Impact of Combined Osmotic Stress and *Prosopis Juliflora* Induced Allelopathy on Germination and Recovery of Selected Crops in Tunisia

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Abstract: Few studies has been conducted on interaction between *P. juliflora* leaf extract sand crop germination under water deficit in arid regions. We conducted laboratory trials in order to evaluate crop species germination and recovery potentials under the combined effects of osmotic stress (0.4, 0.8 and 1 MPa) and allelopathic action of *P. juliflora* leaf extracts (0, 2.5%, 5% and 10%). Four crop species were assessed: Wheat (*Triticum durum*), barley (*Hordeum vulgare*), lettuce (*Lactuca sativa*) and tomato (*Solanum lycopersicum*). Statistical analyses highlighted a selective negative action of allelopathy on monocots compared to dicots. Combination of osmotic and allelopathic stresses exacerbated the negative effects on seed germination of all four species. Osmotic stress was the dominant factor, whereas allelopathy was the secondary factor when the two stresses were combined. While all treatments reduced germination, even at low stress levels, partial and total recovery levels were reported. Tomato, the least stress tolerant species, showed the highest recovery. In short, leaf extracts of *P. juliflora* exacerbated the effect of osmotic stress on seed germination of barley and wheat. In addition, the crops displayed differential adaptive strategies when exposed to the combined stresses.

Keywords: *Prosopis juliflora*, Allelopathy, Osmotic stress, Biotic and abiotic stresses, Monocotyledons, Dicotyledons.

INTRODUCTION

In the arid and semi-arid areas of Tunisia, water is the main limiting factor for the expansion and intensification of crop species [1]. In these areas, the low and irregular precipitation critically limits the proper establishment and spread of both cultivated and natural vegetation [2, 3]. Additionally, invasive introduced species, such as *Prosopis juliflora* (Mesquite), have exacerbated the impact on restoration efforts of both cultivated and natural systems.

In cases where the decline in plant cover has reached an irreversible threshold, natural regeneration does not lead to any level of restoration of vegetation cover [4]. Faced with such widespread degradation cases especially in the arid zones, the Tunisian government has engaged in vast program of forest and range land rehabilitation programs over the last two decades [5]. Consequently, a variety of alien shrub and woody species have been introduced with the aim to improve range land and forest ecosystem health [3]. Leguminous woody species such as *Acacia* and *Prosopis* have also been introduced to the South East of Tunisia (El Fja, Medenine). The aim was to prevent soil erosion and to improve soil fertility. During few short years, many alien species rapidly invaded the arid zones mainly due to their aggressive and high dispersal capacity and occupancy. Features such as vegetative reproduction and high number and germination potentials of their seeds aided in their spread and establishment. *P. juliflora* a native to Central America, for instance, has been naturalized in Tunisia since the 1980s. *P. juliflora* is known to be invasive species, with high dispersal ability through its rapid germination and high pod seed contents [6]. Additionally, it can easily grow in a wide range of semi-arid habitats (coastal marshes, coastal deserts, sand dunes, plains and hilly areas, dry streambed, in land saline flats, degraded and disturbed areas). It has been recommended that exotic species such as *P. juliflora* are to be replaced in order to promote ecosystem richness and diversity [7]. Unfortunately *P. juliflora* has been proven to be highly allelopathic [8, 9].

Currently, the occurring of potential allelopathic interactions between introduced *P. juliflora* and crops may further disturb plant productivity which is often affected by drought in arid regions. Many wild plants have allelopathic effects, while the activities, types and amounts of active compounds depend on plant species [10]. In fact, it was reported that *P. juliflora* roots,

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leaves and fruits contain allelochemicals compounds that inhibited seed germination and growth of neigh boring plants [11]. Numerous allelochemicals such as julifloridin and juliprospine [12], and L-tryptophan and syringin [11] have been isolated from *P. juliflora* leaves. Percent germination and rate of germination were significantly reduced by the extracts [8]. Moreover, extracts from *P. juliflora* fruits and seeds extracts reduced germination, root, shoot and seedling growth compared with root, leaf and flower extracts [13]. While soil extract inhibited germination but did not affect seedling growth. Germination of *Acacia nilotica* and *Acacia tortilis* was not affected by extracts of *P. juliflora*, but leaf and root extracts at high levels inhibited germination of *Cenchrus ciliaris* [9]. In autoxicity trial, both the length of the radicle and the hypocotyl were delayed significantly by *P. juliflora* extracts [14]. The effects of leachates lead to conclude that the effects of allelochemical substances were significant in reducing germination on native plant species [15]. Consequently, plants in arid regions which are obviously exposed to drought stress may be also potentially exposed to allelopathic action of *P. juliflora*. But, the extent of water stress under allelochemical conditions has seen limited scientific attention.

Until now, there are no scientific studies on physiological interaction between *P. juliflora* and crops establishment under water deficit in arid regions. It the present study, we have undertaken laboratory assays in order to evaluate crop species germination potentials under both osmotic stress and allelopathic action of *P. juliflora* extracts. The bottom line was to assess the impact of introducing *P. juliflora* in the Tunisian arid regions.

MATERIALS AND METHODS

Crop Species

The trial was conducted at the Higher Institute of Applied Biology (University of Gabes, Tunisia). For the present study, therefore, we have selected the most common crops in the south eastern part of Tunisia: two monocotyledons; Barley (*Hordeum vulgare*) and wheat (*Triticum durum*) and two dicotyledons; Tomato (*Solanum lycopersicum*) and lettuce (*Lactuca sativa*).

Design of Osmotic Stress and Allelopathy

To simulate osmotic stress imposed by arid region climate, we used an increasing concentration of PEG6000 to create three osmotic stress levels (−0.4 MPa; −0.8 MPa and −1 MPa). The PEG6000 concentration for each osmotic pressure was determined using the method reported by Michel [16].

Allelopathic action of *P. juliflora* was induced by addition of increasing concentration of its aqueous leaf extracts (2.5,5 and 10%). *Prosopis* leaves were collected from there habilitated arid region around the city of Medenine, Tunisia (N33°30'- E10°38'). The leaves were air dried for two weeks and then grinded to a fine powder. Aqueous extracts were prepared by maceration during 24 hours with distilled water at ambient temperature. Before using aqueous extracts for germination assays, the pH using a standard pH-meter (Jenway, 3510 pH meter), and the osmotic pressure, using osmometer (Wescor 5500; Wescor Inc., Logan, UT, USA), were measured.

Germination Assays

For each plant species, the seeds were disinfected by calcium hypochlorite 2.5% for 10 min and then rinsed three times with distilled water. All germination tests were carried out in sterile 9 cm Petri dishes with a double layer of Whatman filter paper moistened with 5 mL of distilled water (control) or the test solution. Each treatment was replicated thrice using 25 seeds per replicate. A seed is considered germinated when the radical emergence reaches or exceeds 1 mm. The germination tests were conducted in incubators set at 25°C in complete darkness during a period of 10 to 13 days.

Germination Parameters

Initial germination capacity (IGC): it estimates the percentage of seed germination under defined initial conditions. (i) $TG_i\% = (n/N)\ 100$, with, n: the number of germinated seeds in the test solution, N: the total number of seeds (25). Non-germinated seeds are rinsed three times to remove the traces of treatment applied, and then transferred to other Petri dishes containing distilled water. The recovery percentage (RP) and final germination capacity (FGC) were determined according to Tlig *et al*. [17]. The recovery percentage of germination (RP) and the final germination capacity (CG_f) were determined by the following formulas: (ii) $RP\%=[(a-b)/(c-b)]100$ and (iii) $FG_c\%=(a/c)\ 100$, with *a* as is the sum of germinated seeds in the test solution and seeds germinated in distilled water after recovery; *b* as the number of seeds germinated only in the test solution; *c* as the total number of seeds (25).

Statistical Analysis

Data were subjected to univariate repeated measures ANOVA [18] using Tukey's HSD test at a 5% significance level using SPSS [19]. Additionally, step wise regression and prediction-equation and analyses between the germination parameters and the two stress factors were used.

RESULTS

Osmotic Stress

The ANOVA analysis indicated that GC was significantly affected by crop species, osmotic treatment and their interaction at P<0.001 (Table **1**). The germination inhibition effects increased as the osmotic pressure increased (Figure **1A**). This was more pronounced for the dicots than was for the monocot species tested. Germination was close to zero at the −0.8 MPa osmotic treatment for tomato seeds. Germination was inhibited by about 85% for wheat and lettuce and 75% for barley, in comparison to the control (Figure **1A**). At the highest osmotic stress (*i.e.*−1 MPa), GC dropped sharply by about 80% for barley and lettuce seeds, and germination was totally prevented wheat and tomato seeds (Figure **1A**). According to GC changes at −0.8 MPa and −1MPa treatments, crop could be ranked for their increasing sensitivity to osmotic stress as follows: Barley, lettuce, wheat and then tomato.

Table 1: F-values from ANOVA Analyses on the Effects of Allelopathy and Osmotic Stress on the Germination Capacity of four Crop Species (Lettuce, Tomato, Barley and Wheat). Significant Levels are at P<0.001 *.**

Source of Variation	Initial Germination (%)
Allelopathy	522,326 ***
Osmotic stress	1316,701***
species	281,865 ***
Allelopathy* Osmotic stress *Species	169,706 ***

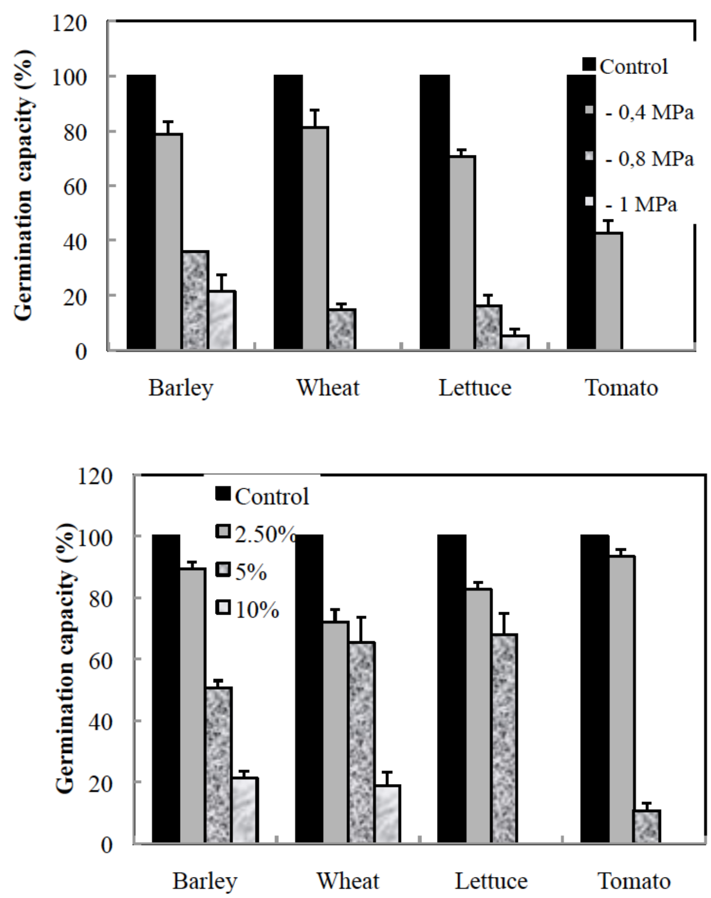

Figure 1: Changes in germination capacity of crop seeds (Barley, wheat, lettuce and tomato) in response to (**A**) osmotic stress (0, -0.4 MPa, -0.8 MPa and -1 MPa) and (**B**) allelopathy of *P. juliflora* (0, 2.5%, 5% and 10%). Data are means of three replicates SD. Means with same letter are not significantly different at P<0.05.

Table 2: General Chemical Proprieties of *Prosopis Juliflora* Aqueous Extracts. Data are Means of Three Replicates ± Standard Deviation

Extract Concentration	2.5%	5%	10%
pH	5,57	5,66	6
Osmotic pressure (MPa)	-0,00045±1.7E-05	-0,0016±1.7E-05	-0,0043±0.0004

Allelopathic Action

Chemical analysis of *P. juliflora* extracts showed that these aqueous preparations have moderate pH and minor osmotic pressure that could not interfere with the potential allelopathic action of this leguminous species (Table **2**). All tested seeds fully germinated in control medium (distilled water) (Figure **1B**). Increasing concentration of *P. juliflora* extracts was associated with decreasing GC (Figure **1B**). Tomato seed germination was not significantly inhibited by *P. juliflora* 2.5% extract concentration. But at the higher concentrations, tomato seeds exhibited the lowest tolerance, relative to the other tested crop species (Figure **1B**). At the highest treatment (10%) the two *Poacea* seeds displayed similar decline in GC (*i.e.* about 80%) when compared to the control (Figure **1B**). At the same treatment, GC was close to zero for lettuce and tomato (Figure **1B**). Based on variations of mean GC for both 5% and 10% treatments and the tolerance of the species, the following ranking is suggested (least to most): Wheat, barley, lettuce and then tomato.

Combination of Osmotic and Allelopathy

At lower *P. juliflora* extract concentration and osmotic pressure (2.5% and −0.4 MPa), seeds germination of lettuce was declined by about 15% GC relative to the control (Table **3**). At the same stress combination, barley and wheat seed germination showed a reduction of approximately 60% and 70% compared to control, respectively (Table **3**). Tomato seeds were found to be the most sensitive with average GC of no more than 11% (Table **3**). The transfer of treated seeds in distilled water was associated with a good germination recovery (Table **3**). We observed higher seeds germination recovery ability for the dicots species (lettuce and tomato) when compared to monocots (barley and wheat) (Table **3**). *P. juliflora* extract at 5% combined with osmotic stress, inhibited seed germination of the *Poacea* species and stopped that of tomato (Table **3**). Seeds of lettuce sustained higher GC value (72%) at the combination (5% / −0.4MPa), but their germination was stopped at higher osmotic pressures (Table **3**). Transferring treated seeds to control medium was associated with a lower germination recovery percentage for barley seeds (Table **3**). Germination of wheat and lettuce seeds was recovered by almost the half while tomato seeds fully germinated in the control medium (Table **3**).

When the highest *P. juliflora* extract concentration (10%) was combined with different levels of osmotic potential, none of the seeds germinated (Table **3**). Except for barley, recovery test showed that all other seeds germinated, at different levels. For instance, about 30% and more than 50% of lettuce and wheat seeds germinated; respectively (Table **3**). Tomato seeds recovered to an average close to that of the control, even after the highest stress combination (10% / −1 MPa) (Table **3**).

As such, the tomato seeds were the most sensitive to the combined stress, exhibited the highest germination recovery (Table **3**). However, barley seeds showed the lesser recovery ability and hence the lower final germination percentage (Table **3**).

Data of the regression analysis (stepwise) between the germination parameters and the two stresses are shown in Table **4**. There was a very high linear correlation between the regression parameters and stress factors ($P<0.001$). The effect of the combination of two stresses on the three parameters differed in magnitude. Among the absolute values of two regression coefficients (1-2), those of osmotic stress are higher for all study parameters and in all species. Our results indicated that the osmotic stress was the dominant factor, while the stress allelopathic had a secondary effect (Table **4**).

DISCUSSION

The present work evaluated the allelopathic impact of *P. juliflora* on the germination of four crop species. The results showed that both stresses impaired germination as the stress intensity increased (Figure **1**). Osmotic stress is known to affect germination by lowering water potential in the medium and consequently restrict seed imbibition and also

Table 3: Changes in Germination Capacity of Crop Seeds (Barley, Wheat, Lettuce and Tomato) in Response to Different Combination Levels of Allelopathy of *P. Juliflora* (0, 2.5, 5 and 10%) and Osmotic Stress (0, −0.4, −0.8, −1 MPa). Data are Means of Three Replicates SD. Means with Same Letter are not Significantly Different at P<0.05

Species	Aqueous extract (%)	Osmotic potentiel (MPa)	Initial GC GC Recovery Final GC		
	Control		100 ± 0 ^{a}	0± 0 ^{f}	100 ± 0 ^{a}
Barley	2,5%	-0,4	28±6,93 ^{d}	10,86±4,78 ^{ef}	36±4 ^{de}
		-0,8	6,67±2,3 ^{fg}	22,83±4,74 ^{e}	28±4 ^{de}
		-1	5,33±2,3 ^{gh}	9,90±4,98 ^{ef}	14,67±6,11 ^{e}
	5%	-0,4	8±4 ^{fg}	0±0 ^{f}	8±4 ^{ef}
		-0,8	1,33±2,31 ^{gh}	0±0 ^{f}	1,33±2,31 ^{f}
		-1	0±0 ^{h}	9± 2 ^{ef}	9±2 ^{ef}
	10%	-0,4	0±0 ^{h}	9,33±2,31 ^{ef}	9,33±2,31 ^{ef}
		-0,8	0±0 ^{h}	10,67±2,31 ^{ef}	10,67±2,3 ^{ef}
		-1	0±0 ^{h}	0±0 ^{f}	0±0 ^{f}
Wheat	2,5%	-0,4	42,67±2,31 ^{d}	25,71±8,69 ^{de}	57,33±6,11 ^{d}
		-0,8	6,67±2,31 ^{fg}	51,45±4,53 ^{cd}	54,67±4,62 ^{d}
		-1	0±0 ^{h}	40±6,93 ^{d}	40±6,93 ^{de}
	5%	-0,4	22,67±4,62 ^{e}	45±5 ^{cd}	57,33±6,11 ^{d}
		-0,8	5,33±2,31 ^{gh}	42,33±5,19 ^{d}	45,33±6,11 ^{de}
		-1	0±0 ^{h}	42,67±4,62	42,67±4,62 ^{de}
	10%	-0,4	0±0 ^{h}	60±6,93 ^{cd}	60±6,93 ^{d}
		-0,8	0±0 ^{h}	50,67±4,62 ^{cd}	50,67±4,62 ^{de}
		-1	0±0 ^{h}	73,33±6,11 ^{c}	73,33±6,11 ^{cd}
Lettcue	2,5%	-0,4	87±2,31 ^{b}	69,44±4,81 ^{c}	96±0 ^{ab}
		-0,8	0±0 ^{h}	86,67±4,62 ^{ab}	88±4,62 ^{ab}
		-1	0±0 ^{h}	86,67±2,31 ^{ab}	86,67±2,31 ^{b}
	5%	-0,4	72±6,93 ^{c}	57,41±8,49 ^{c}	88±4 ^{ab}
		-0,8	0±0 ^{h}	46,67±2,31 ^{cd}	46,67±2,31 ^{de}
		-1	0±0 ^{h}	37,33±2,31 ^{d}	37,33±2,31 ^{de}
	10%	-0,4	0±0 ^{h}	29,33±2,31 ^{de}	29,33±2,3 ^{de}
		-0,8	0±0 ^{h}	30,67±2,31 ^{de}	30,67±2,31 ^{de}
		-1	0±0 ^{h}	33,33±4,62 ^{de}	33,33±4,62 ^{de}
Tomato	2,5%	-0,4	10,67±2,31 ^{f}	79,18±6,44 ^{b}	81,33±6,11 ^{b}
		-0,8	0±0 ^{h}	93,33±2,31 ^{ab}	93,33±2,31 ^{ab}
		-1	0±0 ^{h}	92±4 ^{ab}	92±4 ^{ab}
	5%	-0,4	0±0 ^{h}	100±0 ^{a}	100±0 ^{a}
		-0,8	0±0 ^{h}	89,33±2,31 ^{ab}	89,33±2,31 ^{ab}
		-1	0±0 ^{h}	93,33±4,62 ^{ab}	93,33±4,62 ^{ab}
	10%	-0,4	0±0 ^{h}	96±0 ^{a}	96±0 ^{a}
		-0,8	0±0 ^{h}	80±0 ^{b}	80±0 ^{b}
		-1	0±0 ^{h}	94,67±2,31 ^{ab}	94,67±2,31 ^{ab}

Table 4: Results of Linear Stepwise Regression Between the Germination Capacity and the Two Stresses (Osmotic Pressure and Allelopathy) for Four Crop Species: Wheat, Barley, Lettuce and Tomato

Species	Model	R^2	Test Anova	β_1	β_2
Wheat	$Y=86,75+71,8X_1-3,89X_2$		P<0,001	0,69	-0,37
Barley	$Y=70,75+56,23X_1-3,56X_2$		P<0,001	0,61	-0,41
Lettuce	$Y=104,54+89,29X_1-3,73X_2$		P<0,001	0,71	-0,32
Tomato	$Y=62,69+55,2X_1-2,89X_2$		P<0,001	0,59	-0,33

subsequent germination process at transcriptional and proteomics levels [20]. However, the germination impairment by *P. juliflora* extract was not related to osmotic effects since we measured very low osmotic pressures of its aqueous preparation (Table **1**). Inhibition of crop germination by *P. juliflora* extract was further induced by its potential allelopathic action (Figure **1B**). The inhibition of germination process of the tested crop may be due to the impact of water soluble allelochemicals inside the seeds [21]. Yet, Al-Humaid and Warrag [8] reported that *P. juliflora* foliage contains water-soluble allelochemicals which could inhibit seed germination and retard the rate of germination and seedling growth. Potential interaction between *P. juliflora* allelochemicals released in the soil with drought in arid regions was evaluated by a combined treatment of crop seeds with increasing concentration of PEG and aqueous extracts (Table **3**). We found that seed germination depended on crop species and combination severity. In the present study, moderate stress combination (2.5% and 0.4 MPa), resulted to GC averages between 30% to 70%, while only 10% of tomato seeds germinated (Table **3**). However, the ungerminated fraction of tomato seeds (about 90%) recovered well when transferred to a stress free medium (Table **3**). According to final germination percentage measured at this applied treatment, seeds of lettuce were the most preferment with high initial and final germination, however a fraction of about 65% and 40% of barley and wheat seeds respectively did not recover (Table **3**). Consequently, a monocot seeds seem to be more prone than dicot seeds to *P. juliflora* allelochemicals under water deficit in arid region. Application of *P. juliflora* extract at 5% in combination with increasing PEG stress showed that while a fraction of seeds attempted to germinate for crops, tomato germination was fully inhibited (Table **3**). Under combination of *P. juliflora* extractat 10% with applied osmotic stress, germination was completely inhibited for all crops (Table **3**). What could be remarkably stated is that when germination was prevented, good seed recovery was recorded (Table **3**). It seems that seeds attempt to avoid aggressive external medium by sustaining dormancy, waiting for improvements in germination conditions. This strategy was obvious for tomato seeds (Table **3**). Lettuce, however, adopted a different strategy consisting of a maximum initial germination and only 10% of viable seeds were dormant and then recovered (Table **3**). Barley seeds were the most affected by the stress combination and more than 65% of seeds were irreversibly damaged (Table **3**). Wheat seeds displayed different strategy by keeping about 50% of seeds ungerminated, thus waiting for stress mitigation to germinate (Table **3**).

CONCLUSIONS

The found results suggest the co-existence of osmotic stress and allelopathy highlighted differential strategic adaptations of crops at germination stage when grown in stressful arid conditions. Under these constraints, tomato seeds were the most tolerant by lengthening their dormancy and hence avoiding a risky germination under allelopathy and/or osmotic stress. After conditions recovery, tomato seeds were thus undamaged and kept their germination capacity. Our results showed that barley seeds were the most sensitive and were irreversibly damaged. Barley seeds rapidly started germination process and therefore being exposed to allelochemicals and osmotic harmful effects. Although we have recorded relative tolerance of tomato and lettuce seeds, we are persuaded that introducing the invasive *P. juliflora* potentially aggravated the osmotic stress effects on germination, especially for local cultivated cereals (barley and wheat).

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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