Effect of Invasive and Native Prosopis Plants from Deserts of the UAE on Soil Microbiota and Seed Germination of Desert Plants

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***Abstract*- The effects of the organic and aqueous extracts of the invasive plant *Prosopis juliflora* (Al Ghwaif) and the exotic *P. cineraria* were evaluated on the growth of soil microbiota, four different Gram-positive and Gram-negative bacteria, and on seed germination of two desert plants (*Halocnomum strobilacum* and *Halopoplis perfoliata*). The results revealed that the frequency and abundance of different microbial soil species were higher in control soil, compared with soils from the rhizosphere of the two *Prosopis* species. However, the reduction was significantly greater in the soil of the *P. Juliflora*, compared with that of *P. cineraria*. Well diffusion experiments showed that the methanolic extract of *P. juliflora* dry leaves resulted in the maximum zone of inhibition with a diameter of 31 mm against soil consortium culture when compared to 11 mm by the fresh leaves extract. In comparison, slight inhibition (8 mm) was observed in the extract of *P. cineraria* dry leaves but the fresh leaves extract failed to inhibit the growth of any microbe. The test for the activity of methanolic *P. juliflora* and *P. cineraria* against two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) showed that *P. juliflora* has the highest activity on all four tested bacteria with maximum of 32 mm in diameter against *B. Subtilis*. All experiments indicated that organic and/or aqueous extracts of *P. cineraria* dry and/or fresh leaves showed slight or no inhibition of all tested soil and pathogenic microbes. Germination of the nontreated seeds of both *H. strobilacum* and *H. perfoliata* ranged between 76 and 92%. However, aqueous extract of *P. juliflora* resulted in a complete inhibition of the germination, but that of *P. cineraria* resulted in 20-28 reduction in seed germination. It can be concluded that the organic extract of *P. juliflora* dry leaves displays remarkable activity against soil microbiota, some Gram-positive bacteria, and seed germination of desert plants.**

Keywords- Growth; Inhibition; Invasive plants; Microbiota; Seed germination

I. INTRODUCTION

The use of medicinal plants is continually expanding world-wide. The increasing search for therapeutic agents derived from the plant species is justified by the emergence of diseases, yet natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [1]. According to the latest statistics from World Health Organization (WHO), more than 60% of the world's population depends on traditional medicine for health care [2].

Prosopis juliflora and *Prosopis cineraria* are among few trees growing in the arid deserts of the UAE and currently growing together in the same habitats. They constitute a major ecological feature in the Northern Emirates of the UAE. *P. juliflora* is a major invasive species in India, and has also invaded other regions throughout the world including Saharan and Southern Africa, the Middle East, Pakistan, India, and Hawaii (USA) [3], where it appears to strongly suppress the native species. It is rarely, if ever, seen as a weedy species and has not been successfully introduced into other parts of the world [4]. In last few decades, it has been introduced on a large scale in the afforestation program of the UAE because of its faster growth and soil-binding capacity. It is highly aggressive and grows so densely that it crowds out native vegetation [5, 6]. In its native range, densities of *P. juliflora* can be high relative to other leguminous shrubs and trees, but its canopies can have much stronger facilitative effects on neighbors than other leguminous tree species [7]. The success of *P. juliflora* is largely attributed to the high number of seeds produced and their efficient dispersal mechanisms [8]. The invasion by *P. juliflora* reduces grass availability and stocking density by livestock. It affects the plant biodiversity by creating a physical barrier on seedlings of other plant species, preventing sunlight to reach the vegetation under canopy, lowering the water table and by releasing various chemicals that may have negative effects on the native plant species [8].

P. cineraria is a slowly growing tree native to the dry and arid regions of Arabia and India and is beneficial for the growth and development of other species [9]. Few studies compared the allelopathic effects of invasive and native competitors on the associated native plants [10].

The aim of this research was to study the effects of *P. juliflora* leaf extracts on seed germination and to observe if the same effect can be seen on the inhibition of different microbes. Diversity and frequency of microbiota under the native and exotic plant were also investigated.

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II. MATERIALS AND METHODS

A. Sample collection

Soil and plant samples were collected during April 2013 from Al-Hamidiya area Ajman (UAE). *P. juliflora* is an evergreen shrub 2-3 m tall or small tree from the *Fabaceae* family (Fig. 1). It had a thick grey-green bark. The plants were multi-stemmed with sharp thorns. The tree is deeply rooted. Leaves are bipinnate with mostly two, sometimes more pairs of pinnae, 6-8 cm long. The flowers are fragrant and golden-yellow, dense spikes about 5-10 cm long. The fruit of *P. juliflora* is a cylindrical or slightly irregularly curved green pod which turns yellow upon ripening. *P. Cineraria* is a flowering tree with a height of 6.5m, also, from the *Fabaceae* family. It has bipinnately compound leaves, alternate in arrangement. The leaflets are 15-18 pairs, and shaped oblong with an entire margin.

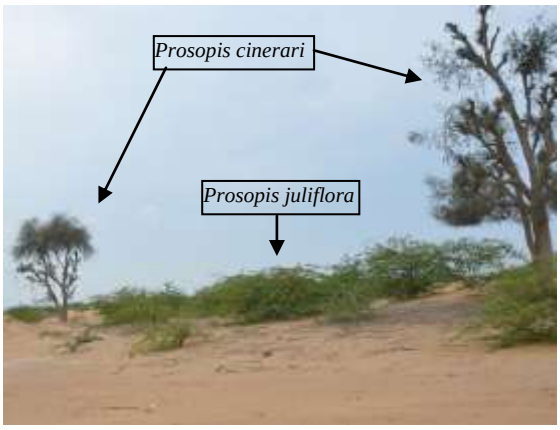

Fig. 1 *P. juliflora* (Al Ghwaif) invasive shrub reaching a height of 2-3 m and *P. cineraria* (Al Ghaf) is a native tree reaching a height of 6-8 m in the deserts of the UAE.

The texture of soil is coarsely grained with predominance of gravel and sand. Three composite soil samples in the area were collected from a depth up to 20 cm. Approximately 750 g of soils and fresh letters were collected from underneath the canopies of both P. juliflora and P. cineraria and from open places away from the canopies. Plant leaf samples were also collected from the two species. The samples were closed tightly in sterile bags, transported to the lab and stored in a refrigerator at 4oC for later use.

B. Sample treatment and processing

All the collected soil samples were passed through a mesh to remove plant and other coarse materials. Then, 10g of each collected soil sample was added to 90 ml of distilled water and incubated at 28 ^{o}C in orbital shaker with shaking at 130 rpm for 30 min. Samples were serially diluted with water up to 10^{-6}. In addition, 0.1 ml aliquots of each dilution were spread on nutrient agar plates, and then plates were incubated at 28 ^{o}C for 4 days. Bacterial colonies were selected according to macroscopic characters (shape, color and size).

All fresh plant leaves were separated then dried at room temperature by spreading them out in a chemical hood. The procedure of Das [11] was followed for aqueous and organic extraction, where 1 g of dried leaves was soaked with 10 ml of methanol then macerated with porcelain mortar and piston, then incubated for 24 hrs at room temperature shaking at 100 rpm. The organic extract was then filtered through a muslin cloth to remove large plant tissues and then centrifuged 3500 rpm to make sure all debris precipitates out of solution. Rotary evaporator (RE100, Heidolph, Germany) was set at a boiling temperature of 70 ^{o}C to evaporate the methanol in the sample and obtain a dried crude extract, 0.1 gm residual extract was dissolved in 1 ml of sterile distilled water. Moreover, water soluble extract was also prepared in same way except without using rotary evaporator. Both organic and aqueous extracts were filter-sterilized through 0.45 µm Millipore filters and stored at 4 ^{o}C prior to use.

C. Seed germination

Seeds of *H. strobilacum* and *H. perfoliata* were provided by Sharjah Seed Bank, Sharjah-UAE. The germination test was carried in two replicates, each with 25 seeds of each species. Germination was conducted in glass Petri dishes with one layer of Whatman filter paper. Five ml of the aqueous and methanolic leaf extracts of *P. juliflora* were added to each Petri dish. Sterile distilled water was used as a control. All plates were incubated at 28 ^{o}C in LEEC Plant germination cabinet (LMS Ltd. Model No. 280A) for 6 days then the germinated seeds were counted. The abnormalities in germinated seedlings were assessed visually by using a dissecting microscope at 40X magnification.

D. Antibacterial assay

Each bacterial isolate recovered from the soil samples was inoculated in 250 ml Erlenmeyer flasks containing 50 ml of

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nutrient broth (NB). Flasks were incubated at 28 ^{o}C with shaking at 230 rpm for overnight. Mixture of all bacterial isolates (consortium) was also cultured in the same way. In addition, four stock bacterial cultures of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* were obtained from Department of Applied Biology, University of Sharjah, Sharjah. Each recovered bacterial isolate was streaked onto individual agar plate and incubated at 37 ℃ for 24 h. After incubation, cultures were transferred to 250 ml Erlenmyer flasks containing 50 ml of NB then incubated at 37 ℃ with shaking at 230 rpm for 24 h prior to tests.

The antibacterial assay was done in triplicates by using the agar well-diffusion method [12]. One ml of each individual, consortium, and identified bacterial culture was aseptically spread over solidified nutrient agar plates. Holes in the agar were made by cutting with a sterile glass serological tube of 6 mm diameter. Each well was filled up with 30 µl crude aqueous and organic extracts. Distilled water was used as a control. The plates were incubated at 28℃ for 24 to 48 hrs, and then the diameter of the inhibition zone formed around each hole was visually observed and recorded in mm. The sensitivities of the test organisms to the crude extracts were measured by a transparent ruler and indicated by clear zone around wells [13].

E. Assessing of bacterial diversity

Diversity of recovered bacterial isolates was assessed by calculating the frequency (No. of occurrence of colonies/total No. of quadrants) and abundance (Average No. of the colonies/total No. of quadrants) of the microbiota using a colony counter.

III. RESULTS

The frequency of occurrence of diverse microbial species in each soil sample from different locations is shown in Table 1. Frequency and abundance of the different species were higher in the control soil sample but the number decreased considerably in *P. cineraria* soil sample whereas more reduction was observed in *P. juliflora* soil sample (Table 1).

TABLE 1 MICROBIAL FREQUENCY AND DENSITY IN THE CONTROL, *P. CINERARIA*, AND *P. JULIFLORA* SOIL SAMPLES

Bacterial Isolate	Frequency	Density
Control		
1	3	11
2	1	11
3	6	16
4	4	8
5	4	7
6	7	22
7	9	270
P. cineraria		
1	2	5
2	3	4
3	10	280
4	7	38
5	4	5
P. juliflora		
1	10	68
2	0	0
3	9	160
4	4	4
5	0	0

Results from agar-well diffusion test indicated that the methanolic extract of *P. juliflora* dry leaves gave the maximum zone of inhibition with a diameter of 31 mm. Whereas, fresh leaves gave moderate zone of inhibition (Table 2, Fig. 2). In comparison, slight inhibition was observed in the extract of *P. cineraria* dry leaves but that of the fresh leaves failed to inhibit the growth of any individual and/or consortium of bacterial isolates (Table 2).

TABLE 2 ACTIVITY OF *P. CINERARIA* AND *P. JULIFLORA* ORGANIC METHANOLIC EXTRACT TOWARDS CONSORTIUM AND SINGLE BACTERIAL CULTURES AS INDICATED BY ZONE OF INHIBITION (MM).

Bacterial Isolate	Dry	Fresh
P. cineraria		
1	10	0
2	8	0
3	8	0
4	10	0
5	9	0

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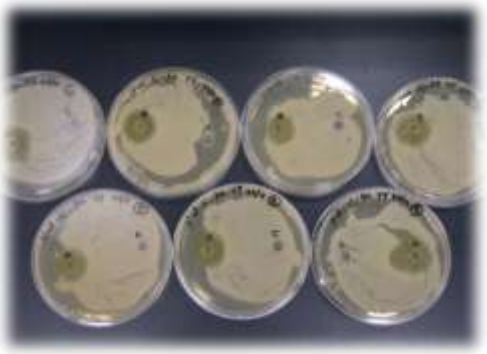
Consortium	8	0
P. juliflora		
1	23	12
2	22	10
3	23	10
4	30	9
5	25	11
6	25	8
7	30	12
Consortium	31	11



a

b

Fig. 2 Activity of *P. juliflora* (a) and *P. cineraria* (b) methanolic extract against different bacterial soil isolates.

Overall dry leaves from *P. juliflora* showed the highest activity on all four tested bacteria with a maximum of 32 mm in diameter against *B. Subtilise* (Table 3). Fresh leaves revealed intermediate inhibition. However, *P. cineraria* dry leaves showed slight inhibition on the tested bacteria, and no inhibition activity was observed by fresh leaves. Moreover, all extracts displayed higher inhibition against Gram positive, compared with Gram negative bacteria (Fig. 3).

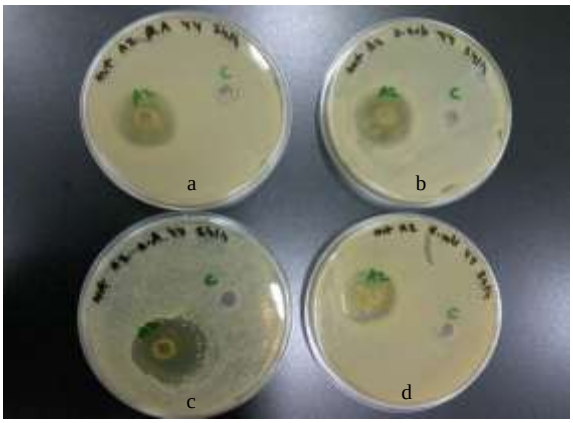


Fig. 3 Agar well diffusion test of *P. juliflora* methanolic extract against *P. aeruginosa* (a), *B. subtilise* (b), *S. aureus* (c) and *E. coli* (d)

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TABLE 3 ACTIVITY OF THE METHANOLIC AND AQUEOUS EXTRACTS OF *P. JULIFLORA* AND *P. CINERARIA* AGAINST THE IDENTIFIED TESTED PATHOGENS. NUMBERS BETWEEN PARENTHESES REPRESENT THE AQUEOUS EXTRACT.

ND: Not Determined

Test Bacteria	*P. juliflora*		*P. cineraria*	
	Dry	Fresh	Dry	Fresh
E. coli	17 (15)	12 (11)	8 (0)	0 (0)
P. aeruginosa	18 (ND)	12 (ND)	8 (0)	0 (0)
S. aureus	22 (17)	11 (14)	9 (0)	0 (0)
B. subtilis	32 (ND)	13 (ND)	11 (ND)	0 (ND)

The aqueous extract of *P. juliflora* dry leaves resulted in less inhibition, compared to the methanolic extract. Inhibition was the highest against *S.aureus* (17 mm) while *P. cineraria* failed to exhibit any inhibition (Table 3).

Effects of *P. juliflora* dry leaf extract were tested on seed germination of two desert plant species (Table 4). Nearly all seeds germinated in control (distilled water) as expected (Figs. 4a and 4b). Moderate seed germination was seen in aqueous extract but difference in seedling morphology was observed as the germinated seedlings were short with dark roots and bended shoots (Fig.4c and 4d). While in methanolic leaf extract, seeds failed to germinate (Fig. 4e and 4f).

TABLE 4 EFFECT OF *P. JULIFLORA* METHANOLIC AND AQUEOUS DRY LEAVES EXTRACT ON SEED GERMINATION OF *H. STROBILACUM* AND *H. PERFOLIATA* (N = 25)

	Halocnomum strobilacum			*Halopoplis perfoliata*		
Time	D. water (control)	Water extract	Methanolic extract	D. water (control)	Water extract	Methanolic extract
Day3	19	1	0	11	1	0
Day6	23 (92%)	20 (80%)	2 (8%)	19 (76%)	18 (72%)	0 (0.0%)

Fig. 4a Seeds of *H.strobilacum* soaked in distilled water (control)

Fig. 4b Germinated seed of *H. strobilacum* soaked in distilled water (control) (40X)

Fig.4c Seeds of *H. strobilacum* soaked in *P. juliflora* dry leaves aqueous extract.

Fig.4d Germinated seed of *H. Strobilacum* soaked in aqueous extract (40X)

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Fig.4e Seeds of *H. strobilacum* soaked in *P. juliflora* dry leaves methanolic extract

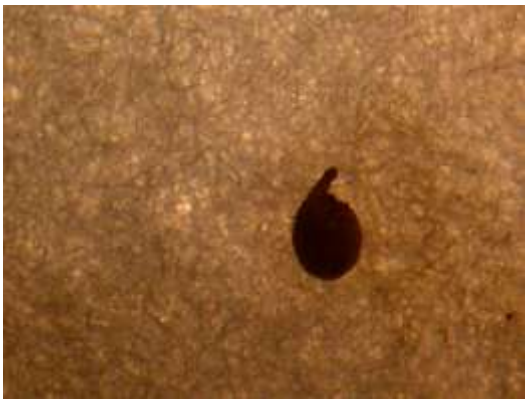

Fig.4f Germinated seed of *H. strobilacum* soaked in methanolic extract (40X).

IV. DISCUSSION

In this research, *P. juliflora* and *P. cineraria* were studied to assess their allelopathic effects as reflected by the reduction of microbiota frequency and plant seed germination. Assessing diversity of recovered bacterial isolates demonstrated higher frequency of microbiota in control and *P. cineraria* soil samples as less allelopathic chemicals are present in that area (Fig. 4a, b), therefore, more microbes can grow in this area. In comparison, less frequency of microbes were observed in *P. juliflora* soil samples (Fig. 4c). This may be because some bacteria are capable of resisting allelopathic effects better than other bacteria.

To observe the allelopathic effects, we choose to test the leaves of the plant on the basic principle of extraction which is to grind the plant material (dry or wet) to increases the surface area thereby increasing the efficiency of extraction. Fresh wet leaves were all dried to prevent enzymatic and microbial activity, thus preserving the product for extending shelf life. Drying is the most common and fundamental method for post-harvest preservation of medicinal plants because it allows for the quick conservation of the medicinal qualities of the plant material in an uncomplicated manner. Some essential nutrients become concentrated in the tissue after drying.

The extracts of dry leaves were tested on seed germination of two desert species that normally grow around the invasive plant: *Halocnomum strobilacum* and *Halopoplis perfoliata.* These species were chosen because they germinate rapidly and are used in many researches regarding seed germination. All seeds germinated healthily as expected in distilled water (Fig. 4a and 4b). In the aqueous extract of the leaves, nearly all seeds germinated reach an average of 19 out of 25 in both species. Nonetheless, difference in morphology was indicated by bended shoots and burnt root tips using dissecting microscope rendering that seeds could not overcome the effect (Fig. 4d). Whereas methanolic extracts of the leaves had an enormous effect since seeds were trying to germinate but were incapable of full germination (Fig. 4f). This experiment concludes that *P. juliflora* has allelopathic metabolites which are responsible for the invasive effect on the seed germination of other plant species.

Furthermore, the alleopathic effects of *P. juliflora* on soil bacterial isolates (single and consortium) and two Gram positive and negative bacteria were noticed. In addition, *P. cineraria* was also tested to indicate the difference between the two species.

A vast difference between *P. juliflora* and *P. cineraria* regarding the zone of inhibition was observed. Analysis of results indicated that dry leaves of *P. juliflora* had a vast effect on microbial growth (Table 4, Fig. 3). The possible explanation behind this may be that fresh leaves of the plant are metabolically active during its life cycle storing only minute amount of the allelopathic metabolites compared with dry leaves. With time, these metabolites accumulate in the entire leaves and when it passes through senescence, these dry leaves fall on the ground with high amount of metabolites, thus exhibiting its allelopathic effect towards other plants and microbes.

Data indicated that Gram positive (*S. aureus* and *B. subtilis)* bacterial exhibited more sensitivity than Gram negative bacteria (*E. coli* and *P. aeruginosa*) to plant metabolites and these results are consistent with their structure (Fig. 4). Gram-negative bacteria were less susceptible because it is related to its outer membrane [14] which endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier [15]. The difference in sensitivity between the Gram-negative and positive bacteria to inhibition by plant extracts is supported by other researchers including Shelef [16]. Overall, *P. juliflora* extracts were proved to have a stronger effect on the microbial growth and it can be considered to have a broad spectrum action. Pasiecznik et al. [3] proposed that the leaves of *P. juliflora* contain various chemicals including tannins, flavonoids, steroids, hydrocarbons, waxes and alkaloids, where tannins and alkaloids were reported to have effects on bacterial growth [17, 18]. The presences of phenolic and flavonoids compounds have been reported to inhibit bacteria growth and are capable of protecting certain plants against bacterial infection [19, 20].

In addition, several studies reported the presence of allelopathic compounds in both *P. juliflora* and *P. cineraria*. For

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example, Kaur et al. [21] detected L-tryptophan in leaf leachates of both *P. juliflora* and *P. cineraria*, but the amounts were 73% higher in leaf leachate of the former than that of the latter. Nakano et al. [22] suggested that L-tryptophan may play an important role in the allelopathy of *P. juliflora* leaves. Therefore, this could be the possible reason behind the difference between the inhibitory actions of these two species. The mentioned metabolites may be potential inhibitory compounds working simultaneously or individually for this property of inhibition. Further tests will be needed to specify the actual inhibitory compound.

V. CONCLUSION

Our research reveals that allelopathic metabolites from *P. juliflora* not only have an abundant influence on seed germination but also on diverse microbial population. Further research need to be conducted aimed at this allelopathic property in similar plant species and an ideal medical discovery in antibacterial active compounds could arise.

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