

# Preliminary Investigations of Allelopathic Effects and Herbicide-based Eradication of Mesquite (*Prosopis juliflora*)

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**ABSTRACT:** Velvet mesquite, locally known as al-ghaf bahri (*Prosopis juliflora*), is a well-known invasive alien plant species in several regions around the world, including Oman, with various environmental effects. The allelopathic effect of *P. juliflora* leaves and seed pods on native ghaf (*P. cineraria*) and a crop species, mung bean (*Vigna radiata*) was demonstrated. Results indicated that *P. juliflora* extracts have inhibitory effects on seed germination and seedling growth of both species, particularly on *P. cineraria* when exposed to pod extracts. For eradication experiments, three herbicides (tribenuron methyl, clethodim and 2,4-D & MCPA) were investigated to determine their inhibitory properties on seed germination, and on the growth and development of young seedlings of *P. juliflora*. Generally, germination time ( $GT_{50}$ , time required for 50% of seeds to germinate) and germination percentage (%) indicated that only 2,4-D & MCPA treatment showed a significant effect on inhibiting seed germination and stopping seedling growth relative to the other two herbicides. The effect of 2,4-D & MCPA was supported by significant reduction in above-ground fresh biomass. The data clearly illustrate the potential negative effects of *P. juliflora* on other plant species and its tolerance of herbicide treatment. On a farm scale, limited application of 2,4-D & MCPA on young *P. juliflora* seedlings would be recommended as an effective way to limit further spread and distribution and consequently to successfully eliminate this invasive alien plant.

**Keywords:** Invasive alien species (IAS); *Prosopis juliflora*; Allelopathic effect; Eradication; Herbicides.

التقصي الأولي لتأثير النباتات الدخيلة (المسكيت) بالتضاد الكيميائي والقضاء عليها بمبيدات الأعشاب

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**الملخص:** المسكيت المخملي أو المعروف محليًا باسم الغاف البحري (*Prosopis juliflora*) هو نوع من أنواع النباتات الدخيلة المعروفة بالأضرار البيئية المختلفة في العديد من المناطق حول العالم بما في ذلك سلطنة عمان. تم إثبات تأثير التضاد الكيميائي لمستخلصات أوراق وحوافظ أوفرون بذور الغاف البحري على كل من الغاف المحلي (*P. cineraria*) وأحد أنواع المحاصيل وهو بقلة الماش أو اللوبياء الخضراء (*Vigna radiata*). أشارت النتائج إلى أن مستخلصات الغاف البحري تعمل على تثبيط إنبات البذور ونمو البادرات لكلا النوعين، خاصة الغاف المحلي عند تعرضها لمستخلصات الحوافظ أو القرون. فيما يتعلق بتجارب القضاء على الغاف البحري، تم فحص ثلاثة مبيدات عشبية (ميثيل التريبينورون وكليثوديم و 2,4-دي إيم سي بي أي) لمنع إنبات البذور ونمو وتطور نباتات أو شتلات المسكيت. بشكل عام، أشارت نتائج وقت الإنبات ( $GT_{50}$ ، الوقت اللازم لإنبات لنسبة 50% من البذور) ونسبة الإنبات (%) إلى أن المبيد العشبي (2,4-دي إيم سي بي أي) كان المبيد الوحيد الذي لديه تأثيرًا فعالاً في تثبيط إنبات البذور ووقف نمو شتلات المسكيت مقارنة بالمبيدات الأخرى. الإنخفاض الكبير في الكتلة الحيوية الخضراء (السيقان والأوراق) دعم التأثير الفعال للمبيد العشبي (2,4-دي إيم سي بي أي). توضح نتائج الدراسة بوضوح التأثير السلبي المحتمل للغاف البحري على الأنواع النباتية الأخرى وكذلك مقاومتها لمبيدات الأعشاب. على مستوى المزارع، قد يكون من المفيد التطبيق الموضعي المحدود للمبيد العشبي (2,4-دي إيم سي بي أي) على شجيرات المسكيت الصغيرة لوقف إنتشار الغاف البحري والمساهمة في القضاء الناجح على هذا النبات الدخيل.

**الكلمات المفتاحية:** الأنواع الدخيلة، تأثير التضاد الكيميائي، إزالة شجرة المسكيت، مبيدات الأعشاب.



## 1. Introduction

Invasive alien species (IAS), according to the Convention on Biological Diversity, is defined as “a species that is established outside of its natural past or present distribution, whose introduction and/or spread threaten biological diversity”. Such species can be introduced to their new habitats accidentally or deliberately and are considered to be a global environmental concern. For example, an IAS may compete with some native species, disturb food webs, degrade habitats and introduce parasites and/or diseases [1]. *Prosopis juliflora* (Sw.) DC, commonly known as velvet mesquite, is a well-known alien invasive plant species worldwide, including in Oman where it is locally called al-ghaf bahri. *P. juliflora* belongs to the Fabaceae family and it is native to Central America and South America [2]. Our field observations indicated that this plant produces copious pods, each of which contains 8–15 seeds. In India, where it is considered an exotic species, *P. juliflora* flowers twice a year (February-March and August-September) [2]. This would contribute significantly to its rapid spread and distribution particularly in the invaded habitats.

*P. juliflora* was probably introduced into the tropics for a variety of purposes including slowdown of deforestation [3] and into Oman for slowing down desertification, for shading and as an ornamental plant. The species has adapted to arid climates and various soil types with a wide range of alkalinity and salinity [2] and is thought to tolerate drought better than other native *Prosopis* species such as *P. cineraria* in Oman [4]. People in areas densely invaded by *P. juliflora* have discovered many benefits of this IAS. For example, they use its wood for fuel and timber, sweet pods as fodder for livestock and woody stems for making charcoal [5, 6].

Nevertheless, *P. juliflora* is a growing threat to ecosystems and their biodiversity [4, 6]. For example, native herbaceous vegetation and native tree diversity under a canopy of *P. juliflora*-trees and in nearby areas tend to be much reduced [7], suggesting the production of allelochemicals. Plant allelopathy refers to the ability of one plant species to influence the seed germination and/or growth of another species through release or secretion of allelochemicals [8]. Studies have demonstrated that *P. juliflora* reduces seed germination percentage of the native ghaf species (*P. cineraria*) when their seeds are planted together [4]. Although leaves of *P. juliflora* are reported to contain a variety of chemicals including alkaloids, flavonoids, hydrocarbons, steroids, tannins and waxes [9], limited studies have been conducted to examine the allelopathic effects of different parts of the plant. Because of the potential threat to native flora diversity and possible effects on invaded farmlands, the concerned authorities (e.g. the Ministry of Environment and Climate Affairs and the Ministry of Agriculture and Fisheries) have launched a national campaign for mesquite eradication.

Eradication of *P. juliflora* is a challenging task since it is spreading rapidly due to its production of a massive amount of seeds. The pods or seeds can be transported or dispersed by a variety of mechanisms including livestock and flooding of the wadis or dry valleys. According to Ilukor *et al.* [7], flooded areas which are likely to be rich in groundwater are usually more prone to *P. juliflora* invasion, relative to drylands. There are several management approaches to controlling the rapid growth and spread of *P. juliflora*, but with wide variability in effectiveness. For example, the national campaign for mesquite eradication is heavily employing a mechanical approach of uprooting adult trees as well as younger seedlings followed by burning of the dry biomass. On a cost-effective basis it is a practical option; however, this approach can facilitate distribution and germination of the seeds buried in the soil if suitable conditions are provided (i.e. enough moisture). Targeting seeds and new seedlings using chemical approaches (e.g. herbicides) is another option to eradicate *P. juliflora*.

Understanding the sensitivity of Omani native species to allelochemicals of *P. juliflora* may help to protect Omani native plants and to manage this IAS especially in the invaded agricultural lands. Therefore, the first objective of this study is to determine the effect of extracts of leaves and seed pods of *P. juliflora* on seed germination and seedling growth of one native Omani plant species (*P. cineraria*) and on that of a crop species, mung bean (*Vigna radiata*). Moreover, an important feature of *P. juliflora* is its ability to produce enormous amounts of seeds with a relatively long dormancy period allowing them to withstand various harsh environmental conditions. Thus, the second objective of this study is to investigate seed germination and seedling growth of *P. juliflora* when exposed to three agricultural herbicides as an alternative approach for eradication, specifically for use on farmlands.

## 2. Materials and Methods

### 2.1 Sample collection and processing

Seedpods of *P. cineraria* and *P. juliflora* were collected from Liwa and Saham (Al-Batinah North Governorate, Oman) from different trees during 2016. Additional *P. juliflora* seedpods were gathered from the SQU botanical garden. Seeds of *V. radiata* were purchased from a local hypermarket. Fresh leaves from *P. juliflora* trees were obtained from Saham. Prior to experimentation with the seeds, they were subjected to the seed viability float test. Briefly, a 250-ml glass beaker was filled with distilled water and then 100 *P. cineraria* or *P. juliflora* seeds were poured into the water and left undisturbed for about 15-20 minutes. After that, the floating and sunken seeds were counted and the viability percentages were calculated (ranging between 92-97%). Floating seeds are unlikely to germinate while sunken seeds are considered viable. To break dormancy, micropyles of *P. cineraria* seeds were scratched against sand paper to improve water imbibition into the seed.

## 2.2 Preparation of aqueous extracts of *P. juliflora* leaves and seed pods

The fresh green leaves were exposed to sunlight until they became dry. Then seedpods (with seeds) and leaves were separately ground to powder mechanically using a grinder. A wide range of concentrations of leaf and seedpod extracts were prepared, based on observations of the presence of copious amounts of leaves and pods under the canopies of these trees. Hence, 2.5, 5.0, 10.0 and 15.0 grams of powdered leaves or pods were weighed out and soaked in 100 ml of distilled water for 24 hours at room temperature. The solutions were filtered through a 2 mm mesh sieve to get rid of undissolved large particles and then centrifuged at 3500 rpm for 15 minutes. The resulting supernatants were dark greenish and yellowish to brownish for leaf and pod extracts, respectively. Aqueous extracts were stored in conical flasks and refrigerated at 4°C until performing exposures.

## 2.3 Seed germination and seedling root growth measurements of *P. cineraria* and *V. radiata*

The exposures to observe seed germination of *P. cineraria* or *V. radiata* were performed in Petri dishes, with five separate treatment levels for leaf and pod extracts (control, 2.5, 5.0, 10.0 and 15.0 grams/100 ml). One cotton pad was placed in each Petri dish and then a small amount (~ 10 ml) of each of the extracts was added for wetting the cotton pads. Distilled water was used as control. Then 10 seeds of *P. cineraria* or *V. radiata* were put in each Petri dish and the dishes were placed in the dark. After 7 days, the Petri dishes were observed by counting the number of germinated seeds and measuring the length of radicle or embryonic root of each seedling. Three replicates were done for each concentration of the extracts.

## 2.4 Eradication experiments of *P. juliflora*

### 2.4.1 Preparation of herbicide exposure solutions

Three herbicides (tribenuron methyl, clethodim and 2, 4-D & MCPA which is a mixture of 2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid) were purchased from a local shop for agricultural materials and chemicals. Following the manufacturers' recommendations, herbicide exposure solutions were prepared by dissolving 0.3 g, 1.0 ml and 15.0 ml of tribenuron methyl, clethodim and 2, 4-D & MCPA, respectively, in 1000 ml of distilled water.

### 2.4.2 Effect of herbicide on seed germination

Seeds of *P. juliflora* were randomly selected for 4 treatments (control, tribenuron methyl, clethodim, and 2, 4-D & MCPA) and 20 seeds were placed in each Petri dish fitted with a filter paper. The seeds were incubated in distilled water (control) or herbicide solution for 24 hours. Pots were prepared using potting peat (Klasmann Potgrond H 70 Blocking substrate, Netherlands) and irrigated using distilled water, and 5 treated seeds were then planted in each pot. The pots were watered and observed for seed germination on a daily basis for 12 days. The data were used to calculate germination time (GT<sub>50</sub> in days required for 50% of the seeds to germinate) and germination percentages (%).

### 2.4.3 Effect of herbicide on seedling growth

A further sample of *P. juliflora* seedlings were germinated and allowed to grow for 14 days, and were then randomly divided into 4 treatment groups (control, tribenuron methyl, clethodim, and 2, 4-D & MCPA) with 10 seedlings taken for each treatment. Next, the prepared herbicide solutions (as tested above for seed germination experiments) were sprayed on the seedlings while control seedlings received distilled water as spray. Changes in the seedlings were observed daily. After 5 days, above-ground fresh biomass was measured by cutting the shoots and leaves from each exposed seedling. The fresh biomass was determined with an analytical balance and expressed in grams.

## 2.5 Statistical analyses

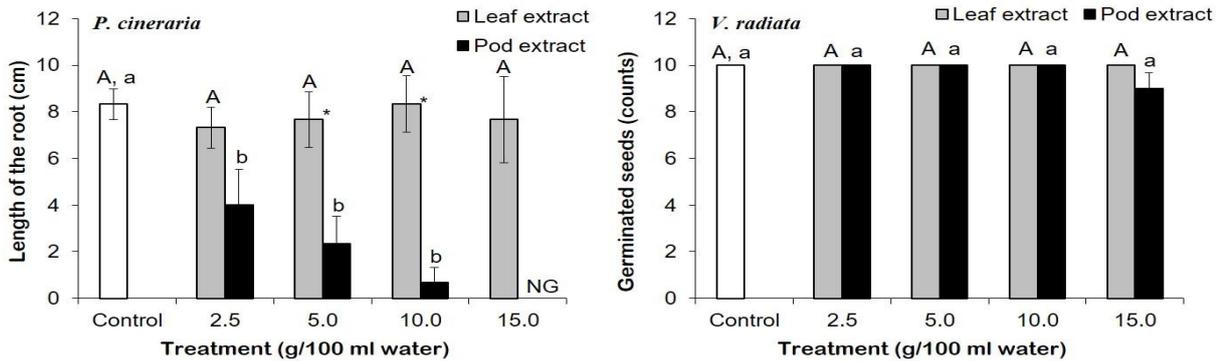
The number of germinated seeds and length of radicles of *P. cineraria* and *V. radiata*, their times to germinate (GT<sub>50</sub>) and germination percentages (%) were processed, graphed and analyzed for descriptive statistics using Microsoft Excel software, as was the above-ground fresh biomass of *P. juliflora*. The values were reported as mean ± standard error (SE). In addition, SigmaStat software was employed to perform one-way analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis ANOVA to detect differences between the control and treatments and among treatments. After significant ANOVA or Kruskal-Wallis ANOVA, *Post Hoc* multiple comparisons were carried out using Tukey's test or Dunn's method. All statistical analyses were established at the significance level of  $P = 0.05$ .

## 3. Results and Discussion

### 3.1 Effect of *P. juliflora* extracts on seed germination and seedling root growth measurements of *P. cineraria* and *V. radiata*

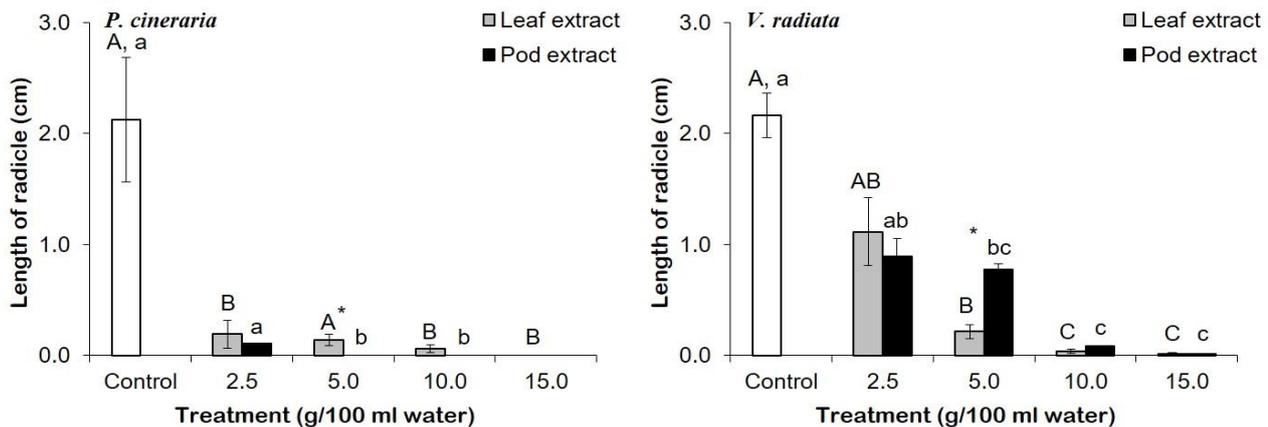
The allelopathic effect of leaf and pod extracts of *P. juliflora* on seed germination and length of radicle or embryonic root growth of the native ghaf (*P. cineraria*) and mung bean (*V. radiata*) was demonstrated (Figure 3.1). Seed germination of *P. cineraria* exhibited concentration-dependent inhibition for the pod extract (Figure 3.1). Seeds

of *P. cineraria* failed to germinate at the highest tested concentration (15 g/100 ml) of *P. juliflora* pod extract (Figure 3.1). Similar numbers of germinated seeds were recorded for both control (extract-free) and all treatments of *P. juliflora* leaf extract (Figure 3.1). Neither leaf extracts nor pod extracts of *P. juliflora* had an effect on the germination of *V. radiata* seeds (Figure 3.1).



**Figure 1.** Effect of leaf and pod extracts of the IAS, mesquite (*P. juliflora*) on the seed germination of the native ghaf (*P. cineraria*) and mung bean (*V. radiata*). The values are means  $\pm$  SE of  $n = 3$  replicates. Capital letters are for leaf extract exposures and lower case letters are for pod extract exposures. Different letters indicate significant differences and asterisks indicate significance between the action of leaf and pod extracts ( $P \leq 0.05$ ). NG: No germination.

Both species (*P. cineraria* and *V. radiata*) showed significant reduction of growth and/or development of their embryonic roots or radicles (Fig. 3.2) with both extract types of *P. juliflora*. While the effect was more pronounced in the case of *P. cineraria* even at low concentration levels, increased reduction in radicles of *V. radiata* was noticed with increased extract concentration (Fig. 3.2). Generally, both species (the native ghaf and mung bean) suffered similarly from both extracts especially at higher concentrations, at which root growth and development was completely inhibited (Fig. 3.2).

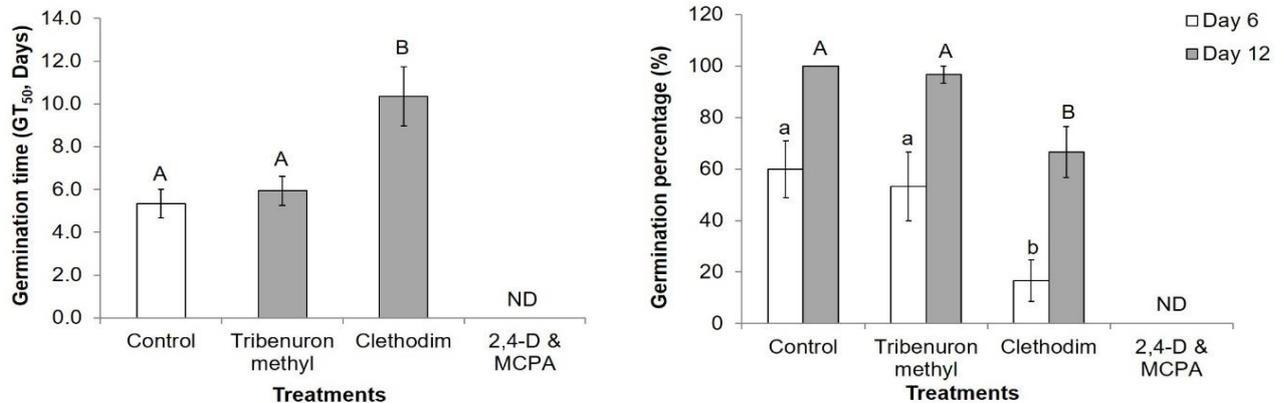


**Figure 2.** Effect of leaf and pod extracts of *P. juliflora* on the growth of embryonic roots of *P. cineraria* and *V. radiata* seedlings. The values are means  $\pm$  SE of  $n = 10-40$  seedlings. Capital letters are for leaf extracts and lower case letters are for pod extracts. Different letters indicate significant differences and asterisks indicate significance between the action of leaf and pod extracts ( $P \leq 0.05$ ).

Relative to the crop species (mung bean), data suggest the native ghaf is likely to be more affected by the allelochemicals released by the IAS even at lower environmentally-relevant levels (e.g. 2.5 grams/100 ml). Earlier studies [4] have suggested the presence of allelochemicals from observations of the germination percentage of the native ghaf species (*P. cineraria*) being greatly reduced when grown together with the IAS (*P. juliflora*). In addition, field surveys [5, 7] have reported that native flora diversity under dense *P. juliflora*-trees tends to be much reduced. The adverse impact of the invasion of arid grazing lands by *P. juliflora* has also been demonstrated [10]. In Oman, *P. cineraria* is a native woody species that provides shade and nutrients for livestock plus other ecosystem services. Soil of areas densely-impacted with *P. juliflora* would be expected to be contaminated by allelochemicals, thus negatively impacting the germination as well as the development and growth of the native species. Moreover, *Prosopis* species have detrimental effects on crop yield or productivity. For example, extracts of *P. juliflora* flowers, leaves and pods affected germination and seedling growth of important cereals such as common wheat (*Triticum aestivum*) and corn (*Zea mays*) [11]. In the present study, despite the fact that the crop species (*V. radiata*) appeared to tolerate the action of allelopathic substances for seed germination (Fig. 3.1), radicle length of its seedlings was markedly affected (Fig.

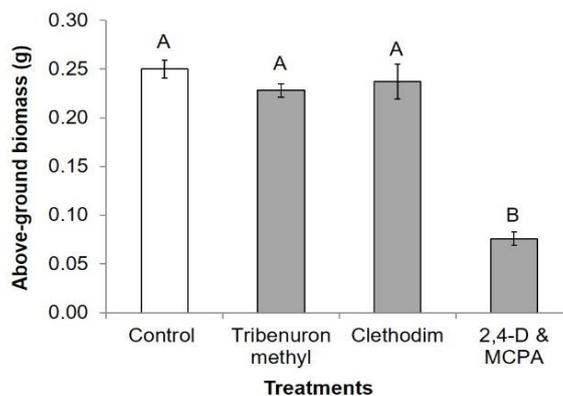
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3.2). Water extracts of dry leaves of *P. juliflora* inhibited germination and delayed the growth of Bermuda grass [12], an important species in tropical and subtropical pastureland. Field surveys have shown that both annuals and perennials in the arid lands are negatively affected by the presence of *P. juliflora*, although this depends on the density and extend of canopy [13]. In the current study, a substantial drop in seed germination of *P. cineraria* was observed, especially when exposed to seedpod extract (Fig. 3.1). The pronounced effect of pod extract may indicate that seed pods of *P. juliflora* act as a storage site or reservoir for allelochemicals. Allelochemicals work individually or in combination to inhibit or reduce germination and growth of the native plant species [9]. Further studies are needed to quantify and characterize the individual substances or mixtures from different parts of *P. juliflora* and identify which would be responsible for the observed reduction in growth and germination of other plant species.



**Figure 3.** Germination time (GT<sub>50</sub>) and percentage (%) for seeds *P. juliflora* after exposure to 3 herbicides. The values are means  $\pm$  SE of n = 6-15 replicates. Different letters indicate statistical significant differences ( $P \leq 0.05$ ). ND: Not determined because seeds exposed to 2, 4-D & MCPA did not germinate even after 12 days.

Figure 3.3 shows GT<sub>50</sub> (the time required for 50% of the exposed *P. juliflora* seeds to germinate) and germination percentage. The seeds in the control had the lowest mean GT<sub>50</sub> of 5.3 days while those of the clethodim treatment had a significantly higher mean GT<sub>50</sub> of 10.4 days (Fig. 3.3). Compared to the control, there was slightly higher mean GT<sub>50</sub> value of about 5.9 for tribenuron methyl, but it was not statistically significant (Fig. 3.3). No GT<sub>50</sub> was calculated for the 2, 4-D & MCPA treatment because no seed germinated even after 12 days (i.e. the duration of the experiment). Clearly, there is an inverse relationship between the GT<sub>50</sub> and the germination rate. In other words, the lower GT<sub>50</sub> values indicate higher germination rates and vice versa. The measurement of GT<sub>50</sub> seems plausible to illustrate inhibition of seed germination by the herbicides. Indeed, GT<sub>50</sub> has been employed to study plant germination under various environmental conditions [14-16]. Despite the fact that the investigated herbicides are usually applied on the seedlings, one objective of the study was to explore an effective way to eradicate *P. juliflora* by targeting seeds. It is not clear how clethodim and 2,4-D & MCPA delayed and inhibited seed germination, respectively. However, it is likely that seed germination and seedling development are affected by the same mechanisms. For example, clethodim acts as inhibitor of acetyl coenzyme A (CoA) carboxylase and 2,4-D & MCPA produces an action mimicking growth hormone auxins [17, 18]. Interestingly, an increase in auxin biosynthesis was reported to enhance seed dormancy via abscisic acid stimulation [19].



**Figure 4.** Above-ground fresh biomass for 14-day old seedlings of *P. juliflora* exposed to the 3 herbicides. The values are means  $\pm$  SE of n = 5 replicates. Different letters indicate statistically significant differences at  $P \leq 0.05$ .

For germination percentages, *P. juliflora* seeds in the herbicide-free condition (i.e. control) scored 100% germination after 12 days (Fig. 3.3). Germination did not differ much between the control and the tribenuron methyl treatment (Fig. 3.3). Relative to the control and tribenuron methyl treatments, the clethodim treatment had a very low germination percentage, with only 17% of the seeds germinated after 6 days and about 67% of them germinated after 12 days (Fig. 3.3). After exposure to the 2, 4-D & MCPA treatment, seeds failed to germinate during the duration of the experiment (Fig. 3.3). With the exception of exposure to 2, 4-D & MCPA, the above-ground fresh biomass of the 14 day old of *P. juliflora* seedlings did not appear to be affected by the herbicides (Fig. 3.4). On average, seedlings in the control, tribenuron methyl and clethodim treatments had statistically similar above-ground fresh biomasses of 0.25, 0.23 and 0.24 g, respectively. On the other hand, the above-ground fresh biomass of *P. juliflora* seedlings treated with 2,4-D & MCPA dropped by more 50% percent (less than 0.1 g on average) relative to that of the control. While *P. juliflora* seeds and seedlings exhibited tolerance to tribenuron methyl and clethodim, 2,4-D & MCPA was the most powerful in inhibiting seed germination and stopping seedling growth of this IAS. The latter herbicide is a mixture of two active chemical ingredients (2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid) and which probably act additively or synergistically to result in complete inhibition of seed germination and delay of seedling growth. Tribenuron methyl and 2,4-D & MCPA are herbicides for controlling broad-leaved weeds by inhibition of acetolactate synthase and mimicking growth hormones auxins [17], respectively. On the other hand, clethodim is a selective herbicide for narrow-leaved weeds and grasses and works as inhibitor of acetyl coenzyme A (CoA) carboxylase [17, 18]. Herbicides have been used in controlling mesquite for quite a long time [20].

In general, one disadvantage of the chemical approach for controlling alien invasive plant species and weeds is the emergence of resistant individuals. For example, some weeds have already been shown to have resistance to tribenuron methyl and clethodim [17, 20]. Interestingly, neither herbicide was effective for controlling *P. juliflora*, although clethodim did influence seed germination significantly in the present study. *P. juliflora* has been reported to tolerate several adverse environmental conditions such as copper-contaminated soils [21] as well as drought. For instance, *P. juliflora* exhibited a higher tolerance to drought conditions relative to native *Prosopis* species (e.g. *P. cineraria*) [4]. This feature seems to play a major role in the widespread distribution of this IAS in habitats with highly variable environmental conditions. Conversely, few resistant weeds have been reported for auxin-like herbicides (e.g. 2,4-D & MCPA) [17]. In this study, 2,4-D & MCPA worked very well for controlling seedling growth of *P. juliflora*, but this does not make it a better chemical option for controlling mesquite particularly in the wild or open areas. Large-scale application of such powerful herbicides would be associated with detrimental consequences on the native flora. On the farm scale, localized application of 2,4-D & MCPA on young *P. juliflora* seedlings may be recommended as an effective way to limit further spread and distribution. However, the use of pesticides including herbicides in agriculture may be generally associated with effects such as herbicide accumulation and residuals in produce. For example, residues of 2,4-D have been detected at levels of up to 0.030 mg/L in marketable watermelon [22].

#### 4. Conclusion

The study aimed to quantify the germination and growth of the native ghaf species (*P. cineraria*) and mung bean (*V. radiata*) when exposed to water extracts of leaves and seed pods of the IAS (*P. juliflora*) and to investigate the effectiveness of 3 agricultural herbicides to stop seed germination and seedling growth of *P. juliflora*. While seed germination of *V. radiata* was not affected by the extracts, seedling growth of native ghaf and mung bean was substantially reduced by both leaf and pod extracts, emphasizing earlier reports of the negative effects of this IAS. Seeds and seedlings of *P. juliflora* did not appear to be affected by the herbicides tested, except by 2, 4-D & MCPA which would be effective for restricted use on agricultural lands for successful elimination of this IAS.

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