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Effects of *Prosopis juliflora* on germination, plant growth of *Sorghum bicolor*, mycorrhiza and soil microbial properties

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ABSTRACT

We studied the effects of *Prosopis juliflora* on seed germination and growth of *Sorghum bicolor*, on Arbuscular Mycorrhizal Fungi (AMF) colonization and soil microbial properties. Aqueous extracts of this plant significantly inhibited the germination of *Sorghum bicolor*. Its litter also inhibited the plant growth and their AMF colonization roots. Results showed that *P. juliflora* stimulated the soil microbial biomass carbon, soil metabolic quotient and soil enzymes activities.

Key words: Allelopathy, arbuscular mycorrhizal fungi, invasive legumes, *Prosopis juliflora*

INTRODUCTION

Plant invasions cause gradual disappearance of native plant species. Invasive plants are very effective competitors against endemic species, reducing their growth and biomass (20). In addition, invasive plants can also alter the soil rhizosphere microflora, by increasing the pathogens levels or affecting the relationships between the native plants and symbiotic microbes. (6) Invasive species also produce allelochemicals, toxic to native plants that cannot be detoxified by indigenous soil microflora (50). Many studies (4,33) have confirmed the inhibitory allelopathic effects of invasive plants through interference (allelopathy + competition) from the invasive plants on native species.

Interactions between the plants and soil microbes have major consequences on the functioning of both agricultural and natural ecosystems (42). There is increasing evidence to suggest that invasive alien species (IAS) cause major changes in the composition of soil microbial communities and to the soils health and productivity (12). Currently, research is foccussed on the mechanisms related to the performance of introduced species (23) as these non-native plants can be considered desirable for forestry and agriculture or undesirable as biological invaders. The good performance of introduced species can be related to climate or edaphic conditions and particularly to genetic differences between the native and non-native species (23). The interactions of these plants with soil biota can be considered as the main mechanism underlying their success in their new habitat (38).

The success of invasive legumes may also depend on their ability to form effective symbiotic relationships with resident AMF populations of native ecosystems. Recently, Mahdhi *et al.* (30) indicates that mutualistic interactions between the *Prosopis* species and native rhizobia and AMF facilitate its establishment in new habitats and makes it a stronger competitor than native plant species. Changes in soil quality can be assessed through physical, chemical and biological processes. Biological indicators (enzymes activities, microbial biomass carbon and soil respiration) are good indicators of soil quality

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(15). The soil microbiological status is important to establish the reforestation programmes and to select the type of plants suitable for restoration of degraded lands (47).

Prosopis juliflora, (Family Mimiosoideae, subfamily Leguminosae) is an evergreen subtropical legume woody plant that dominates the local plant species, especially in wadi beds and rain- flooded depressions in Saudi Arabia. This tree is native to northern South America, Central America and the Caribbean and is extremely drought-tolerant. In Saudi Arabia, it has been introduced to slow down the desertification and as wood resource. However, despite its good qualities and its ecological role, it is considered as serious invading plant in many Saudi Arabian ecosystems (30).

Sorghum bicolor crop is cultivated as fodder and grain crop. It is cultivated in areas, where invasive plant *P. juliflora* is dominant. Considering the importance of sorghum crop, this study aimed to (i) evaluate the effects of *Prosopis juliflora* on seed germination and plant growth of *S. bicolor* and (ii) effects on AMF colonization *of Sorghum bicolor* and soil microbiological properties.

MATERIAL AND METHODS

Plant material and preparation of aqueous extracts

Litter, fresh leaves and roots of *Prosopis juliflora* were collected in November 2017 from the Jazan region, Saudi Arabia. (16.8894° N, 42.5706° E). Approximately 500 g of each plant material was collected from 4-5 years old trees of *P. juliflora*.

Ten g fresh plant material was soaked in 100 ml distilled water for 24 h, homogenized and filtered through filter paper. The extract was diluted with distilled water to 1: 2 (50 %) and 1: 4 (25 %) to obtain 3 concentrations: 25, 50 and 100 %. Aqueous extracts were stored at -20° C. Part of leaf litter was stored at 25° C until used in pot experiments

Germination bioassay

To determine the effects of this plant extracts on seed germination, bioassays were done in sterile Petri dishes containing 1 % agar and 12 seeds per plate. Four ml of each concentration of aqueous extracts was added to each Petri dish as per treatments. The control was treated with 4 ml sterile distilled water. Each treatment was replicated thrice. Germination (%) was determined after 5 days after incubation at **25** ^{0}C and germination speed was calculated as per Einhellig *et al.* (10).

$$S=[N1+((N2-N1)/2)+ ((N3-N2)/3)+\ldots..+ ((Nn-Nn-1)/n)]x100$$

Where, N1, N2, N3, Nn represent the proportion of germinated seeds on day 1,2,3…n after the start of the experiment.

Pot experiments

Two germinated seedlings of *S. bicolor* were aseptically transplanted to pots containing autoclaved vermiculite. In each pot, 200 g dry crushed leaf litter was mixed with autoclaved vermiculite. Treatments were replicated 8-times. The Controls pots had no litter. The pots were placed in growth chamber at 25 °C and watered once a week with a sterilized nutrient solution (49). Two months later plants were harvested, shoots and roots were separated, dried at 70 °C in oven and weighed.

Root colonization by Arbuscular Mycorrhizal Fungi

The effects of *P. juliflora* on AMF colonization of *S. bicolor* roots was investigated after seedlings were raised on soils collected from under *P. juliflora* trees. Bulk soil (Soils from Outside) was used as control. Soil was collected from three locations in Jazan (Sabia : 17.20° N, 42.62° E, Wadi Jazan: 16.98° N, 42.63° E and Abu Areesh: 17.02° N, 42.92° E). It was sieved through 2-mm sieve. The composite soils mixture from three sites was used in this experiment. Three replicates were maintained for each soil type i.e. bulk soil and soil collected from under *P. juliflora.* Two months later, plant roots were collected, washed with sterile water and processed as described by Phillips and Hayman (37). Stained roots were examined microscopically for AMF infection (19). Ninety root pieces per plant were examined. The intensity of mycorrhization (M) was assessed as per Trouvelot *et al*. (49).

Isolation, enumeration of AMF spores

Soil samples were collected from three sites (described above). From each site, soil from under the canopy of *P. juliflo*a trees was collected. Soils from outside the area of the trees was also collected. AMF spores in the soil samples were extracted by the wet sieving and sucrose centrifugation technique (16). The supernatant was decanted into a 32 mm sieve, washed with running tap water and transferred to Petri dishes. Spore density was expressed as the total number of spores per 100 g of soil (31).

Soil microbiological and biochemical properties

Soil samples were collected from three sites (described above). For each site, we collected bulk soil and soil from rhizospheric zones of *S. bicolor* and from under *P. juliflora*. Soil microbial biomass carbon (Cmi) was determined by the fumigation extraction method (1) using ninhydrin-N reactive compounds extracted from the soils with KCl after a 10-day fumigation. Soil respiration was determined by the titration of CO_2 emission (35). Soil phosphatase, β- glucosidase and dehydrogenase were determined by colorimetric methods (14).

Statistical analysis

Statistical analyses were done with SAS statistical package. The data were subjected to ANOVA test. Comparisons among means were made using the Least Significant test at the 5% level of significance ($P< 0.05$).

RESULTS AND DISCUSSION

Seed germination and seedlings growth of *S. bicolor*

The litter and leaf extracts of *Prosopis juliflora* significantly inhibited the germination of *S. bicolor* (Table 1). With 100 % leaf extract, germination speed decreased from 43 % (control) to 17 %. Sorghum seeds germination was reduced with increasing concentrations of the aqueous extracts as per Laosinwattana *et al*. (25). Previous studies (26,32) have confirmed the allelopathic effects of *P. juliflora* on seed germination of native plant species. For example, Noor *et al.* (34) showed that aqueous extracts of canopy soil and fruit and seed extracts of *P. juliflora,* inhibited the germination and early seedling growth of *Zea mays*. The allelopathic effects of *P. juliflora* were presumed due to the presence of phenolic compounds, alkaloids and amino acids in the extracts (26). In the present studies, growth of *S. bicolor* seedlings was slightly affected by the litter of *P. juliflora* (Table 2). The shoot length and the root dry weight of *S. bicolor* were decreased

only 7% and 8%, respectively, than control seedlings. Similar results were reported by Shaik and Mehar (41) regarding the effects of *P. juliflora* on germination and growth of rice. However, *P. juliflora* litter and leaves are very inhibitory to growth of some native plant species in Ethiopia (17).

Table 1. Effects of concentrations of aqueous extracts of *P juliflora* on sorghum seed germination

Treatment	Germination (%)	Germination Speed (%)
Control	100	43.9±1.5
Litter extract concentration (%)		
25 (%)	63.8±2.7	25.4 ±0.7
50(%)	52.7±2.7	20.8±0.8
100(%)	44.4±2.7	17.8±1.3
Mean	53.63*	21.33*
Leaf extract concentration (%)		
25 (%)	72.2±2.7	29.5 ±0.8
50(%)	63.8±2.7	26.7±1.2
100(%)	**52.7±2.7**	20.7±0.9
Mean	**62.9***	25.63*
Root extract concentration (%)		
25 (%)	97.2±1.2	43.7±0.4
50(%)	91.6±1.2	38.8±1.5
100(%)	**80.5±1.2**	33.7±0.4
Mean	89.76	38.73

Data are means± standard error of three replicates
*Values varied significantly at ($P<0.05$) level across treatments in ANOVA.

Table 2. Effects of *P. juliflora* litter on seedling growth of *Sorghum.*

Parameter	Control	Treatment	Inhibition (%) over control
Shoot length (cm)	89.1 (±5.1)	82.6 (±3.4)	**7.29**
Root length (cm)	21.5 (±2.1)	20.3 (±3.2)	**5.58**
Shoot dry weight(g)	17.3 (±0.7)	16.7 (±0.6)	**3.46**
Root dry weight(g)	2.9 (±0.2)	2.65 (±0.1)	**8.62**

Assessment of root colonization by AMF and the number of spores

The mycorrhizal fungi affects the plant invasion process and thus may have crucial role in success of invasion (39). Our results showed that AMF root colonization was seen in all roots of *P. juliflora* and *S. bicolor.* Vesicles, intraradical hyphae and arbuscules were observed in the cortex of roots, although not necessarily in the same root segment. The intensity of mycorhization in *P. juliflora* was significantly higher than *S. bicolor* (Figure 1). The higher mycorrhizal intensity in roots of *P. juliflora* confirmed the findings of Lekberg *et al*. (27) that the success of plant invasion is enhanced by the better mutualists. Hawkes *et al*. (21) showed that invasive plant species influenced the network of AMF in the soil of native species through early root activity.

Our results showed that *P. juliflora* negatively affects the AMF colonization of *S. bicolor* roots. The intensity of mycorrhization in *S. bicolor* was significantly lowered i.e. 24.6 % (plants growing on bulk soil) to 17.1 % in plants growing on soil collected from

under *P. juliflora* tree canopies. These results are in agreement with previous studies which showed that invasive plant species changes the composition of AMF community (8,52,53). Invasive plants suppresses the native plants growth by disrupting their mutualistic associations through production of secondary metabolites that directly limits their AMF development (43).

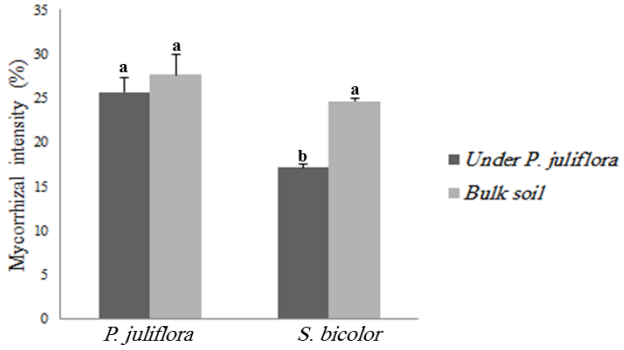

Figure 1. Mycorrhizal intensity in *P. juliflora* and *S. bicolor* roots in bulk soil and soil collected from under *P. juliflora*.
Different letters on top of bars indicate significant differences (P <0.05, mean and standard error, n=3).

In comparison to bulk soil and native species (*S. bicolor*), the highest number of AMF spores were found in the rhizosphere of *P. juliflora*. The highest number of AMF spores (153 spores/100 g soil) were recorded in soil samples collected under *P. juliflora* from Sabia, while the lowest were from bulk soil taken from Wadi Jazan (80 spores per 100 g soil). Similar results were also reported by Shah *et al*. (42) about the positive effects of invasive plants in India on the abundance of AMF spores than on native vegetation. However, other reports (2,5) have reported that invasive plants inhibited the AMF hyphal growth and spore germination.

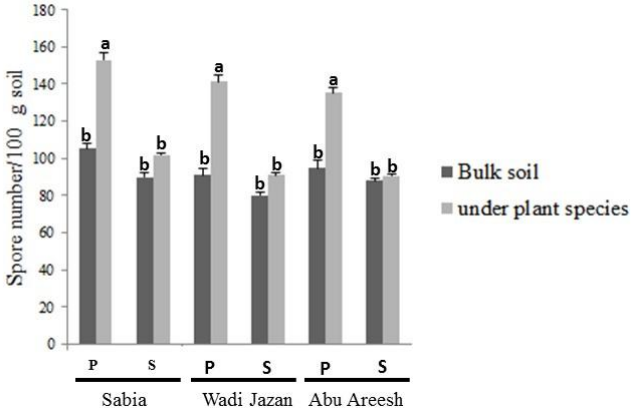

Figure 2. Spore number of Arbuscular Mycorrhizal Fungi in Jazan soils. Different letters on top of bars indicate significant differences (P <0.05, mean and standard error, n=3). P: *P. juliflora*, S: *Sorghum bicolor*

Soil Microbial biomass carbon and respiration

Plant invasions considerably changes the diversity and abundance of soil microbial communities (28). The present study showed that *P. juliflora* positively affects the microbial

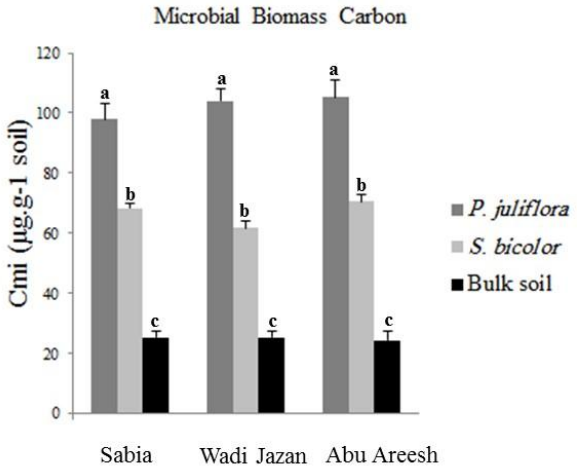

Figure 3. Microbial biomass carbon (Cmic) of *P. juliflora* and S. *bicolor* soils collected from 3-sites in Jazan regions (Sabia, Wadi Jazan and Abu Areesh). Different letters on top of bars indicate significant differences (P <0.05, mean and standard error, n=3).

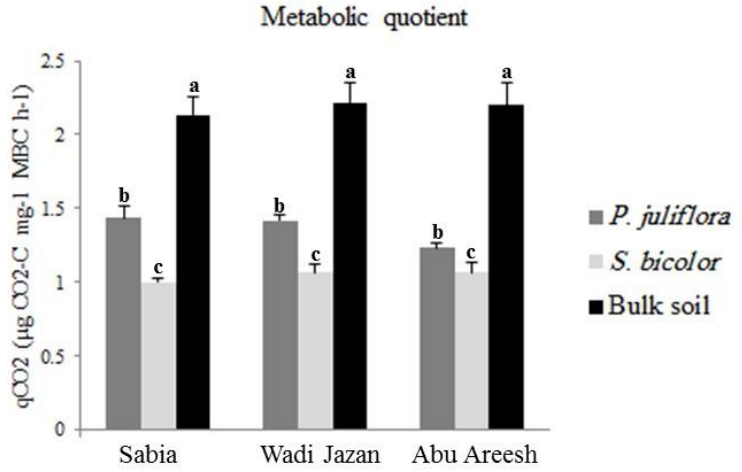

Figure 4. Metabolic quotient (qCO2) of *P. juliflora* and *S. bicolor* soils collected from Jazan regions. (Sabia, Wadi Jazan and Abu Areesh). Different letters on top of bars indicate significant differences (P<0.05, mean and standard error, n=3).

biomass carbon the contents of microbial biomass carbon significantly differed between the soils from under *P. juliflora* and *S. bicolor* (Figure 3). In all sites, Cmi was greater in the rhizosphere of *P. juliflora* than in the rhizosphere of *S. bicolor*. The highest value was recorded in the rhizosphere of *P. juliflora* from Abu Areesh (105.3±5.7) and the lowest in the rhizosphere of *S. bicolor* also from Abu Areesh (44.6±2.5). The soil microbial community below the *P. juliflora* tree were beneficial because of their ability to mobilize nutrients.

Metabolic quotient is measure of how effective microorganisms are in the studied site. In our study, a lower metabolic quotient was seen in soil collected under *P. juliflora* (Figure 4) indicating higher microbial activity, with less carbon per unit biomass being lost through respiration. There is regular replenishment of the organic matter under the *P. juliflora* trees due to leaf fall which does not occur in soil away from the trees. While in the bulk soil, the higher metabolic quotient suggested that more carbon per unit biomass was lost through respiration of substrates incorporated into microbial biomass.

The strong effects of *P. juliflora* on the microbial biomass carbon and metabolic quotient could be explained by the changes in the quantity of litter added by invasive plants (29). Previous studies (40,51), suggested that invasive plants can change the above ground (leaf litter) and below ground (root litter) inputs. Recently, Kuglerova *et al*. (24) found that litter from invasive plants decomposed faster than from native species. In our study, the changes in microbial biomass by *P. juliflora* perhaps occurred due to its leguminous leaf litter. The secondary metabolites excreted from the *P. juliflora* may be beneficial to the growth and reproduction of some soil micro-organisms (18). However, previous reports (33,46) mention negative effects of invasive plants on soil microbial properties and soil enzymes activities. The effects of invasion on soil microbial properties perhaps depends on the invasive plant species. Recently, Mahdhi *et al*. (30) found that *P. juliflora* positively affected the soil microbial properties. The higher microbial biomass carbon was recorded in soil collected from under *P. juliflora*.

Soil enzymatic activities

Dehydrogenase, Phosphatase and β-glucosidase enzymes are indicators of microbial activity (9). Therefore, an increase in their activities reflects an increase in microbial activities in the soil (14). In this study, we found that Dehydrogenase, phosphatase and β-glucosidase enzymes activities were significantly influenced by the plants canopy. Enzyme activities were higher in the plant habitat (Figure 5) than in open area. There were no effects of sites on enzymes activities. Among the three sites, the highest activities of three enzymes were in the rhizosphere of *P. juliflora* and the lowest were in bulk soil**.** Increase in soil enzyme activities under *P. juliflora* suggest that this invasive plant may play accelerate the soil organic matter decomposition and enhance the mineralization rates. The higher foliage shedding, increases the soil organic matter content and is responsible for the increased enzyme activities. In our study, the increase in enzyme activities in soil collected from under *P. juliflora*, showed increased microbial activity in the rhizosphere of this invasive plant. Such high activities helps in nutrients cycling and regulates the *P. juliflora* competition in terrestrial ecosystems. This is in agreement with previous studies (7,44), which reported that changes in soil microbial community and enzyme activity play an important role in plant invasion.

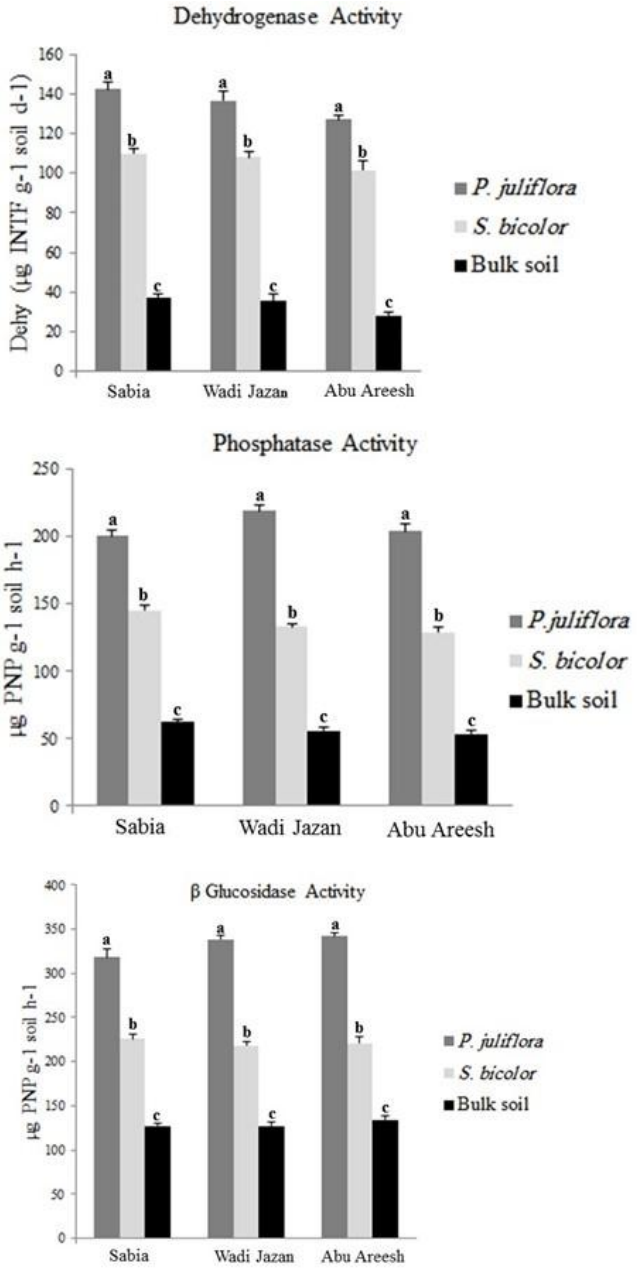

Figure 5. Dehydrogenase, phosphatase and β- glucosidase activities of *P. juliflora* and *S. bicolor* soils collected from Jazan regions. (Sabia, Wadi Jazan and Abu Areesh). Different letters on top of bars indicate significant differences ($P<0.05$, mean and standard error, n=3).

CONCLUSIONS

This study showed that *Prosopis juliflora* had inhibitory allelopathic effects on seed germination of *S. bicolor*. The inhibition of germination increased with extract concentrations**.** The *P. juliflora* stimulated the mycorization, the microbial biomass carbon, metabolic quotient and soil enzymes activities.

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