

# Evaluation Of The Antifungal And Antioxidant Activity Of *Citrullus Colocynthis* Against The Dieback Of Olive Tree In South Tunisia

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**Abstract**—To evaluate antioxidant activity and quantify total content of polyphenols, lipid and protein in two organs of plant extract of the *Citrullus colocynthis* (*C. colocynthis*) fruits and seeds. An qualitative and quantitative analysis of methanolic extracts, prepared from *C. colocynthis* fruits and seeds was carried out using standard methods. Estimation of their antioxidant activity was determined by free radical scavenging activity assay using 2,2-diphenyl-1-picrylhydrazyl and ferric reducing power and antibacterial activity from this three compounds. against a pathogens from olive a *Fusarium solani* and *Fusarium oxysporum* cause a new dieback of branches and roots of olive trees was observed in several orchards in southern Tunisia. Preliminary phytochemical screening of the methanol extracts showed that the content of the compounds varies among the two organs of cucurbits with the presence in different concentration of polyphenols, lipid and protein. Polyphenols from the bitter apple seeds have power over important antioxidant with a rate equal to 0.128295 g EAA / 100g monitoring the activity of polyphenols from fruit with a rate roughly equal (EAA 0.997614 g / 100g) in trote, there are antioxidant activity outcome of seed proteins and fruit with the lowest value (0.5568 g EAA / 100g). While the lipid extract from seed lipid (0.99 g EAA / 100g) shows strong compared to that of fruit (0.089 g EAA / 100g) and relative to the protein with a slightly lower rate than that of polyphenols. These phytoconstituents ensure an interesting antifungal and antibacterial activity with a maximum inhibition rate of lipid, protein and polyphenolic extracts from the seeds that reaches 95%.

**Keywords**—antioxydant activity, antifungal activity, polyphenolic extracts, lipid and protein, antibacterial, , *Citrullus Colocynthis*, dieback, olive

## 1. Introduction

Current olive pest and infectious diseases caused by fungi are still a major threat to agriculture despite of the huge progress in of plant protection industry and pesticides. The disease control and management strategies are difficult to implement and so expensive (Pinto and Morais, 2000), (Trapero and Blanco, 1999, USEPA, 1996), since the pathogen lives in the ground and the application of conventional fungicides did not show any effectiveness (Sinclair and Hudler, 1998). The treatments applied are fundamentally cultural (Stipes, 2000). A simple method to protect the plant against these pathogens is particularly needed in developing countries due to relative widespread drug resistant microorganisms. Therefore, new antimicrobial substances must be developed and novel strategies should be investigated (Clardy and Walsh, 2004). Current research ocused on natural plant products as an effective source, based on their ethnobotany uses (Verpoorte and al, 2005). *Citrullus colocynthis* belongs to the family of Cucurbitaceae. Members of this family are generally dioeciously herbs which may be prostrate or climbing. *Citrullus colocynthis* had been extensively used in medicine since ancient times. Traditionally, its fruit was used for the treatment of microbial diseases, jaundice, inflammation, ulcer, diabetes and urinary diseases in Asian and African countries (Saganuwan, 2010) and as insecticidal agent (Rahaman and al, 2008). The aqueous extract of *Citrullus colocynthis* possesses an antidiabetic effect and antioxidant activity (Gurudeeban and Ramanathan, 2010). The purpose of this study is to highlight the beneficial effect of using of natural substances extracted from *Citrullus colocynthis* as bioprotective agents against olive dieback. Liquid medium dilution and direct contact methods were applied on potentially toxin-producing fungal strains isolated and identified from the olive trees. In this respect, phytochemicals from fruits and seeds have been shown to possess significant antioxidant

capacities. The antioxidant properties of fruit and seeds are depending on their content of phenolic compounds (flavonoids mainly), proteins and lipids. Polyphenols from the bitter apple seeds have power over important antioxidant with a rate equal to 0.128295 g EAA / 100g monitoring the activity of polyphenols from fruit with a rate roughly equal (EAA 0.997614 g / 100g) in total, there are antioxidant activity outcome of seed proteins and fruit with the lowest value (0.5568 g EAA / 100g). While the antioxidant from seed lipid (0.99 g EAA / 100g) shows strong compared to that of fruit (0.089 g EAA / 100g) and relative to the protein with a slightly lower rate than that of polyphenols. Thus they could be utilized to control the quality of herbal preparations to ensure their antifungal and antimicrobial activities. Moreover, their effects of controlling of olive tree dieback of plants caused by *Fusarium oxysporum* et *Fusarium solani*.

## 2. Materials and methods

### 2.1. Plant material

The fruits of *C. colocynthis* were collected at the stage of maturity, from September to November in a south location of Tunisia. Seeds were salvaged, dried, sheltered from light and stored at ambient temperature till analysis. Five hundred grams of each plant organ (fruit pulp and seeds) were powdered and passed through a 40-mesh sieve. The plant material was extracted with 95 % ethanol in a Soxhlet apparatus and ethanol was removed and dried at 40°C by a rotary evaporator.

### 2.2. Bacterial and fungal strains

#### 2.2.1 Fungal support

PDA plates with the mycelium of the two strains of *Fusarium oxysporum* and *Fusarium solani* isolated and identified by microscopic and molecular techniques were incubated at 35 ° C for 4 to 5 days. In order to obtain pure cultures, each colony was subcultured repeatedly in new boxes of PDA and incubated at 35 ° C, in order to ensure growth and production of clean mycelium.

#### 2.2.2. Microbial support

This support is composed of bacterial strains and reference strains as following: *Salmonella typhimurium* ATCC 1408 L2, *Enterococcus faecalis* A1 ATCC 29212 L3 A2, *Staphylococcus aureus* ATCC 25923 L6 A3 and *Escherichia coli* ATCC 35218 L5 A4.

### 2.3. Phytoscreening of *Citrullus Colocynthis*

#### 2.3.1. Determination of total polyphenols by spectrophotometry

The concentration of total phenolics was measured by the method described by (Kim and al), with modifications. Briefly, an aliquot (1 ml) of standard solutions of gallic acid (20, 40, 60, 80 and 100 mg/ml) was added to a 25 ml volumetric flask containing 9 ml of deionized water (H<sub>2</sub>O). After 5 min, 10 ml of 7%

Na<sub>2</sub>CO<sub>3</sub> solution was added with mixing. One milliliter of Folin - Ciocalteu's phenol reagent, adjusted to 25ml with distilled water and was shaken thoroughly. After incubation for 90 min at 23 °C, absorbances of both blank and standard solutions were read at 750 nm. Total phenolic contents of samples were expressed as mg gallic acid equivalents (GAE)/100 g fresh weight. Absorbance of the pink color developed was determined at 765 nm. A calibration curve was prepared with quercetin and the results were expressed as mg quercetin equivalents /100g sample.

#### 2.3.2. Radical-Scavenging Activity

DPPH radical-scavenging activity of sample extracts and standards (quercetin and BHT) were determined. The capacity of samples of *Citrullus* seeds and fruit to scavenge the lipid-soluble 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, which results in the bleaching of the purple color exhibited by the stable DPPH radical, is monitored at an absorbance of 515 nm. Radical scavenging activity of extracts and standards, which was interpolated from graph, constructed using percent inhibition against concentration of standards. An amount of 0.5 g of cold sample is added to 3 ml of methanol in a test tube, left in the dark and placed in an ice bath for one hour after making the samples. After one hour, the sample was taken from the tube with a Pasteur pipette, placed in 2ml Eppendorf and centrifuged at a speed of 15,000 G, temperature of 4°C and for 10 min. The supernatant was recovered and put in two 5ml tubes. The DPPH was expressed as percentage inhibition by comparison with ascorbic acid standards (calibration curve constructed from 1.0 to 8.0 µg/mL).

#### 2.3.3. Lipid extraction

Mature fruits were cut to get the seeds that have been finely ground and also conducted with crushed fruit extraction; hot using the mounting Soxhlet and hexane as solvent. After three hours, the recovered hexane is evaporated under reduced pressure at 50 ° C using a rotavapor [5], a viscous yellow oil having a foul odor was obtained.

#### 2.3.4. Lipid assay

Total lipids were determined according to the method (Goldsworthy et al, 1972) using vanillin as reagent. The lipids form with hot sulfuric acid, together with the presence of vanillin and orthophosphoric acid, a pink complex. The assay is done on 100 µl aliquots of the lipid. The tubes are closed, shaken and placed for 10 min in a water bath. After heating to 100 ° C for 5 min, 200µl of this mixture was added a 2.5ml of sulphophovanilline and vigorously stirred. After 30 min incubation in the dark, absorbances were read at 350 nm against a blank. The sulphophovanillin is prepared as follows: dissolve 0.38 g of vanillin in 55 ml of distilled water and add 195 ml of orthophosphoric acid 85%. This reagent is stable for 3 weeks at 4 ° C in the dark. The lipid stock solution is freshly prepared from 2.5ml edible oil.

### 2.3.5. Protein Extraction

From one gram of delipidated meal, proteins are extracted by a cold maceration for 24 hours

using 25 mL of three solvents (0.5 M NaCl, a tompam solution pH = 7.4 and distilled water). After filtration, the volume of the extract is adjusted to 100 mL per each solvent.

### 2.36. Protein Assay

The protein assay was performed according to the (Bradford method 1976) in a 100 .ml liquot of 4 ml of Commassie Brilliant Blue (BBC; G 250; Merck) reagent is prepared in 50 ml of ethanol 95 °, then added by 100 ml of ortho phosphoric acid at 85% and made up to 1000 ml with distilled water .The shelf life of the reagent is 2 to 3 weeks at 4 ° C. The presence of protein in is revealed by the formation of blue dyes. Absorbance is read at 595 nm against a blank. Calibration curve is made from a bovine serum albumin solution titrating 1mg / ml.

### 2.4. Antifungal activity of phenolic, protein and lipid extracts of *Colocynthis* in vitro

The antifungal activity of the studied extracts was assessed by different methods namely Well's method, disc method and direct contact method.

#### 2.4.1. Wells' method (Double Layer)

In a Petri dish containing PDA medium, a thin layer of 0.6% PDA medium (3ml) containing 1ml of the spore suspension to be tested was spread on the surface. After solidification, 100 .ml of the sample is deposited into wells formed in the agar (Achemchem.F and al, 2004.2)

#### . Discs' method

9 mm diameter discs cut from Whatman No. 1, sterilized and impregnated with different concentrations of aqueous extracts of the tested biological materials (proteins, lipids and polyphenols) have been deposited gently on the surface of a previously inoculated medium. The inhibition diameters are measured around the disks after incubation in an incubator at 30-35 ° C for 24 to 48 h.

#### 2.4.2. Direct contact method

Choice of solvent recovery polyphenolic extracts, lipid and protein ; DMSO seems to be the solvent that has no powerful antifungal potency over other solvent recovery (Yrjönen , 2004) , ( Alvi, 2005), (Mohammedi , 2006,) and ( Owagh and al ,2010). After incubation for 48 h, the culture medium (DMEM) of each cell was aspirated and replaced by 1 mL of a solution containing the following concentrations of DMSO (Dimehtylsulfoxide, DMSO; Mallinckrodt Baker Inc, Phillipsburg, NJ, USA): 0.05 mM (0.0004%); 0.1 mM (0.0008%); 0.3 mM (0.0024%); 0.5 mM (0.004%); or 1 mM (0.008%) mixed in plain DMEM. The control group was represented by DMSO-free DMEM. The cell were kept in contact with the solutions for 24 h and maintained in a humidified incubator at 37°C

under a 5% CO<sub>2</sub> and 95% air atmosphere during that period of time.

#### 2.4.3. Antifungal test

The antifungal activity of the polyphenolic, lipid and protein extracts were carried out on two strains: (*Fusarium Oxysporum*, *Fusarium Solani*). They have been tested in vitro by direct contact method on PDA agar medium to determine the inhibition rate, comparing their actions to various concentrations of mycelial growth ( Hussin and al, 2009), and a PDB liquid medium to determine the minimum inhibitory concentration (MIC), the fungicidal concentration and fungistatic concentration (FCS) ( Derwich and al, 2010).

Determination of inhibition rate dilutions of *Citrullis Colocynthis* with a were prepared. To determine the inhibition rate, 3g of polyphenolic, lipid and protein extracts extract were introduced into a tube containing 10 ml of DMSO (300 mg / ml), 5 ml of the solubilized extract is then added to 05 ml of DMSO (150 mg / ml). The same procedure was applied for the preparation of concentrations from 18,75mg / ml to 300 mg / m . One ml of each extract is added to tubes containing 19ml of sterile liquid PDA medium. The mixture is homogenized at 45 ° C ( Subrahmanyand al, 2001). Then, the mixture was put in Petri dishes of 90 mm (20ml / box) (Satish and al, 2010).

After solidification of the agar, the Petri dishes were divided into two parts, where a fungal strain was inoculated by a mycelial disk, taken from the youth culture of the fungus. The PDA without extract served as a control for each strain (Mishra and Dubey, 1994), ( Khallil, 2001). The final concentrations of polyphenolic extracts used were calculated using the following equation

$$\text{With : } Cf = Ci / 20$$

Cf: final concentration of polyphenolic extracts, proteic and lipid in 1 ml of the PDA;

Ci = initial concentration of the polyphenolic extract, protein and lipid solubilized in DMSO [22]

Strains were incubated for 2 days to *Fusarium solani* and *Fusarium oxysporum* at a temperature of 30 ° C

The percentage inhibition of mycelial growth compared to the control, was calculated by the following formula:  $PI(\%) = (A-B) / A \times 100$

With:

PI (%): inhibition rate expressed as percentage;

A: Diameter colonies in the "positive control" boxes;

B: Diameter of colonies in plates containing extract of polyphenols, lipids and proteins [23] Bajpai and al, 2010. The polyphenolic, lipid and protein extracts are considered very active when it has an inhibition between 75 and 100%, the fungal strain is considered

very sensitive when it has an inhibition between 50 and 74%, the fungal strain is called sensitive, moderately active when it has an inhibition between 25%, limited or not active when it has an inhibition between 0 and 24%, the fungal strain is called insensitive or resistant(Alcamo, 1984).

**2.4.3.1.Liquid medium dilution method**

Serial dilutions were made from the culture medium of the sample stock solution . To these dilutions was added a suspension of fungal microorganismThe preparation of this suspensions of fungal strains selected after sporulation of fungal strains (inoculated into 20 ml of PDA in Petri dishes after incubation at 30°C for 4 days), the sporecultures were recovered by adding 10 ml of sterile distilled water under vigorous stirring [ (Solis-Pereira and al, 1993). A spectrophotometric assessment at 625nm (read at a wavelength of 625 nm) of the fungal suspension was carried out; to standardize the spore suspension to 106 spores / ml (Hussain and al, 2008).

**2.5. Statistical Analysis**

Data collected from all the experiments in this study were analyzed for statistical significance using analysis of variance (ANOVA). All samples were analyzed in three replications.

A. Means of treatments in each experiment were separated using Duncan’s multiple range tests.

**3. Results and discussion**

**3.1. Phenolic compounds, lipid and proteins contents of *Citrullus Colocynthis***

The seeds and fruits of *Citrillus* depict high contents of polyphenols, lipids and proteins, especially for the content of lipids assessed for the fruit

**Figure 1:** Content of phenolic compound from Coloquinte seeds and fruits

mg/ml Sample	Polyphenols	Proteins	Lipids
<b>Seeds</b>	36.63*±0,033	15.952*5±0,0083	51.666*5±0,018
<b>Fruits</b>	55.146*5±0,023	8.751 5±1,33	93.333*5±0,016

**Table 1:** Mean concentrations of extracted compends

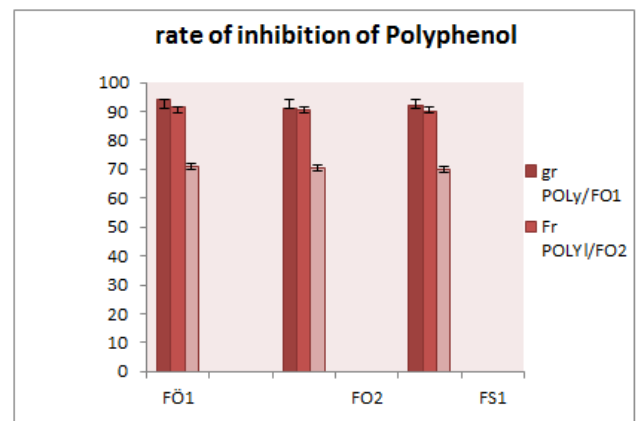
Values are the average of triplicate samples analysed individually. Star indicate significant differences.

Also, the contents are varying in seeds and fruits. Lipids are predominants compared to proteins and polyphenols in the two organs of *Citrillus* , fruits

showed the highest lipid contents .In another hand, the seeds and fruits have lowest protein contents. , however fruits exhibited lowest content compared to seeds with a threshold 0.00 795 g/ml  $P < 0.05$ . The contents of polyphenols and proteins of *Colocynthis Citrullus* obtained in this study are similar to the results reported by ( Pravinand al, 2013). However,lipid contents obtained in this study are different from those reported by (Simmons and al, 19(2006)

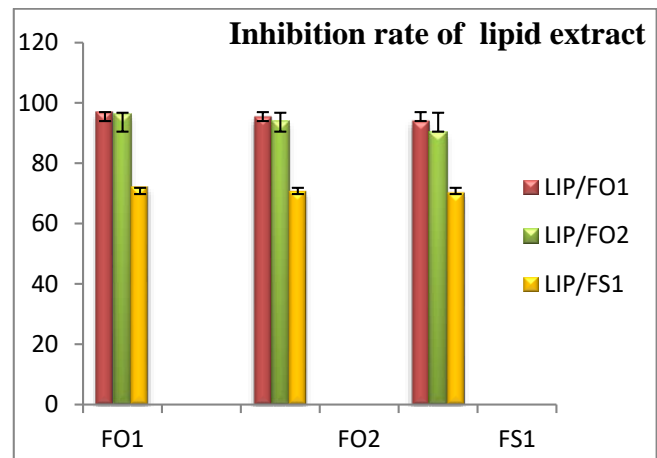
**3.2.Determination of the antioxidant power of polyphenols3.3.Antifungal tests with phenolic extracts, protein and lipid colocynthe**

The results of the antifungal activity of phenolic compound are presented in( Figure 2).



**Figure 2:** percentage inhibition of polyphenols - fungus by the method well polyphenols extracts of seeds, : polyphenols extracts of fruits,FO : *Fusariumoxysporum,Fusariumsolani*)

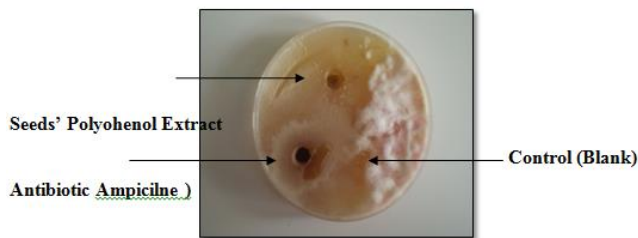
The analysis results showed that the antifungal activity is more important with the protein and lipid extracts from the seed of the fruit. The results of the antifungal activity of the lipid extract are presented in the following histogram(Figure 3)



**Figure 3:** Percent inhibition of lipid fungus by the method well

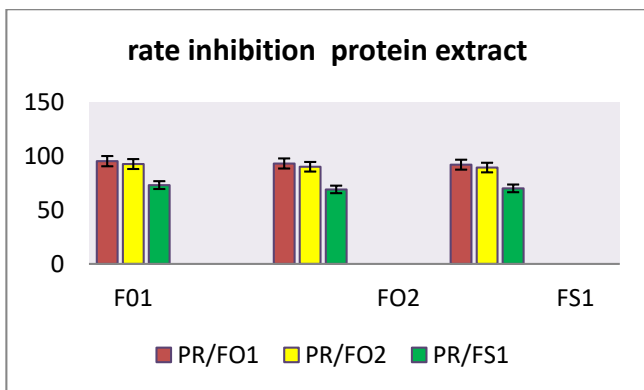


Polyphenolic extract from different parts including fruit a have been tested seeds on fungal strains (*Fusariumsolani*, *F.oxysporum*) after 5 days of incubation at 35 ° Cfor the antifungal activity, using the well method. Results obtained showed that the antifungal activity is higher with the phenolic fruit extract, followed by the extract of seeds and finally that of the antibiotic, showing moderately reduced mycelial growth.From the results shown in Figure4), there is a degradation of the pathogenic mycelium as it was depicted in Petri dishes



**Figure 4:** Inhibitory effect of polyphenol on *Fusarium solani*.

The results of the antifungal activity of the protein extract are presented in the following histogram(Figure 5)



**Figure 5 :** Percent inhibition of protein -fungi by the method wells.

The results Showed that the antifungal activity is higher with the seeds' protein extract than those of fruit and the antibiotic(Ampiciline), wherethe mycelial growth was reduced moderately.

the Minimum Inhibitory Concentrations (MICs) must be calculated in order to define the antifungal efficacy. (Tiwari et al, 2009) Results of calculations for polyphenolic , protein and lipid extracts results are shown in (Table 2).

**Table 2:** Minimum inhibitory concentrations (mg / ml) polyphenolic, lipid and protein extracts of *Colocynthis Citrullus* in liquid medium.

Fungal strains	MICs (mg/ml)		
	Lipids	Proteins	Polyphenols
<i>Fusariumoxysporum</i>	1.87	7.5	0.94
<i>Fusariumsolani</i>	0.94	3.75	0.94

The minimum inhibitory concentration (MIC), defined as the lowest concentration of polyphenols that has resulted in the complete inhibition of the growth of fungi [39] Jong and al, 2010, is ranging from 7.5 to 3.75 mg / ml for protein extract, from 1.87 to 0.94 mg / ml for the lipid extract and 0.94mg / ml for the polyphenol extract. *Solani* species strains have proven that fungal strains were more sensitive to the polyphenolic extract than the other two extracts.

### 3.4. Fungistatic and Fungicidal concentrations

The fungistatic (FCS) and fungicidal concentrations (FC) expressed in mg / ml are shown in (Table 3).

**Table 3:** Fungistatic (FCS) and fungicidal concentrations (FC) in mg / ml polyphenolic, lipid and protein extracts of *Citrullus Colocynthis*

Fungal strains	CF		CFS	
	Lipids	Protein	Polyphenols	
<i>Fusariumoxysporum</i>	∅	7.5	∅	7.5
<i>Fusariumsolani</i>	15	7.5	∅	7.5

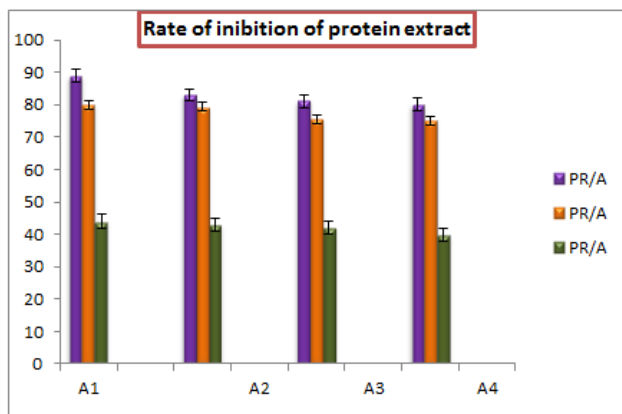
∅ :empty set

Crops grown after obtaining there observed in the presence of polyphenolic, lipid and protein extracts of *Citrullus* on the strains tested. Protein extracts reveal fungistatic activity on fungal strains at a concentration of 7.5 mg / ml and no fungicidal activity while lipids exhibit fungicidal activity at a minimum concentration equal to 15mg / ml for the strain *Fusarium solani* only and fungistatic activity for the two fungals strains. As for the polyphenols, the fungicide concentration is 15 mg / ml and fungistatic concentration is 7.5 mg ml for the *Fusarium solani* strain. The studied strains do not have the same sensitivity. This could be due to the nature of the fungal strains and/or the wall, consisting of a complex network of proteins and polysaccharides and which varies in composition depending on the fungal species .Some polyphenolic compounds bind to microorganisms' proteins, blocking their enzymatic activities, (Mohamed Sham *et al.*, 2010) but also any DNA and RNA synthesis inhibition et d'ARN (Hadi, 2004).

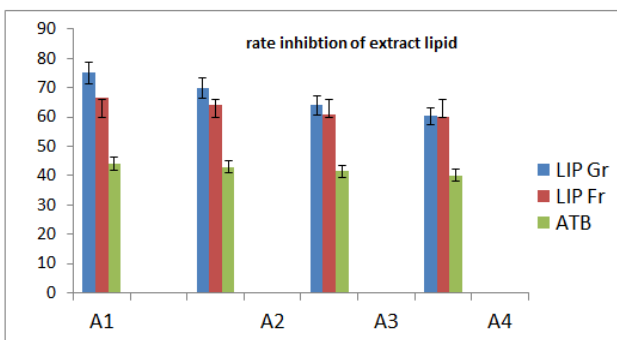
### 3.5. DiskTest of antibacterial power

The results of the antibacterial activity in the following histogram (Figure 6)of the protein extracts using the disc method on agar medium showed that the proteins exhibit activity against four bacterial strains, namely. *Salmonella typhimurium* ATCC 1408 L2 A1, *Enterococcus feucalis* ATCC 29212 L3 A2, *Staphylococcus aureus* ATCC 25923 L6 A3 and *Escherichia coli* ATCC 35218 L5 A4.

A1 is more sensitive towards the protein extract. While there is no difference between the antibacterial activity of other bacterial strains.  $P < 0.05$ .

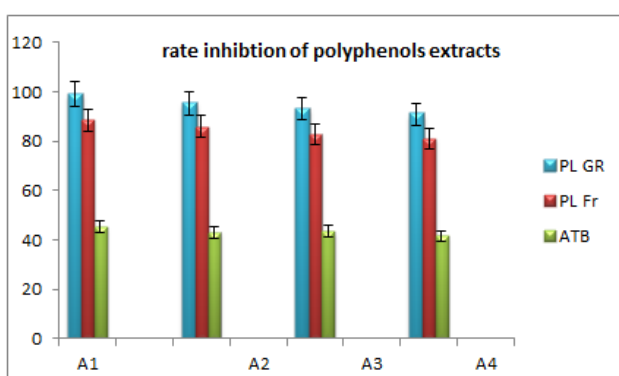


**Figure 6:** Percentage inhibition of the protein / bacteria Disk method (PR : protein, A : bacteria)



**Figure 7:** percentage inhibition of the lipid / bacteria by the method of discs (Gr : seeds, Fr : fruit, ATB : antibiotique, LIP : lipid)

From (Figure 7), the results of the antibacterial activity of the lipid extracts of *Citrullus* using the agar medium on disk method showed that lipids exhibit activity against four Bactrian strains. A1 bacterium is more sensitive lipids recovered from seeds than fruits. While there is a slight difference between the values of the antibacterial power  $P < 0.05$ . Moreover, the susceptibility of bacteria is almost equivalent to the antibiotic.



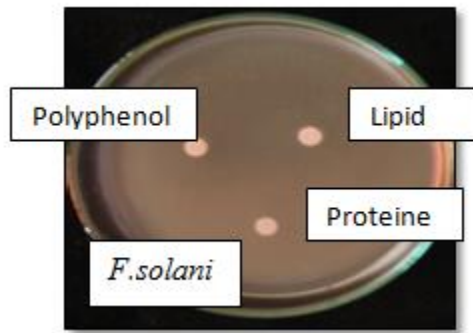
**Figure 8:** Percent inhibition of polyphenolic extracts versus bacteria by disc method

The results of the antibacterial activity of polyphenolic extracts using the PDA environment on disk method showed that polyphenols depicted antibacterial activity against 4 bacterial strains. A1 is

more sensitive to the polyphenolic extract from seeds than fruits. On the other hand, there is a slight difference between the antibacterial power for the for bacterial strains while a low inhibition rate was observed for the antibiotic. From the previous data the polyphenol extracts gourd seeds showed a remarkable fungicidal effect against *Fusarium oxysporum* and *Fusarium solani* isolated from olive. In addition to that, these two strains do not show the same sensitivity, confirming the occurrence of non-phytotoxic and potentially effective compounds against pathogenic fungi (PRABAVATHY and al, 2006), (Chang and al, 2008).

This sensitivity is explained by the inhibition of fungal growth after transplanting discs in PDA media (direct contact method), which is similar to the findings of fungal strains of molds. The strong antifungal activity of the methanolic extract was also reported by (Hadizadeh and al, 2009) where the alcoholic extracts of *Citrullis Colocynthis* showed good antifungal activity (Figure 9).

According to (Yen Chang, 2008) In fact, the inhibition is due to the nature of the fungal strains of the wall composed of a complex network of proteins and polysaccharides and which varies in composition depending on the fungal species. (Domineco and al, 2005) reported that the disruption of this matrix by adsorption of phenolic compounds following the complexation polyphenols / membrane polysaccharides, may result in a reduction in the flow of internal and external layers of the wall which becomes defective and sensitive to osmotic lysis of antifungal agents. In addition, the antifungal activity of polyphenolic extracts could also be due to the fact that certain polyphenolic compounds bind to microorganisms proteins, blocking their enzymatic activities (Mohamed Sham and al, 2010). It may also add the inhibition of DNA synthesis and RNA [47] Hadi, 2004. In light of the results obtained, we can say that the lipid extracts of the seeds of *Citrullus colocynthis* exhibited slow mycelial growth. The work of (Amrouche A. and al, 2013) have shown that bitter apple oils are powerful natural antifungal and can be a very important source of constituents for plant protection used to eradicate fungal infections. The antimicrobial capacity of polyphenols act on the bacteria by the denaturation of cellular proteins (Hamdi and Ellouz, 1993) and alteration of the membranes. They may also inhibit the activity of symbiotic nitrogen-fixing bacteria by inhibiting the activity of digestive enzymes and / or precipitating nutritional proteins (Hättenschwiler and Vitousek, 2000)



**Figure 9:** Antifungal effect polyphenolic, lipid and protein extracts by the disk test

The antimicrobial capacity of polyphenols to inhibit the bacteria is explained by a fact of denaturation of cellular proteins (Hamdi and Ellouz, 1993) and alteration of the membranes (Ranalli, 1991). They can also inhibit the activity of symbiotic nitrogen-fixing bacteria by inhibiting the activity of digestive enzymes and / or precipitating nutritional proteins (Hättenschwiler and Vitousek, 2000).

### Conclusion

Despite being a highly recalcitrant species, the olive tree is subject to a wide range of attacks of pathogens such as fungi, bacteria and viruses, which affect both the root, vegetative or reproductive system. However, fungal diseases in general and especially dieback cause the most significant damage often leading to the decline of the entire tree. The main causative agents of dieback disease are soil fungi, identification and purification of pathogens is the first step made in the context of our project is to classify each fungal strains and determine the range of the infecting the olive tree. The results of extraction and assessment of polyphenols, lipids and protein of *Citruscolocynthis* showed that their contents vary within the organs (fruit and seeds). It would be interesting to conduct further study on the various pathogenic fungal strains of the genus *Fusarium* responsible for certain deadly diseases of the olive tree and to appeal certain other biological products ensuring sustainable production of olive oils and very high quality and safety with a contamination level approaching zero tolerance. The purpose of studying the interaction of fungi and polyphenols, fats and proteins is to use these substances as biological agents in various cosmetics, pharmaceutical and drug alternative biopesticides against different pathogens of olive tree, as a part of a biological control.

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