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Arbuscular mycorrhizal fungi affect total phenolics content and antimicrobial activity of *Tamarix gallica* in naturel semi-arid Algerian areas

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Abstract:

Polyphenolic compounds gained interest in the pharmaceutical research due to their beneficial bioactive compound for a healthy diet. Arbuscular mycorrhizal fungi (AMF) are known to interact with host plant metabolism, inducing accumulation of health-promoting phytochemicals and polyphenolic molecules. *Tamarix gallica* is a halophytic species having hepatotonic and stimulant properties. Leaf, flower and root infusion have anti-inflammatory and anti-diarrheic properties. The purpose of this study was to assess the presence and the diversity of arbuscular mycorrhizal fungi associated to *T. gallica* rhizosphere in Algerian steppic semi-arid site and to evaluate antimicrobial activities of leaf and root phenolic extracts. Four glomoid and one gigasporoid AMF spore type were isolated from rhizosphere of mycorrhizal *Tamarix* specimens. Total phenolic compounds were higher in roots than in leaves. Furthermore, *T. gallica* extracts showed significant antimicrobial properties against human pathogen bacterial strains. The mean inhibition zone was from 0 to 15mm when concentration increased from 1 to 100mg.l^{-1}. The highest significant activity for leaves and roots methanolic extract was observed against *Proteus mirabilis*. Positive correlation was recorded between AMF root colonization and antimicrobial activity. Significant effect of plant type, soil characteristics and AMF root colonization on polyphenolic content was noticed. These findings highlight the complexity of *Tamarix gallica*/AMF symbiosis and their effect on polyphenolic compounds and suggest that *T. gallica* inoculated with arbuscular mycorrhizal fungi may be considered as an interesting source of antioxidants for therapeutic or nutraceutical industries and for food manufactures.

Key Words:

Mycorrhizal colonization, AMF biodiversity, polyphenolic extract, human pathogens.

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Introduction:

There is in our days, an increasing interest in the evaluation and use of plant polyphenol for scientific research and industrial (dietary, pharmaceutical and cosmetic) purposes. Several groups of polyphenols (anthocyanins, tannins, flavones, isoflavones, flavonols) were currently used in the industry as antioxidants by way of nutraceuticals and/or functional foods (Ksouri et al., 2009; Tabet and Boukhari, 2018). The use of phenolic compounds is currently scientifically and rationally managed in order to develop new products for various medical, veterinary and cosmetic purposes (Avio et al., 2017). Phenolic compounds have an important effect on plant oxidative stability and on microbial safety especially under environmental abiotic stress such as high calcareous level and soil salinity (Ksouri et al., 2009). Recent studies revealed protective effect of natural biophenols, such as resveratrol, caffeic and rosmarinic acids against hydrogen peroxide oxidative cytotoxicity by the regulation of the endogenous antioxidant defense system and the modulation of signaling pathways (Bettaib et al., 2017). These bioactive compounds can delay the lipids and proteins oxidation by inhibiting the initiation or/and propagation of oxidative chain reaction. Thus, they may prevent or repair cell damage caused by oxygen (Ksouri et al., 2009). In front of the increased resistance of bacteria to antibiotics (Mota-Fernandez et al., 2011; Tabet and Boukhari, 2018), researchers pointed out the antibacterial efficiency of polyphenolic compounds which makes them a valuable and effective alternative to conventional antibiotics. Indeed, the phenolic molecules exhibits a wide spectrum of medicinal properties, such as antiallergic, anti-arthero-genic, anti-inflammatory, antimicrobial, anti-thrombotic, cardio-protective and vasodilatory effects (Balasundram et al., 2006; Tabet and Boukhari, 2018).

In Algeria, there is many halophytic species with therapeutic properties among which *Tamarix gallica*. This species belongs to a genus that includes about 65 species natives from Asia, Africa and Europe (Baum, 1978). *T. gallica* is a widespread species in coastal Mediterranean areas deciphering a high adaptability to different environments and a high tolerance to hard environmental conditions (Abou Jouadé et al., 2012). This species tolerates maritime windy conditions which makes it a good windbreak in coastal gardens; (Chiej, 1984). Moreover, its extensive root system makes it suitable to control erosion in sandy soils (Abou Jouadé et al., 2012). In Asian countries, it's known for their hepatotonic and stimulant properties, as it was traditionally used in the treatment of various liver disorders. Leaf and flower infusion have anti-inflammatory and anti-diarrheic properties (Ksouri et al., 2009; Bettaib et al., 2017).

Symbiotic fungi constitute important components of ecosystem diversity, due to the mutualistic interaction with roots of the terrestrial plants (Smith and Read, 2008). It has been clearly demonstrated that a diversity of mycorrhizal fungi co-exists in natural communities and can have different effects on the plant growth, soil stability and stress resistance (Smith and Read, 2008; Wilson et al., 2009). The arbuscular mycorrhizal fungi (AMF) root colonization varies according to plant species, fungal and bacterial communities as well as soil environmental and plant physiological conditions (Smith and Read, 2008). Recent study showed that *T. gallica* is naturally associated with arbuscular mycorrhizal fungi (Bencherif et al., 2016). It was proven that AMF induce plant tolerance to biotic and abiotic stress by altering plant secondary metabolism (Bruisson et al., 2016). Thus, AMF may lead to enhanced biosynthesis of health-promoting phytochemicals (polyphenols, carotenoids, flavonoids, and phytoestrogens) and to a higher activity of antioxidant enzymes (Avio et al., 2017). Some researchers suggest that food plants with higher contents of polyphenols, carotenoids or mineral nutrients may be obtained through the biotechnological use of AMF mycorrhization (Castellanos-Morales et al., 2010; Farmer et al., 2007; Giovannetti et al., 2012; Avio et al., 2017). In addition, Toussaint et al., (2007) reported that *Ocimum basilicum* L., a medicinal plant, colonized by AMF yields higher concentrations of phenols in their shoots compared to non-colonized plants.

Relatively little is known about the effect of AMF colonization on the accumulation of active phytochemicals in medicinal plants (Monta-Fernandez et al., 2011), such as *Tamarix* plants. The present study aims to investigate the AMF soil diversity associated with the rhizosphere of *Tamarix gallica* in Algerian semi-arid planted site after 30 years of plantation. Effect of natural AMF colonization on total polyphenolic content and their antimicrobial activity on some human bacteria and yeast have been studied and discussed.

2. Material and methods:

2.1. Site description and soil analysis

Tamarix gallica plants used in this study were extracted from Algerian semi-arid site known as: El Mesrane (N: 34. 90 359°; E 003. 05 973, with 880 m of altitude) (Table 1). In this site *T. gallica* was planted in 1984 in order to fight against soil desertification. Soil texture was estimated using textural triangle (USDA, 2013), Organic matter and organic carbon were determined by Walkley-Black method according to CEEAEQ (2003) using soil moisture and total calcareous content as described by Petard (1993). Soil samples were analyzed for pH on

(1:5) soil: water suspension and soluble salt were determined by measuring the electrical conductivity (1:5) soil: water suspension at 25°C (AFNOR, 1995). Soil salinity was established according to USDA (2013) description. Available phosphorus (P) was measured according to Olsen method (1954). Total soil Nitrogen (N) was determined using the Kjeldahl method followed by titration of the distillates after Kjeldahl sample preparation and analysis (USDA, 2013). Available potassium (K), magnesium (Mg) and sodium (Na) were determined according to Nathan et al. (2012).

2.2. Evaluation of AMF colonization

2.2.1. Soil sampling

Five mature *T. gallica* trees at 20 m intervals were selected. Soil samples were collected over area of 100 m^2. One Kg of soil was collected from each plant rhizosphere at 25-50cm depth and kept in labeled plastic bags at 4°C until processing.

2.2.2. Spore extraction and AMF root colonization

AMF spores extraction was performed on 100g of sampled soil according to wet sieving procedure described by Gerdemann and Nicolson (1963). Spore morphology was studied under a compound microscope (Nikon Eclipse 800). Specimens were described and identified using original descriptions of species and identification keys according to Blaszkowski (2012) and specialized websites *http://www.zor.zut.edu.pl/Glomeromycota/index.html* and *http:// invam.caf.wvu.edu.*

T. gallica roots were washed under water to remove soil debris and cut into 10 - 15 mm fragments to allow better staining performance. Phillips and Hayman (1970) method modified by Dalpé and Seguin (2013) was used to stain *T. gallica* roots and AMF root colonization was evaluated according to McGonigle et al. (1990).

2.3. Extraction and determination of total phenolic content

Green leaves and roots were collected during the growing season (April), placed in plastic bags, transported to the laboratory and allowed to air dry. A portion of the root systems was collected from each plant at 25-50cm depth and kept in labeled plastic bags at 4°C until processing. The leaves and roots of *T. gallica* were rinsed with distilled water, left at room temperature for 5 days in the dark, oven-dried for 1 hour at 60°C. The dried leaves of our plants were crushed into small parts and then powdered finely. Extracts were obtained by magnetic stirring of 10g dry powder in 100 ml pure methanol. Extracts were kept for 24 h at

room temperature, filtered through a filter paper (Whatman N°4). At last, the filtrates were concentrated under vacuum. Then, they were stored at 4°C (Falleh et al., 2008). The total phenolic content was described by Singleton and Rossi (1965), using the Folin-Ciocalteu method (Folin and Ciocalteu, 1927). It was determined by spectrophotometer at 750nm. The mixture was left to stand at room temperature in the dark for 2h. The total phenol concentration was determined using a standard curve for gallic acid and expressed as mg equivalent gallic acid g^{-1} dry wight (Wu et al., 2006).

2.4.1. Flavonols quantification

From the root methanolic extract, 0.5 ml aliquot was amended with 1.5 ml ethanol 95%, 0.1ml $AlCl_3$ at 10%, 0.1 ml potassium acetate (1M) and 2.8ml distilled water. The mixture was incubated at room temperature and the absorbance at 415nm measured after 30 min (Woisky and Salatino, 1998). The flavonols concentration was determined using quercetin (Merck) as standard and a calibration curve with 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ($mg.l^{-1}$) quercetin in ethanol 80% was used.

2.4.2. Flavones quantification

A 1ml aliquot of the leaves methanolic extract was mixed with 2ml of 2,4-dinitrophenilhidrazin (1%) reagent (Merck) and 2ml methanol at 50°C for 50 min. The mixture was added with 5ml 1% KOH in 70% methanol and kept at room temperature for 2 min. After that, 1 ml aliquot was added with 5ml methanol, centrifuged for 10 min and 1ml supernatant was added with 4 ml methanol. The absorbance was determined at 495 nm (Nagy and Grancai, 1996). The flavones concentration was determined using a naringenin standard (Merck) and a calibration curve of 0, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 ($mg.l^{-1}$) of naringenin in methanol was used.

2.5. Antimicrobial activities

2.5.1. Microbial germ characteristics

The antimicrobial potential of Tamarix polyphenols has been tested over six bacterial strains and one yeast identified as: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Salmonella thyphi*, *Shigella dysenteriae* and *Candida albicans* yeast. They were chosen for their high pathogenicity for human and animals and for their development of antibiotic resistance. These strains were obtained from microbiology Djelfa hospital laboratory. Strains activities are presented in supplementary data (S1).

2.5.2. Screening of antimicrobial activity

The antibacterial activity of plant extracts (leaves and roots) was assessed by the agar disk diffusion assay (Bagamboula et al., 2003). The bacterial strains were first grown on Muller

Hinton medium at 37°C for 24 h prior to seeding onto the nutrient agar. One or several colonies of the indicator bacteria were transferred into API suspension medium (BioMérieux) and adjusted to the 0.5 McFarland turbidity standard with a Densimat (BioMérieux). A sterile filter disc with 6mm in diameter (Whatman paper N°3) was placed on the infusion agar seeded with bacteria, and 10µl of several extract concentrations were dropped onto each paper disc, representing 2, 4, and 100 mg per disc. The treated Petri dishes were kept at 4°C for 1h, and incubated at 37°C for 24h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. Standard discs of gentamycin (10 µl) and Ampicillin (10µl) served as positive antibiotic controls according to CASFM 2005 guidelines. Discs with 10µl of pure methanol were used as negative controls. For the antifungal activity of the same organ extracts, the agar-disc diffusion method was used as previously described. *Candida albicans* was first grown on Sabouraud chloramphenicol agar plate at 30°C for 18–24 h. Several colonies were transferred into Api suspension medium and adjusted to two McFarland turbidity standard. The yeast inoculate was streaked onto Sabouraud chloramphenicol agar plates at 30°C using a sterile swab and then dried. A sterile filter disc, diameter 6 mm (Whatman paper N°3) was placed in the plate. Ten microlitres of different extract concentrations (representing 1, 10, and 100 mg per disk) were dropped on each paper disc. The treated Petri dishes were placed at 4°C for 1–2 h and then incubated at 37°C for 18–24 h. As for the antibacterial activity, the antifungal one was evaluated by measuring the diameter of the growth inhibition zone around the discs. The susceptibility of the standard was determined using a disc paper containing 20µg of amphoterecin B. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

2.6. Statistical analysis

Data were analyzed statistically by means of comparison by ANOVA or the Kruskal-Walis tests (with Bonferroni correction of P value) were used as appropriate, to analyze data on metabolic activity. Percentage colonization data were arcsine-transformed before analysis. Pearson correlations were also used to determine whether AMF colonization was positively, negatively or not associated with polyphenol content and correlation with their antimicrobial activity. Statistical tests were performed using Prism 5.0. The ecological attributes were calculated in the following manner: species richness (S), Shannon's biodiversity index (H') and equitability indices of Pièlou (Moreira et al., 2007) using Decorana program of PC-Ord 5.0 (McCune and Mefford, 2011).

3. Results

3.1. Pedological characteristics

Globally soil pH was mostly alkaline (7.85), electrical conductivity indicated that El-Mesrane soil is not considered saline soil, but classified as calcareous soil. Textural triangle classified the soil as sandy-loamy-clayey (Table 2).

3.2. AMF native spore population in *T. gallica* rhizosphere and root colonization

Spore isolated from the rhizosphere of *T. gallica* in El-Mesrane site belong to *Septoglomus*, *Funneliformis* and *Gigaspora* with a total of five species: *Septoglomus constrictum*, *Funneliformis mossea*, *Gigaspora gigantea*, and two non-identified glomoid species. Shannon biodiversity index and equitability index were positively correlated with AMF root colonization rate at $p<0.05$ (Table 3).

All sampled roots showed arbuscular mycorrhizal structures. *T. gallica* AMF root colonization rate was about 13.89% (Table 3). Significant effect of soil and plant species was recorded on *T. gallica* AMF mycorrhizal colonization at $p<0.001$ (Table 6).

3.3. Quantification and identification of phenolic compounds

Based on the absorbance values after reaction with Folin–Ciocalteu reagent, results of the colorimetric analysis are given in figure1. The methanolic extracts of the *T. gallica* contain polyphenols and flavonoids. The total polyphenol content of the methanolic extract of *T. gallica* obtained by maceration method was expressed as gallic acid equivalent per gram of dry weight. The amount of total polyphenols was higher in roots (356.26 ± 2.01 mg of GAE.g^{-1} dray weight) than in shoots and leaves (339.61 ± 4.04 mg of GAE.g^{-1}). Flavonoids were higher in root than in shoot of *T. gallica* plants. In leaves, flavones represent 91% from total shoots flavonoids, while in roots, flavonols represent 98% from total roots flavonoids. Positive correlation was recorded between AMF mycorrhizal colonization and total leaves phenolic content ($r=0.8493$, $p<0.01$) and with total roots phenolic content ($r=0.8777$, $p<0.0001$). In addition positives correlation was recorded between AMF mycorrhizal colonization and flavonoid content of leaves and roots ($p<0.01$), flavones and flavonols content ($p<0.05$) (Table 5). Multi analysis of variance (ANOVA) indicated that polyphenolics extract, flavonoids, flavones and flavonols are influenced by plant species (*T. gallica*) ($p<0.001$), soil agropedological characteristics ($p<0.001$) and AMF root colonization ($p<0.001$) (Table 6).

3.4. Antimicrobial activity

The results of antimicrobial activity of *Tamarix gallica* methanolic extracts measured by the agar diffusion method against selected pathogenic bacteria are showed in table 4. The mean inhibition zone for all bacteria treated with *T. gallica* shoots extracts increased from 0 to 15mm when the concentration increased from 1 to 100mg.l^{-1}. Roots extracts were more active against bacteria as compared to leaf ones. In the two plant tissues evaluated for their effect on bacterial growth, the inhibition zones around bacteria increased with the extract concentration. The most significant activity of *T. gallica* extract was recorded against *P. mirabilis* (Figure 2) and the lowest activity was observed against *S. thyphi*. Interestingly, polyphenol extract have a positive effect on *Klebsiella pneumonia* showing increased antibiotic resistance (Table 4). Leaves and roots extract exhibited positive activity against *Candida albicans* with higher extract concentration (100mg.ml^{-1}) as compared to standard Amphoterecin B (Table 4). Positive correlation was recorded between AMF root colonization and antimicrobial activity of *T. gallica* leaves and roots extract at $p<0.05$. Multi way analyses of variances indicated that antimicrobial activity of *T. gallica* extract was positively influenced by plant species ($p<0.05$ and $p<0.01$), soil agropedological characteristics ($p<0.001$), AMF mycorrhizal colonization ($p<0.05$) and by the combination of plant species x soil characteristics x AMF mycorrhizal colonization ($p<0.001$ and $p<0.0001$) (Table 6).

Discussion

The first objective of this study related with the biodiversity of indigenous AMF species associated with *T. gallica* rhizosphere. AMF species isolated from rhizosphere were *S. constrictum*, *F. mosseae*, *Gi. gigantea, Glomus sp1* and *Glomus sp2*), species previously found associated with Tamarix trees. (Chaudhry et al., 2013; Bencherif et al., 2016). Several studies have shown that *glomoid spore* species are abundant under semi-arid Mediterranean ecosystems and recognized to be well adapted to environments subjected to disturbance and stress Such as elevated temperature (Gavitto and Azcon-Aguillar, 2012), and elevated soil calcareous level (Labidi et al., 2015). The few studies dedicated to the AMF population of *Tamarix* rhizosphere confirmed the abundance of Glomeraceae (Meinhardt and Gerhing, 2012; Chaudhry et al., 2013; Bencherif et al., 2016). Among species found in *T. gallica* rhizosphere, *S. constrictum* was the most abundant in comparison to the other identified species. This may indicate a better adaptation of this particular AMF to calcareous soils. Indeed, *S. constrictum* has also been frequently detected in neutral and alkaline soils from

both agricultural and non-agricultural areas Oehl et al., 2009; Labidi et al., 2015; Bencherif et al., 2016).

T. gallica roots sampled from the studied Algerian semi-arid ecosystems are found to be naturally colonized with AMF. This mycotrophic status has been earlier mentioned by Bencherif et al. (2016). *T. gallica* has a specific extensive root system, with thick lignified primary root and secondary fine roots (Belarouci, 1991) in which AMF hypha penetrate and differentiated vesicls and a few arbuscules. However, except Bencherif et al. (2016), no other studies were devoted to *T. gallica* AMF roots colonization. For that, the present investigation study highlights the Tamarix mycotrophic status of 30 years old plantation. Positive correlation recorded between AMF mycorrhizal colonization and AMF biodiversity index suggest that the AMF species isolated from *T. gallica* rhizosphere are those who form the arbuscular mycorrhizal symbiosis with this host plant.

The second objective of this study aimed to evaluate the polyphenolic content of plant tissues, (leaves, shoots and roots) and estimate their antimicrobial potential. This approach is pioneer at recording the production of polyphenolic compounds in *T. gallica* plants associated with AMF under field conditions. Results showed that the total polyphenol content was higher in roots than in leaves. That is in agreement with previous study indicating that *Tamarix gallica* was an accessible source of natural phenolic compounds, with high content of total phenolics in shoots, leaves and roots metabolic extract (Espin et al., 2007; Ksouri et al., 2009; Hmidene et al., 2017; Bettaib et al., 2017; Tabet and Boukhari, 2018).

It revealed also that the *T. gallica* AMF root colonization had a positive effect on the production of flavonols and flavones. The recorded increase in flavonols and flavones might be due to a decrease in cell carbohydrates content due to the mycorrhizal association (Toussaint et al., 2007; Ferrol and Pérez-Tienda, 2009). Indeed, Solfanelli et al. (2006) explained that the flavonoid biosynthetic pathways are strongly up-regulated by regulation of sucrose content. These sugars regulated by AMF colonization act as signaling molecules, whose signal transduction pathways may lead to the activation or inactivation of gene expression in phenols biosynthesis (Mota-Fernandez et al., 2011). In the other hand, the production of secondary metabolites changes with the concentrations of auxins and cytokinines (Jeong et al., 2007) and the production of those plant hormones might have been directly affected by the AMF that colonized the roots (Mota Fernandez et al., 2011). The present study on *Tamarix* and others involving different AMF-colonized plants, e.g. *Fragaria*

vesca, *Allium cepa* and *Vitis vinifera*, showed enhanced phenolics content in the different parts of studied plants (Lingua et al., 2013; Torres et al., 2016; Avio et al., 2017).

The methanolic extracts of *T. gallica*, containing active molecules of phenolic components, were efficient to inhibit most bacterial and fungal strain growth. In several studies, the inhibitory effects of plant extracts against bacterial pathogens were attributed to their phenolic composition (Baydar et al., 2004; Rodriguez Vaquero et al., 2007, Ksouri et al., 2009; Tabet and Boukhari, 2018). In the present study, the inhibitory levels measured with leaves methanolic extract on *S. aureus* was higher than those observed by Ksouri et al. (2009) and by Tabet and Boukhari (2018), these authors have used non-mycorrhizal *T. gallica* methanolic extract. That suggested that AMF enhanced positive effect of leaves methanolic extract to inhibit infection caused by *S. aureus*. In addition, leaves and root methalonic extract of *T. gallica* showed a better inhibition level on *Proteus mirabilis* than Ampicillin and Gentamycin antibiotics. This result is important because *Proteus mirabilis* represents 90% of all *Proteus* infections in humans because it is very present in water and soils (CTCB, 2018). Our results showed that the root methanolic extract recorded more efficient antimicrobial effect than leaves ones. That suggests the higher value of root for the exploitation of phenolic extract in pharmaceutical products. It might be more interest to inoculate *T. gallica* roots in vitro culture for more economic production. Indeed, the inhibitory effect of these phenolics could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation (Ksouri et al., 2009; Mota-Fernandez et al., 2011; Tabet and Boukhari, 2018). Ours results suggests that for better *T. gallica* extract antimicrobial activity more concentration is needed with even purification of phenolic compounds. Purified components may be used as natural antimicrobials in food systems, as well as to prevent the growth of food borne bacteria resulting in extension of the shelf life of processed foods (Ksouri et al., 2009). In addition, present study, focused on AMF effect on polyphenolic extract and explained the positive influences of AMF mycorhization on antimicrobial activity of *T. gallica* polyphenolic extract. However, Waśkiewicz et al. (2013) assessed that phenolic content and antimicrobial activity of halophytic plants such as *T. gallica* depending on biological (species, organ, physiological stage and symbiotic microorganisms), environmental and technical factors. Taken together this information my-confirms the interesting potential of *T. gallica* associated to AMF symbiotic species as a valuable source for natural antioxidant and medicals molecules.

Conclusion

The investigation on AMF associated with *T. gallica* rhizosphere in Algerian steppic semi-arid areas revealed interesting AMF diversity and root colonization levels. Moreover, our findings showed that AMF root colonization affects positively *T. gallica* polyphenols compounds and antimicrobial activity of studied plant methanolic extract. This study highlighted the ability of natural AMF community to positively influence the biosynthesis and quality of phenolic compounds and the interesting potential of this halophyte as a valuable source of natural bioactive molecules. In the future, it will be interesting to use the indigenous AMF isolates from naturally steppic soils in order to exploit more efficiently the AMF function in semi-arid Algerian steppes to promote production of *T. gallica* and other steppics plants natural bioactive molecules.

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Table 1 Bioclimatic characteristics of studied site

Studied Site Name	***Tamarix* species**	***Tamarix* Status**	**M (°C)**	**m (°C)**	**R (mm/y)**	**Altitude (m)**	**Coordinates**	**Q_3**	**Bioclimatic stage (Emberger classification)**
El-Mesrane (Djelfa)	*T. gallica*	Planted (1984)	36.21	1.59	165.4	830	34°20'09''N 3°47'02'' E	25.63	Upper semi-arid with mild winter

M: maximum temperature. m: minimum temperature (M and m temperature data taken during the period of study). R: rain. (R: means of annual Rain way). Q_3: Emberger pluvio-thermic quotient.

Table 2 Agropedological characteristics of the field studied site soil:

Studied parameters	Results in El-Mesrane studied site
pH water	7.85
EC (ds m^{-1})	0.8
Salinity (g l^{-1})	0.29
Available P (mg g^{-1})	0.37
Moisture (%)	10.18
N (mg g^{-1})	0.26
C (mg g^{-1})	1.9
C/N	34.23
Mg (meq 100 g^{-1})	1.32
K (mg g^{-1})	250.0
Na (meq 100 g^{-1})	1.39
MO (%)	0.32
Total calcareous (%)	13.83
Active calcareous (%)	6.6
Clay (%)	25.01
Silt (%)	13.55
Sand (%)	61.45
Texture	Sandy-loamy

Data are presented as means obtained from the four studied seasons for each site. -: not detected.

Table 3: AMF species isolated from the studied site according to different seasons

AMF species	Studied site El-Mesrane
Total AMF spore number/100g	165
S	5
H’	0.921
H_{max}	1.60
E	0.838
Funnneliformis mosseae	60
Septoglomus constrictum	73
Gigaspora gigantea	09
Glomus sp1	12
Glomus sp2	11
Total mycorrhizal rate (%)	13.89

Data are presented as means obtained from five replicates from the studied site.

Table 4: Antimicrobial activity of *Tamarix gallica* leaves and roots methanolic extracts at different concentrations (mg.l-[1]). Inhibition zone calculated as diameter around the disc (mm).

Extract concentration/microbial strains	**Leaves extracts (mg.l-[1])**			**Roots extracts (mg.l-[1])**			**Ampicillin (µl)**	**Gentamicin (µl)**	**Amphoterecin B (µg)**
	1	10	100	1	10	100	10µl	10µl	20µg
Staphylococcus aureus	na	na	10±0.00	na	na	11±0.02	18±0.01	40±0.01	na
Escherichia coli	na	na	6.5±0.1	na	na	10±0.01	17±0.01	16±0.01	na
Klebsiella pneumonia	na	na	9±0.01	na	na	8±0.001	na	na	na
Salmonella thyphi	na	na	Na	na	na	na	20±0.01	28±0.01	na
Shigella dysenteriae	na	na	9±0.03	na	na	9±0.00	21±0.01	na	na
Proteus mirabilis	na	8±0.01	14±0.01	na	5±0.02	15±0.00	na±0.01	24±0.01	na
Candida albicans	na	na	7±0.00	na	na	8±0.01	na	na	10±0.02

na: non antimicrobial activity.

Table 5 Pearson's correlation coefficients between the different studied parameters and *T. gallica* AMF root colonization

Parameters	**Pearson r**	**P Value**
Biodiversity index	0,8915*	0,0211
Equitability index	0,8360*	0,0389
Leaves polyphenol content	0.8493**	0.0041
Roots polyphenols content	0.8777****	0.0004
Leaves flavonoids content	0.8902**	0.00135
Roots flavonoids content	0.8749**	0.00106
Flavonons	0.7749*	0.0115
Flavonols	0.8133*	0.0115
Leaves antimicrobial activity	0.7153*	0.0512
Roots antimicrobial activity	0.7672*	0.015

Note: *:p<0.05, **: p<0.001, ****:p<0.0001, Pearsonr: Coefficient of parametric correlation.

Table 6 Influence of *T. gallica* species, soil, AMF mycorrhizal colonization and *T. gallica* extract antimicrobial activity on polyphenols according to multi-way ANOVA with Bonferroni correction The initialized value are significant at $p<0.0001$ (Same error degrees of freedom for root and leaves =10).

	Leaves polyphenols ($mg.g^{-1}$ DW)				Roots polyphenols ($mg.g^{-1}$DW)			
	Flavonoids		Flavonons		Flavonoids		Flavonols	
	F	P	F	P	F	P	F	P
Plants species (1)	29.144	<0.001	29.144	<0.001	28.25	<0.01	28.25	<0.01
Soil (2)	77.0186	<0.001	78.16	<0.001	75.25	<0.001	76.15	<0.05
Mycorrhizal colonization (3)	49.438	<0.001	42.632	<0.001	50.192	<0.001	49.206	<0.001
Antimicrobial activity (4)	50.882	<0.01	45.878	<0.001	116.44	<0.001	50.583	<0.01
(1)*(2)	2.68	<0.01	2.17	<0.05	2.81	<0.01	2.64	<0.01
(1)*(3)	29.144	<0.001	28.563	<0.001	27.15	<0.01	29.214	<0.01
(1)*(4)	6.55	<0.0001	4.43	<0.0001	6.44	<0.0001	4.56	<0.001
(3)*(4)	22.4	<0.05	24.5	<0.05	21.16	<0.05	21.24	<0.05
(1)*(2)*(3)*(4)	36.98	<0.001	36.8	<0.001	0.828	<0.0001	1.2	<0.0001

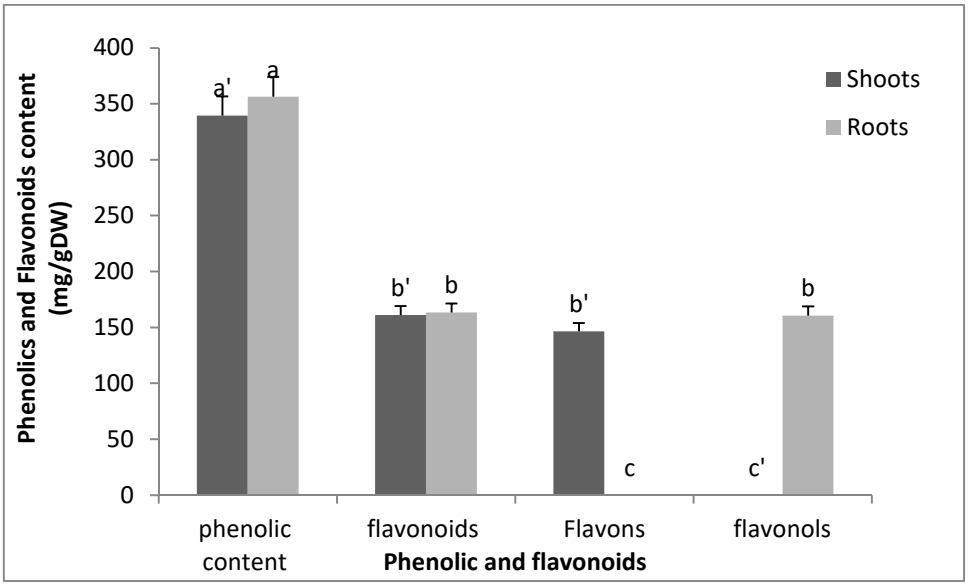


Figure 1 : Phenolic, Flavonoids, Flavonols and Flavons content in *T. gallica* leaves and roots.

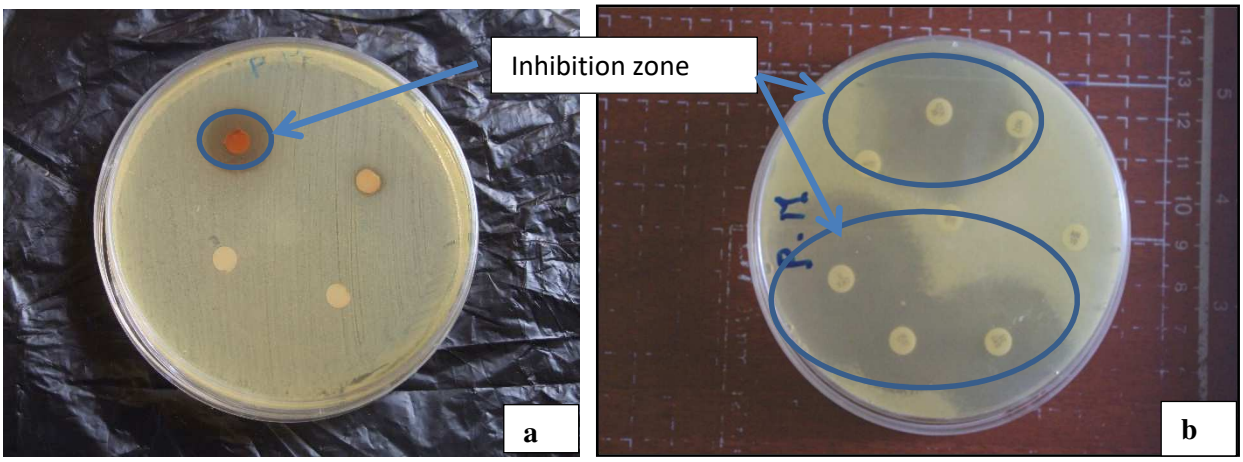
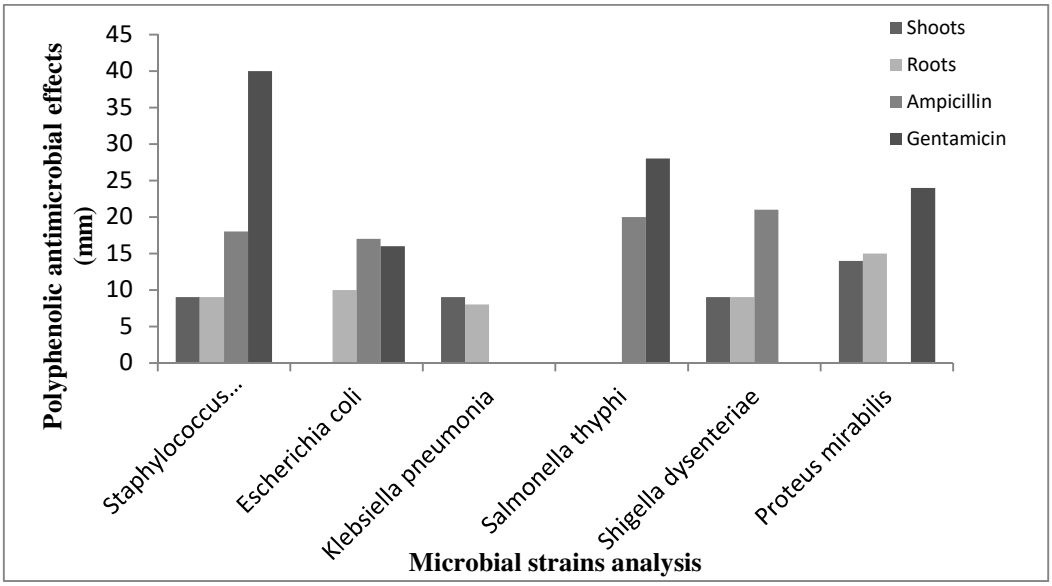


Figure 2: Methanolic extract effect on *Proteus mirabilis*. **a**: Antimicrobial effect of leaves extract. **b**: Antimicrobial activity of root extract.



Graphical abstract: Antimicrobial activity of polyphenolic extract of horticultural plant *Tamarix gallica* as compared to antibiotics.