doumbouya *et al.*, 2012). According to the European and Mediterranean Plant Protection Organization (2003), the main means of transmission of *Foa* are spores and mycelium in the soil.

The negative correlation that was observed between the inhibition percentages and concentrations in some tests was reported also in the work of Gaceb-Terrak (2010), which showed that a phenolic acid extract of 0.48 mg/ml resulted in a growth kinetics of *Foa* similar to that obtained to 0.12 mg/ml and it was less active than to 0.24 and 0.36 mg/ml. Gaceb-Terrak (2010) justified this phenomenon by the activation of the power of detoxification of these molecules that the 0.48 mg/ml concentration could represent. A similar work concerning the action of a salicylic acid on the same strain and another on the action of the oil of *Melaleuca quinquenervia* on the sporulation of *Fusarium oxysporum* f. sp. *radicis-lycopersici* led to a similar result (Touam *et al.*, 2006; Doumbouya *et al.*, 2012).

It appears from the results of the efficacy of cell-wall polysaccharide extracts on the population density of *Foa* in the soil that the leaf extracts seem to be more active than those of flowers and the cellulose of leaves has led to the best reduction of the *Fusarium* population in the soil. In comparison with other work, our extracts have exercised a good reduction of the *Foa* population compared with that obtained in the presence of the powder of *Anacyclus valentinus; Eucalyptus sp; Rosmarinus officinalis L, Inula viscosa; Artemisia herba alba; Laurus nobilis; Mentha piperita; Salvia officinalis; Tetraclinis articulata; Thymus vulgaris* (Simoussa *et al.*, 2010). By contrast, the powder and the essential oils of these same plants appeared to be active against *Fusarium oxysporum* f. sp. *lentis* (Belabid *et al.*, 2010). Moreover, several studies have shown the effectiveness

of medicinal plants and their extracts against fusarium vascular (Mebarki, 2015). Indeed, the leaf extracts of *Piper betle* L, the powder and the essential oils of *Xylopia Aethiopica*, the powder of neem seeds, oil of *Melaleuca quinquenervia* and of *Ocimum gratissimum* proved an effectiveness against *Fusarium oxysporum* f. sp. *radicis-lycopersici*, Fungal parasite of cultures of tomato and in the control of fusarium wilt caused by this pathogen (Singha *et al.*, 2011; Soro *et al.*, 2010; Hadian *et al.*, 2011; Doumbouya *et al.*, 2012). Similarly, the extracts of *Chromolaena odorata* were active against Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubens* (Krad *et al.*, 2009). Moreover, according to Bowers and Locke (2000), the clove and neem oils, the pepper extract and the essential oil of mustard have reduced the population density of *Fusarium oxysporum* f. sp. *chrysanthemi* in soil and protected chrysanthemum plants.

The results obtained show that the antifungal activity on the different stages of the development of Foa varies from one extract to another. Cell-wall polysaccharide extracts of *Cotula cinerea* deserve further study, to exploit their antifungal properties in the control of date palm fusarium wilt.

References

Alabouvette, C. (1999). *Fusarium* wilt suppressive soils: an example of disease-suppressive soil. *Australas Plant Pathol.*, 28, 57-64.

Amraoui, H., Sedra, M., Hamdaoui, A. (2004). Etude des sécrétions protéiques et enzymatiques du *Fusarium oxysporum* f. sp. *albedinis*, agent causal du bayoud, fusariose vasculaire du palmier dattier. *Al awamia*, 109-110, 47-62.

Baracat, M.S., Bertran, R.E., Ride, J.P. (1989). Chitin oligosaccarides elicit lignification in wounded wheat leaves. *Physiol. Mol. Plant Pathol.*, 34, 3-12.

Belarbi, H., Dexheimer, R., Mangenot, J. (1984). Le pneumatode chez *phoenix dactylifera*. II ultrâstructure de la paroi de la cellule aérifère chez des pneumatodes jeunes provenant de cultures axéniques. *Can. J. Bot.*, 62, 972-981.

Benlarbi, L., Bellahcene, M., Mebarki, L., Vander, W.C., Cornu, B., Moussaoui, A. (2018). Pathogenic and molecular detection of *Fusarium oxysporum* f. sp. *albedinis* isolates from different areas in southwest Algeria. *Trop. J. Pharm. Res.*, *17*(8), 1629-1635.

Bounaga, N., Djerbi, M. (1990). Pathologie du palmier dattier. *Options Méditerranéennes Sér A*, 11, 127-132.

Bowers, J.H., Locke, J.C. (2000). Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the greenhouse. *Plant Dis.*, 84, 300- 305.

Chanda, S.K., Hirst, E.L., Jones, J.K.N., Percivale, E.G.V. (1950). The construction of xylan from esparto grass (*Stipa tenacissima* L.). *J. Chem. Soc.*, 50, 1289-1297.

Deese, D.C., Stahmann, M.A. (1962). Pectic enzymes and cellulase formation by *Fusarium oxysporum* f. sp. *cubens* on stem tissus from resistant and susceptible banana plants. *Phytopathology*, 52, 247-255.

Djerbi, M. (1982). Bayoud disease in North Africa: history, distribution, diagnosis and control. *Date Palm Journal*, 1, 153-198.

Djerbi, M. (1990). Méthodes de diagnostic du bayoud. Bulletin OEPP/EPPO, 20, 607-613.

Doumbouya, M., Abo, K., Lepengue, A., Camara, B., Kanko, K., Aidara, D. & Kone D. (2012). Activités comparées in vitro de deux fongicides de synthèse et de deux huiles essentielles, sur des champignons telluriques des cultures maraîchères en Côte d'Ivoire. *Journal of Applied Biosciences*, 50, 3520–3532.

Enokibara, S., Mori, N., Kitamoto, Y. (1992). Purification and some properties of a carboxyl methyl cellulase from *Favolus arcularus*. *Journal of Ferment and Bioeng.*, 73, 230-232.

European and Mediterranean Plant Protection Organization. (2003). Diagnostic protocols for regulated pests. *Bulletin EPPO*, 33, 265-269.

Gaceb-Terrak, R. (2010). Contribution à la connaissance des interactions palmier dattier (*Phoenix dactylifera* L) – agent causal du bayoud (*Fusarium oxysporum* f. sp. *albedinis*) par analyses phytochimiques des lipides et des phenylpropanoides. Algiers, Algeria, University of Sciences and Technology Houari Boumediene, PhD Thesis.

Garibaldi, A., Guglielmone, L., Gullino, M.L. (1990). Rhizosphere competence of antagonistic *Fusaria* isolated from suppressive soils. *Symbiosis*, 9:, 401–404.

Hadian, S., Rahnama, K., Jamali, S., Eskandari, A. (2011). Comparing Neem extract with chemical control on *Fusarium oxysporum* and *Meloidogyne incognita* complex of tomato. *Advances in Environmental Biology*, 5, 2052-2057.

Harche, M., Tollier, M.T., Monties, B., Catesson, A.M. (1991). Caractérisation comparée des constituants (polyosides, lignine et acides phénoliques) des parois cellulaires de trois graminées; *Stipa tenacissima*, *Lygeum spartum* et *Aristida pungens*. *Cellulose Chem. Technol.*, 25, 11-17.

Hassikou, R., Hassikou, K., Ouazzaoui, T.A., Douira, A. (2002). Effet in vitro et in vivo de quelques fongicides sur *Curvularia lunata*. *Actes Inst. Agron. Vet.*, 22, 205-213.

Kra, K.D., Diallo, H.A., Kouadio, Y.J. (2009). Activités antifongiques de l'extrait de *Chromolaena odorata* (L.) King & Robinssur deux isolats de *Fusarium oxysporum (E.F. Sm.)* responsables du jaunissement mortel des feuilles des bananiers. *Journal of Applied Biosciences*, 24, 1488–1496.

Lekchiri, S., Moueqqit, M., Lekchiri, A. (2006). Mise en évidence d'une activité cellulase chez *Fusarium oxysporum* f. sp. *albedinis* induite par une nouvelle forme d'hydrocellulose purifiée. (ed) *International Congress of Biochemistry*, 09-12 May 2006. Agadir, Morocco, 175-178.

Maiza, K., De La Perriere, R.B., & Hammiche, V. (1993). Pharmacopée traditionnelle saharienne: Sahara septentrional. In *Actes du 2 ème Colloque Européen d'Ethnophmacologie et de la 11 ème Conférence internationale d'Ethnomédecine, Heidelberg*.

Maouni, A., Lamarti, A., Douira, A., Badoc, A. (2001). Effet de dérivés calciques sur le développement de moisissures lors de la conservation des poires. *Bull. Soc. Pharm. Bordeaux*, 140, 79-88.

Markouk, M., Redwane, A., Lazrek, H.B., Jana, M., Benjama, A. (1999). Antibacterial activity of *Cotula cinerea* extracts. *Fitoterapia*, 70, 314-316.

Mebarki, L., Kaid Harche, M., Benlarbi, L., Rahmani, A., Sarhani, A. (2013). Phytochemical Analysis and Antifungal Activity of *Anvillea Radiata. World Applied Sciences Journal, 26*(2), 165-171.

Mebarki, L., Kaid Harche, M., Benlarbi, L., Kasmi, H., Matrouine, M. (2015). *Bubonium graveolens* extracts for controlling *Fusarium oxysporum* f. sp. *albedinis*. *Romanian Biotechnological Letters, 20*(1), 10026-10035.

Mebarki, L., Kaid-Harche, M., Benlarbi, L., Makhloufi, A. (2010). *Cotula cinerea*, une plante de la pharmacopée Nord Africaine. (ed) *International Symposium, plant and microbial species described in Algeria from 1962 to 2010*, 18-20 October 2010. Oran, Algeria, 71.

Mebarki,L. (2018). Traditional uses, phytochemistry and biological activities of *Cotula cinerea* Del: A review. *Trop. J. Pharm. Res., 17*(2), 365-373.

Mebarki, L. (2020). The biological activities of flavonoids and plant cell wall polysaccharides: a minireview. *Advances in Biology & Earth Sciences, 5*(2), 69-75.

Munier, P. (1973). Le palmier dattier. Paris, France, Maisonneuve et Larose.

Ortega, J. (1990). Production of extracellular cellulolytic enzymes by *Fusarium oxysporum* f. sp. *lycopersici*. *Tex. J. Sci.*, 42, 405-409.

Redgwell, R.J., Selvendran, R.R. (1986). Structural features of cell- wall polysaccharides of onion (*Allium cepa). Carbohydr. Res.*, 157, 183–199.

Robert, A., Benkhalifa, A. (1991). Progression de la fusariose du palmier dattier en Algérie. *Sécheresse,* 2, 119-128.

Roussos, S., Raimbault, M. (1982). Hydrolyse de la cellulose par les moisissures – Screening des souches cellulolytique. *Ann. Microbiol. Inst. Pasteur.*, 133, 455-464.

Saaidi, M. (1990). Amélioration génétique du palmier dattier Critères de sélection, techniques et résultats. *Options Méditerranéennes Sér A,* 11, 133-154.

Si Moussa, L., Belabid, L., Tadjeddine, A., Bellahcene, M., Bayaa, B. (2010). Effect of Some Botanical Extracts on the Population of *Fusarium oxysporum f.* sp. *albedinis*, the Causal Agent of Bayoud Disease in Algeria. *Arab Journal of Plant Protection*, 28, 71-79.

Singha, I.M., Kakoty, Y., Unni, B.G., Kalita, M.C., Das, J., Naglot, A., Wann, S.B., Singh, L. (2011). Control of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* using leaf extract of Piper betle L.: a preliminary study. *World J. Microbiol. Biotechnol.*, 27, 2583-2589.

Soro, S., Ouattara, D., Zirihi, G.N., Kanko, C., N'guessan, E.K., Kone, D., Kouadio, J.Y., Ake, S. (2010). Effet Inhibiteur in Vitro et in Vivo de l'extrait de Poudre et de l'huile Essentielle de *Xylopia Aethiopica* (Dunal) A. Rich. (Annonaceae) sur *Fusarium oxysporum* f. sp. *Radicis-lycopersici* (Forl), Champignon Parasite des Cultures de Tomate. *Eur. J. Sci. Res.*, 39, 279-288.

Thibault, J.F. (1980). In Characterization and valorization of cell wall polysaccharides of *Urginea pancration* (Steinh) Phil. from Tessala Mountain (north-west of Algeria). *Les Technologies de laboratories*, 5, 23-29.

Touam, D., Gaceb-Terrak, R., Rahmania, F. (2006). In Contribution à la connaissance des interactions palmier dattier (*Phoenix dactylifera* L) – agent causal du bayoud (*Fusarium oxysporum* f. sp. *albedinis*) par analyses phytochimiques des lipides et des phenylpropanoides. Algiers, Algeria, University of Sciences and Technology Houari Boumediene, PhD Thesis.

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: INNIS (Innis M, Gelfand D, Sninsky J, White T.) (Ed.) *PCR Protocols a Guide to Methods and Applications San Diego*. Academic Press, pp 315322.

Zhang, Y.J., Zhang, S, Liu, X.Z., Wen, H.A., Wang, M. (2010). A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. *Lett. Appl. Microbiol.*, 51, 114–118.