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Full Length Review Paper

Phytoconstituents and biological activities of *Opuntia-ficus indica* L. mucilage grown in tree Tunisian provenances

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ABSTRACT

In recent years, the use of natural products became a big necessity in order to prevent human health and to protect the environment besides risks caused by using synthetic chemicals. However, *Opuntia-ficus-indica* mucilage had an ample application for ethno medicine, there is no reports focusing on their chemical composition and biological activity. In this work, we aimed to investigate, for the first time, the antioxidant activity, the total phenolic and the flavonoid contents, the antimicrobial and allelopathic activities of the mucilage extracted from *Opuntia-ficus-indica* rackets collected at three different ages (1, 2 and 3 years old) originated from three Tunisian provenances (Sbeitla, Matmata and Ouslatia).

Total phenolic, flavonoid and tannin contents in the tested extracts were determined using Folin-Ciocalteu, Aluminum trichloride and Vanillin reagent, respectively.

In vitro susceptibility to human pathogenic was evaluated against three bacterial strains (*Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniea*) and three fungal strains (*Fusarium culmorum*, *Fusarium solani* and *Botrytis cinerea*), according to well Agar diffusion method. The allelopathic effects tested on *Triticum durum*, *Lens culinaris* and *Trigonella foenum-graecum*. The germination percentage, plumule and radicle lengths were recorded after seven days.

Biochemical analysis showed that Ouslatia provenance was the richest one on phenols (51.692 ± 0.78 microgram equivalent acid gallic per milligram dray weight µg=EAG/mg DW), flavonoids (13.77 ± 1.77 µg QE/mg DW=microgram quercetin equivalent per milligram dray weight) and tannins (37.61 ± 0.62 µg EC/mg DW=microgram of catechin equivalent per milligram dray weight). Similarly, this provenance was the most efficient against all tested microorganisms especially against *K. pneumoniae* (25 mm) and *Fusarium solani* (19 mm). Similarly, the largest capacity to neutralize 22-diphenyl-1-picrylhydrazyl (DPPH) radical (0.003 ± 0.002 mg/ml=milligram per milliliter) was found in Ouslatia provenance.

Opuntia-ficus-indica mucilage of Ouslatia successfully inhibited *Triticum durum* at levels of -25% (seeds germination) and -93.60% (shoot length).

Tunisian mucilage originated from rackets of Ouslatia provenance could advantageously used as a therapeutic agent, pharmaceutical industry, cosmetic and biopesticide products.

Key words: Antioxidant activity, allelopathic effect, biochemical analysis, *Opuntia-ficus indica,* racket, mucilage

INTRODUCTION

The cactus (*Opuntia-ficus-indica*), commonly called prickly pear, is belongs to the Cactaceae family and is characterized by its adaptation to difficult climatic

conditions of arid and semi-arid regions (El-mostafa et al., 2014). It is currently integrated into many strategies such as soil fixation and the fight against desertification in different countries (Ennouri et al., 2014). For this purpose, cactus cultivation becomes very important to the survival of agriculture especially in arid and semi-arid areas (Abdel-farid et al., 2013).

The incorporation of allelopathic substances into agricultural management could reduce the use of herbicides, fungicides, insecticides and decreased the deterioration of the environment (Elaloui et al., 2017; Prasad et al., 2016). Indeed, many studies had shown the presence of an important allelopathic power in the different parts of *Opuntia ficus-indica* (Corrales-Garcia et al., 2004).

Opuntia-ficus-indica cladodes had wild uses such as anti-diabetic, anti-ulcerogenic and cicatrizing effects (Park et al., 2001; Rsaiss et al., 2013). They offered also an extraordinary freeze widely investigated in pharmaceutical industry, cosmetic, as a hair treatment and as water purifying agent.

These interested results promoted the use of OFIM (*Opuntia-ficus-indica* mucilage) and encouraged us to develop its culture and its valorization (Feugang et al., 2013). Tunisian people were interested in fruits, the edible organ, known as Sultan el Galla due to their richness on fiber minerals, and many nutritive compounds (Singleton et al., 1965). Previously, studies had performed taking into biochemical compositions or several therapeutic activities (antioxidant, antimicrobial activities of the essential oil and organic extracts...). However, reports validated the medicinal potential on mucilage, were contradictory and lacked precision. Therefore, this work was conducted in order to establish biochemical composition of mucilage originated from three, Ouslatia). The antioxidant, antifungal, antibacterial and allelopathic effects of this extract against provenances (Sbeitla, Matmata *Triticum durum* (durum wheat), *Lens culinaris* (lentil) and *Trigonella foenum-graecum* (fenugreek) were also determined.

MATERIAL AND METHODS

Plant Material

Rackets of three provenances (Sbeitla, Matmata, Ouslatia) were sampled in summer 2017. For each shrub, three rackets of different ages (1, 2 and 3 year olds) were selected. The identification of the plant material was done by Professor Mohamed Boussaid (Laboratory of Plant Biotechnology (01/UR 0-9-10), INSAT, University of Carthage, Tunisia) and a voucher specimen of the plant was deposited at the Herbarium of INRGREF (National Institute of Research in Rural Engineering, Water and Forests). Rackets were crushed in a blender (Moulinex) with rotative knives type 320, homogenized with 500 ml water and then filtered through a fine cloth and centrifuged for 15 min. The yields extraction of the obtained supernatant (mucilage) was determined, dried and stored until analysis: 1 g of powder was submitted to maceration with 10 mL of pure methanol for 30 min. The extracts, filtered through Whatman No 1 filter paper, were pooled and concentrated under vacuum.

Chemical Reagents

Folin-ciocalteu, phenol, DPPH, Gallic acid, catechin sodium carbonates, hydrochloric acid and methanol were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used were of the highest purity.

Total Phenol Flavonoid and Tannin Contents

A colorimetric method was used to evaluate the total phenolic contents (Earp et al., 1981). From each sample, 0.5 mL of methanolic solution was added to 2.5 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate (75 g/L) solution. The reading of the absorbance was done at 765 nm using a Shimadzu 1600-UV spectrophotometer after incubation during 30 min. The same process was repeated for Gallic acid used as standard. Total phenols of each fraction, expressed into mg GAE/g DW, were measured using the regression equation of a calibration curve y=0.0114+0.518, R2=0.9932.

The total flavonoid contents were assayed using the aluminum trichloride method (Elaloui et al., 2018). The absorption was measured by a Shimadzu UV-1600 (Tokyo, Japan) spectrophotometer at 420 nm. Flavonoid contents were expressed as mg QE/g DW. The calibration curve range was 0-50 µg/mL (R^2=0.981). All measurements were performed in triplicate. Tannins contents were determined flowing the vanillin method as described by (Rsaissi et al., 2013) with slight modifications. In brief, in darkness conditions, 200 µL of leaf extracts were added to a mixture of vanillin (1 mL) and 4 mL of HCl. After incubation for 20 min, absorbance was read at 500 nm using a Jenway 6100 spectrophotometer.

Antioxidant Activity

The ability of the plant extracts to scavenge DPPH free radicals was assayed using the standard method with some modifications (Tomas-Menor at al., 2013). So 2.36 mg of DPPH dissolved in 100 mL of ethanol, mixed in test tubes and incubated in obscurity. The absorbance was read at 490 nm after 30 min of incubation in dark place. Measurements for each experiment were done in triplicate. The percentage inhibition was calculated by the following equation:

$I\ (\%)=((A_0- Ac)/A_0) \times 100$

A_0 was the absorbance of the control and Ac was the absorbance of the plant extract.

The IC_{50} value, were determined graphically by the linear regression.

Antimicrobial Activity

Fusarium culmorum, *Fusarium solani* and *Botrytis cinerea* used in the experiments were obtained from the culture collection of the Tunisian National Institute of Agronomic Research (INRAT). *Escherichia coli* (ATCC10536), *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniea* (ATCC 10031) were generously provided by Laboratory of Ecology, Technology and Microbiology (INSAT).

The bacterial and fungal stock cultures, adjusted to suspension of 10^6 cells, were incubated for 24 hours at 37°C on potato dextrose agar (PDA) medium and were refrigerated at 4°C. Antibacterial tests were evaluated using well Agar diffusion method under strict aseptic conditions. So 100 µL of suspension was put in 5 mL of melted cool test agar (Hadj et al., 2008). It was inoculated by flooding with Petri dishes containing agar culture medium BTCS. After agar solidification, three wells (10 mm in diameter) were bored using a sterile cork borer. Three concentrations of each leaf extract (5, 60 and 100 mg/mL) were prepared and dripped directly into the first, the 2nd and 3rd well, respectively with micropipette. Sterilized distilled water was used as a negative control, which was introduced into the 4th well. After 24 h of incubation at 37°C, the diameter of inhibition zones surrounding each well was measured.

Allelopathic Activity

25 seeds of *Triticum durum (T. durum), fenugreek* and *lens culinaris* (*L. culinaris*) were sterilized with sodium hypochlorite (5%) for 2 min, rinsed with distillate water then arranged in Petri plates (9 cm in diameter) lined with two discs of Whatman N°1 filter paper. Each Petri plate was moistened with 2 mL of the aqueous extract tested. Control seeds were similarly treated using 2 mL of deionized water. Bioassays were conducted under laboratory conditions during 7 days.

Parameters recorded were:

- The germination percentages PG (%)=((Treated seeds - control) × 100)/25
- The shoot length in cm (SL)
- The root length in cm (RL)
- The rate of inhibition or stimulation (%):

Inhibition (-)/stimulation (+)=((Treated seeds-control) × 100)/Untreated seeds.

Statistical Analysis

Results were statistically evaluated using STATISTICA (Statsoft,1998). Data from three samples was reported as means ± standard deviation. Differences were tested for significance with the ANOVA procedure using the Duncan test with a significance level of $p<0.05$

RESULTS AND DISCUSSION

Water and Mucilage yields of *Opuntia-ficus-indica* Rackets

The water yields ranged from 89.33% (Matmata provenance) to 91.4% (Sbeitla provenance) based on dry weight. This variability could be explained by genetic inconsistency. These results were compared to these obtained (93%) for Algerian provenances (Hadj et al., 208). For mucilage yields obtained from OFI rackets, Sbeitla provenance had the highest average (0.98%) and Matmata had the poorest mucillage contents (0.364%).

Secondary Metabolites Contents

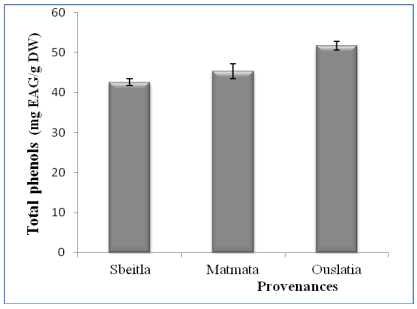
The comparison between provenances, Figure 1 showed that Ouslatia mucilage's was the richest one (51.692 ± 0.78 µg EAG/mg DW) flowed by Matmata (45.377 ± 1.88 µg EAG/mg DW) and Sbeitla provenance (42.614 ± 1.09 µg EAG/mg DW).

Phenolic contents obtained in this study were higher than those showed by Rsaissi et al., 2013. The variation in phenol levels observed between provenances could be explained by environmental conditions (light, ripeness...). This idea had been confirmed by other authors (Elaloui et al., 2017). This richness on the phenolic composition of OFIM impelled the greatest potential value for exploitation and utilization especially in cosmetic industries, foods and pharmaceutical fields (Cheboua et al., 2001). Therefore, the identification and the purification of these phenolic compounds become a big need for this investigation (Bondet et al., 1977).

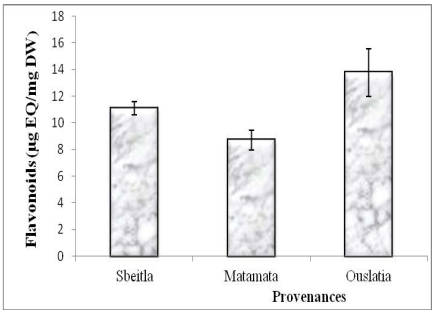
Flavonoid rates decreased slightly to 13.77 ± 1.77 (Ouslatia); 11.08 (Sbeitla) and 8.70 µg QE/mg DW (Matmata), respectively. High tannin yield was observed in Ouslatia provenance with a level of 37.61 ± 0.62 µg EC/mg DW. These results showed that the extraction and drying procedures affected the biological activity and the composition of the mucilage (Bimal et al., 2017).

Antioxidant activity

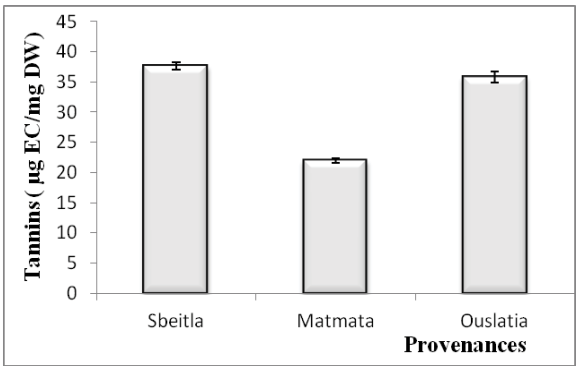
As shown in Table 1, the antioxidant activity of the OFIM followed this order: Ouslatia (0.003 mg/mL)>Sbeitla (0.0107 mg/mL)>Matmata (0.024 mg/mL). The DPPH assay used in this study was almost near or even higher than that of ascorbic acid (0.013 mg/mL). This antioxidant performance was measured at ambient temperature



Total phenols



Flavonoïds



Tannins

Figure 1. Total phenol, flavonoid and tannin contents of *Opuntia ficus-indica* mucilage (Sbeitla, Matmata, Ouslatia).

thus; the risk of the thermal degradation of the molecule tested was eliminated. These findings were in accordance with these announced by Bondet et al.,1977. The Ouslatia provenance exhibited higher levels of radical scavenging activity as privileged amounts of phenolic compounds. These data could justify that antioxidant activity was due to these compounds (Bouyahya et al., 2017). Nevertheless previous studies have suggested that the antioxidant activity of plant extracts have been partly associated with the high levels of ascorbic acid (Bukar et al., 2015).

Table 1. Antioxidant activity (mg/ml) of *Opuntia ficus-indica* mucilage originated from three tunisian provenances (Sbeitla, Matmata and Ouslatia).

Extracts	Ouslatia	Matmata	Sbeitla
IC_{50}	0.003 ± 0.002	0.024 ± 0.0007	0.011 ± 0.0002
Ascorbic acid	0.013 ± 0.01		

The data are the mean values of three measurements ± SD (standard deviation).

The OFIM could be considered as an excellent agent to neutralize free radicals and so to prevent organisms for many diseases such as diabetes, cancers, cardiovascular (Ennouri et al., 2014).

Antimicrobial activity

In the present study, all tested mucilage extracts produced an antimicrobial effect compared to the control, (well containing only solvent). This activity differed from a sample to another and from one strain to another. Bacteria were more sensitive against mucilage extracts than the fungal strains. This idea was also confirmed by (Bouyahya et al., 2017). Statistical analysis shown in Figure 2, undoubtedly showed a big correlation between the age and the antimicrobial activities. The oldest rackets (3 years old) were more potent against all tested organisms. Thus, results could be explained that those rackets produced the big levels of phenolic compounds.

Results showed that tested extracts exhibited the strongest antibacterial activity (25 mm) against *K. pneumoniae* especially when they were used at the concentration of 0.4 g/L, while the minimum zone of inhibition (20.5 mm) was obtained for *S. aureus* shown in Figure 3. Antibacterial activity against *S. aureus* were almost near that of Gentamicin (20 mm). The *E. coli* was also potent to mucilage extracts with a zone of inhibition of 20.5 mm. Thus, those extracts could be to treat some diseases such as diarrhea, vomiting (Chebouat et al., 2001).

Another study showed that *Opuntia-ficus-indica* flower extracts had great potential against *S. aureus* (Ennouri et al., 2017)

The Ouslatia provenance exhibited the strongest

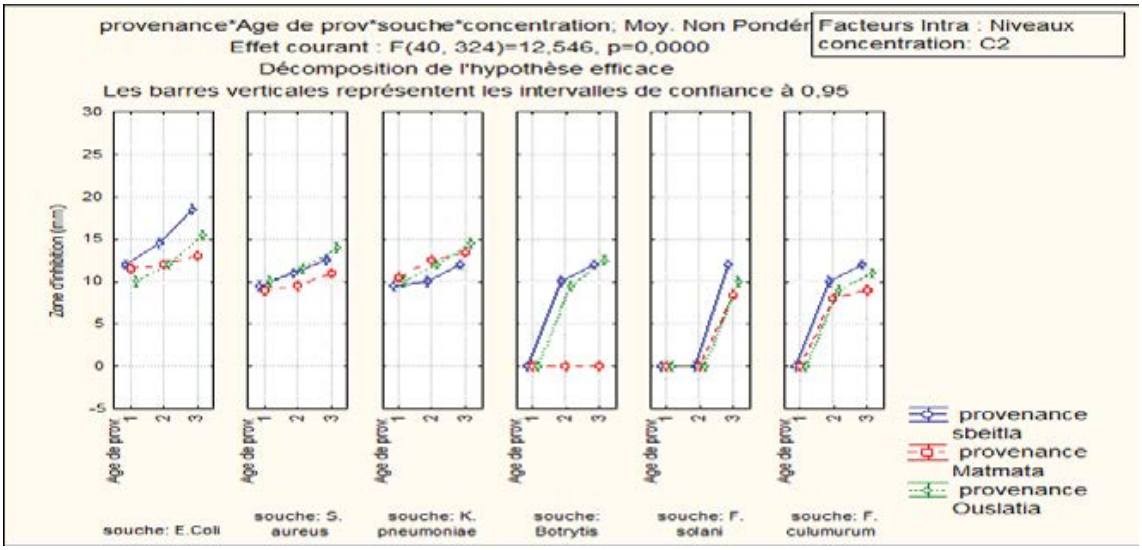


Figure 2. Antimicrobial activity of *Opuntia ficus-indica* mucilage originated from three tunisian provenances (Sbeitla, Matmata and Ouslatia).

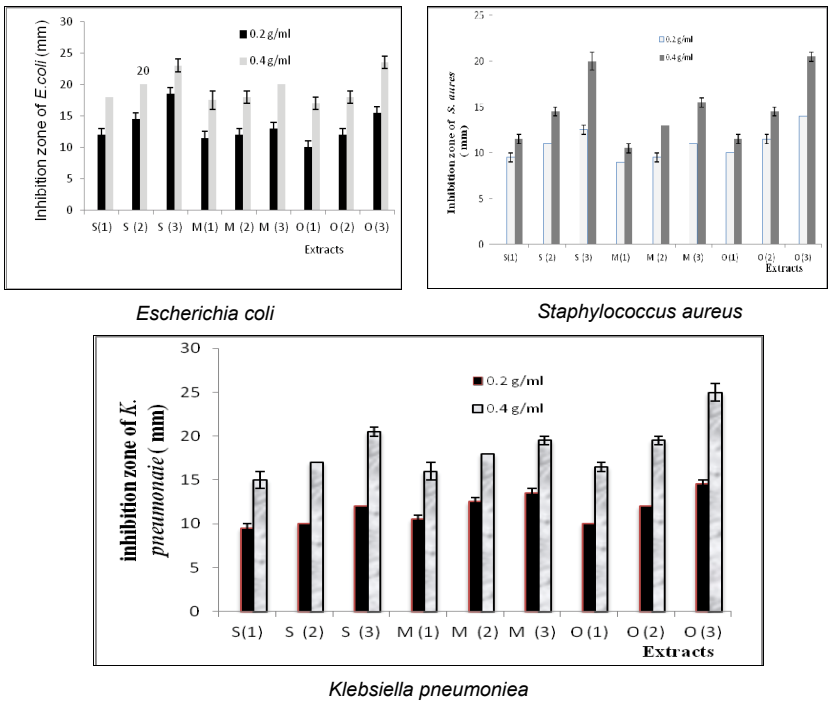


Figure 3. Diameter zone of inhibition (mm) produced by *Opuntia ficus-indica* mucilage originated from three tunisian provenances (Sbeitla :S, Matmata: M and Ouslatia:O), (1) : racket extract aged 1 year, (2) : racket extract aged 2 years, (3) : racket extract aged 3years). The data are the mean values of three measurements ± SD (standard deviation); the confidence intervals were calculated at the threshold of 5%.

antifungal activity and the highest phenolic contents. The same tendency was shown with the fungal strains with a diameter of inhibition zone ranging between 18.5 mm and 19 mm (*Fusarium solani*).

A comparison between all provenances shown in Figure 4, showed that the Ouslatia extracts were the most efficient against all tested microorganisms. This richness was due to the high level of tannins. In fact, this compound had been reported to prevent the development of bacteria by precipitating microbial protein and making nutritional proteins unavailable for them (Elaloui et al., 2017).

For provenance Matmata, mucilage used at concentration of 0.2 g/L had no effect on *Botrytis cinerea*.

This study showed also that variation in age could lead to chemical and biological activities variations among the same organ of the same species. Such antimicrobial activity could be contributed to alkaloids, lectins, terpenes and saponins (Li et al., 2010). OFIM could be a source of alternate drugs to prevent the body against these organisms (Li et al., 2010).

Allelopathic Activity

OFIM of Ouslatia provenance used at concentration of 60 g/L successfully inhibited the fenugreek and *T. durum* seeds at rates of -60% and -25%, respectively. These effects became less obvious (-10 %) for *L. culinaris* seeds. Concentrations 5 g/L and 20 g/L were without any inhibitor effects on all tested seeds.

Allelopathic effect of *Opuntia ficus indica* Mucilage on Root Length

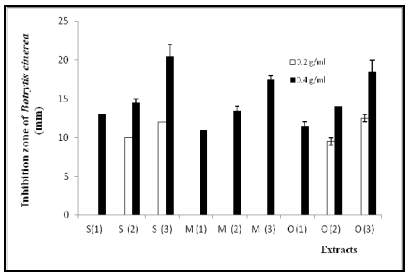
The 5 g/L concentration stimulated the *T. durum* (6.81%) and *L. culinaris* (16.66%) root lengths especially when treated by the Sbeitla provenance shown in Figure 5.

The Ouslatia provenance, used at the same concentration, could stimulate the fenugreek seeds at levels of 50%. Others concentrations had great inhibition ranging from 84.09% (Matmata provenance) to 92.04% (Sbeitla provenance).

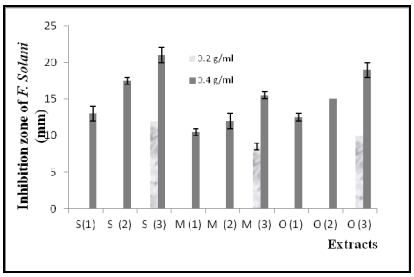
Allelopathic effect of *Opuntia ficus indica* mucilage on shoot length

Exception done for the concentration 5 g/L that stimulated traget species, *T. durum* shoot lengths were inhibited by the concentrations 20 g/L, 40 g/L and 60 g/L. The 60 g/L gave the muximum of inhibition. Inhition levels flowed this order Ouslatia (93.60%)>Sbeitla (92.26%)>Matmata (90.02%). The Sbeitla provenance showed potent inhibition on *L. culinaris* shoot (82.35%). Alleloathic effects of *Opuntia-ficus-indica* extracts were more important than these of *Ziziphus* leaf extracts. In fact, these extracts could suppress root growth at levels of -84 and -86% for *Ziziphus jujuba* and *Ziziphus lotus* respectively as shown in Figure 6.

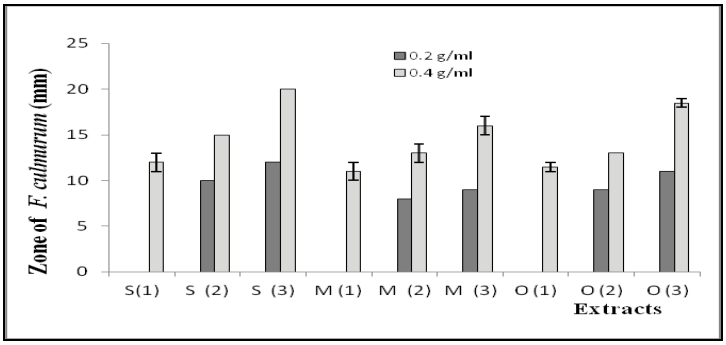
Inhibitory effects produced by *Opuntia ficus-indica* mucilages suggested that these extracts contained several allelochemicals such as cinnamic and phenolic acids (Bukar et al., 2015). In fact, these compounds could suppress the target plant species depending on their concentration. These phenolic allelochemicals could inhibit plant root elongation, cell division, change cell ultra-structure.



Botrytis cinerea



Fusarium solani



Fusarium culmorum

Figure 4. Diameter of zone inhibition (mm) produced by *Opuntia ficus-indica* mucilage originated from three tunisian provenances (Sbeitla :S, Matmata: M and Ouslatia:O); various concentrations, (1) : racket extract aged 1 year, (2) : racket extract aged 2 year, (3) : racket extract aged 3 years). The data are the mean values of three measurements ± SD (standard deviation); the confidence intervals were calculated at the threshold of 5%.

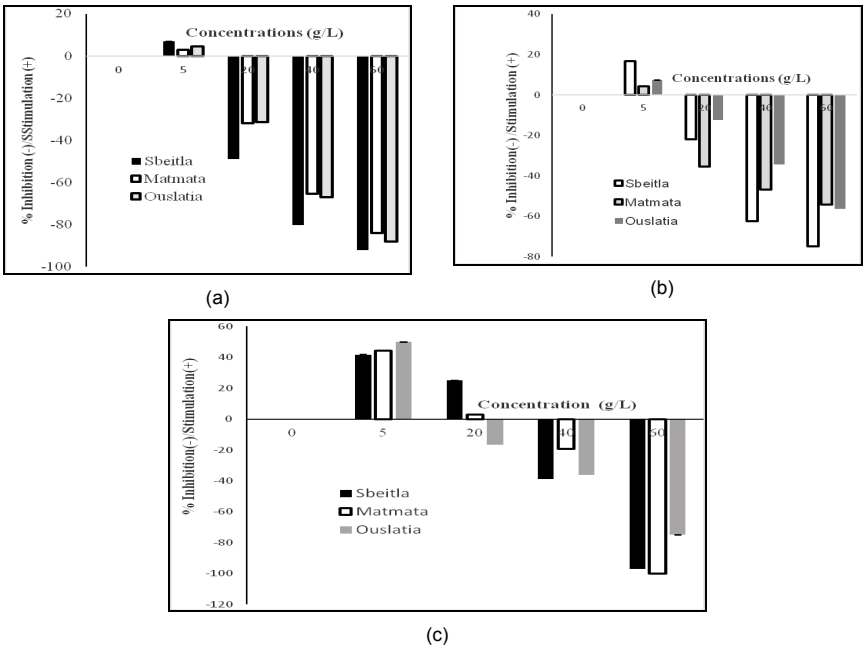


Figure 5. Inhibitory effects produced by Opuntia Ficus-indica mucilage originated from three tunisian provenances (Sbeitla, Matmata and Ouslatia) on Triticum durum (a), Lens culinaris (b) and fenugreek (c) root lengths noted after 7 days of incubation. Data are mean values of three replicates ± SD (standard deviation); Confidence intervals were calculated at the threshold of 5%.

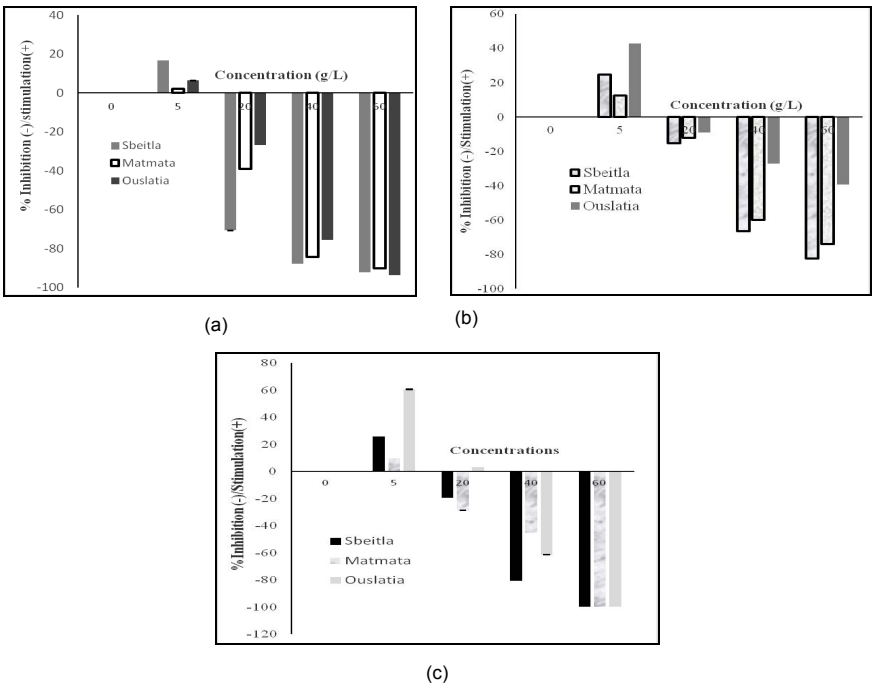


Figure 6. Inhibitory effects produced by *Opuntia ficus-indica* mucilage originated from three tunisian provenances (Sbeitla, Matmata and Ouslatia) on *Triticum durum* (a), *Lens culinaris* (b) and fenugreek (c) shoot length noted after 7 days of incubation. Data are mean values of three replicates ± SD (standard deviation); Confidence intervals were calculated at the threshold of 5%.

CONCLUSION

These results showed that *Opuntia ficus-indica* mucilage could be used to treat against fungal attack, bacterial infections and as biopesticides and replace chemicals compounds of a high degree of toxicity such as glyphosate (roindop) in order to improve and preserve our health and our environment. Further, it would be important to intensify such biological treatments on other strains and other plants at various concentrations. Thus, mucilage could be used to evaluate their effect in the treatment of polluted water, all optimizing the treatment conditions.

Abbreviations: µg EAG/mg DW: Microgram Equivalent Acid Gallic per Milligram Dray Weight; µg EC/mg DW: Microgram of Catechin Equivalent per Milligram Dray Weight; µg QE/mg DW: Microgram Quercetin Equivalent per Milligram Dray Weight; mg/ml: Milligram per Milliliter; DPPH: 22-Diphenyl-1-Picrylhydrazyl

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