

# Impacts of bracteole removal and seeding rate on seedling emergence of halophyte shrubs: implications for rangeland rehabilitation in arid environments

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**Abstract.** Direct seeding techniques often result in unsatisfactory outcomes in rangeland rehabilitation, primarily because of low seedling emergence and poor establishment. Seed processing techniques aimed at improving seedling emergence have gained interest by pasture managers. The purpose of this study was to investigate the combined effects of bracteole removal and seeding rate on seedling emergence in seven halophytic species: *Atriplex halimus*, *A. canescens*, *A. leucoclada*, *A. nummularia*, *A. lentiformis*, *Salsola vermiculata* and *Haloxylon aphyllum* under semi-arid conditions in Tel Hadya (Syria). Each of these species was evaluated for seedling emergence under two seed treatments (bracteoles removed and non-removed bracteoles) with three seeding rates (10, 30 and 60 seeds per pot), in a completely randomised block design. The results showed a positive effect of seed treatment on seedling emergence for all studied species. The native *A. halimus* had the highest emergence percentages whereas the introduced *A. nummularia*, had the lowest. However, there were no significant effects of seeding rates on seedling emergence. These results showed that bracteole removal could improve germination and seedling emergence, and potentially increase the rate of establishment of the species studied. Therefore, when implementing rangeland rehabilitation projects, bracteole removal needs to be considered. The native *S. vermiculata* should be recommended for direct seeding in the West Asia and North Africa region given its high seedling emergence, known high palatability, nutritive value, and high auto-regeneration performance.

**Additional keywords:** arid and semi-arid ecosystems, direct seeding, indigenous plants, rangeland restoration, seed scarification treatments.

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## Introduction

Most rangelands in the West Asia and North Africa (WANA) region are poorly managed, resulting in land degradation, reduced productivity, and loss of plant biodiversity (Hudson *et al.* 2017). Overgrazing, conversion of rangelands into rainfed cropping systems, land tenure issues, and climate change are major drivers for this degradation (Ates and Louhaichi 2012; Ouled Belgacem and Louhaichi 2013). Several government agencies have becoming increasingly aware of the magnitude of the problem and strive to provide solutions to address rangeland degradation. However, given the low and slow return on investment, governments are not able to finance large-scale projects to effectively restore and develop rangeland resources

(Louhaichi *et al.* 2016a). Therefore, cost-effective techniques for slowing down and eventually reversing the trend of rangeland degradation are needed.

Transplanting shrub species has been extensively used for rangeland rehabilitation in the WANA region due to high transplant survival, effective soil stabilisation, and reduced soil erosion (Louhaichi and Tastad 2010; Louhaichi *et al.* 2016b). To improve establishment rates, shrub seedlings are usually grown in nurseries and then transplanted in the field (Franco *et al.* 2006). However, this process is often expensive and time consuming (Douglas *et al.* 2007) as it requires facilities and labour to grow seedlings for several weeks or months in nurseries. Furthermore, establishing shrubs from nursery-grown

seedlings is particularly challenging in arid environments and/or remote rangeland sites due to lack of irrigation and adequate site preparation (e.g. establishment of contour furrows). Besides the irrigation cost, maintenance is needed to replace dead seedlings during the first year of establishment (Palmerlee and Young 2010).

Direct seeding might be a more effective alternative to transplantation of seedlings to improve plant species richness and the productivity of rangelands (Fernández *et al.* 2012). Direct seeding techniques are common practices for the establishment of most herbaceous pasture species and have been used to successfully revegetate large areas (Malcolm 1994). Although direct seeding is a cost-effective technique to implement large-scale restoration projects, its success in arid regions is limited by harsh environmental factors and poor soil properties, in addition to seed characteristics such as seed dormancy and physical structures (e.g. bracteoles) that interfere with seed germination (Ungar and Khan 2001; García-Fayos *et al.* 2010; Busso *et al.* 2012; Raizada and Juyal 2012; Prats *et al.* 2016).

Many halophytes, in particular *Atriplex* species, have chemical and morphological characteristics that regulate seed dormancy and germination (Ungar 1978; Khan and Ungar 1985). *Atriplex* seeds are enclosed within bracteoles that control the timing of water imbibition, a crucial step in the seed germination process (Ungar and Khan 2001). The bracts surrounding the seeds may contain chemical compounds that interfere with germination (Young *et al.* 1980; Jefferson and Pennacchio 2003). Removal of these bracts has been suggested as a mean of improving seed germination of *Atriplex halimus* and *Salsola vermiculata* (Osman and Ghassali 1997).

In most rehabilitation projects, managers tend to increase seeding rate assuming that seeds could have a low germination rate or could be subject to predation by wildlife (Florentine *et al.* 2013). Direct seeding with a higher seeding rate increases input costs and may lead to inter-plant competition for nutrients and space (Gordon *et al.* 1989). However, using low seeding rate greatly decreases plant population and results in low productivity (Florentine *et al.* 2013).

Understanding the factors that affect seedling emergence and plant establishment under rangeland conditions is critical for improving our ability to address the important rehabilitation problems such as low succession and poor spatial distribution of perennial plants (Lesica and Allendorf 1999; Ghassali *et al.* 2012; Kemp *et al.* 2013; Porqueddu *et al.* 2016). Therefore, the objective of this study was to investigate the combined effects of bracteole removal and seeding rate on seedling emergence of seven native and introduced halophytic shrub species that are widely used for rehabilitating degraded rangelands in the WANA region.

## Materials and methods

### Study site

The study was conducted in Tel Hadya (Syria) at the headquarters of the International Centre for Agricultural Research in the Dry Areas (ICARDA) (36°01'N, 36°56'E) using outdoor plastic pots of 14 cm diameter and 25 cm depth. Each pot contained 3.5 kg of a 1:1 mixture of farm soil and sand topped with a layer of

organic sheep manure. The average annual rainfall over two decades in the study site is 340 mm (ICARDA headquarters meteorological station), occurring predominantly from October to May.

Seeds of *Atriplex halimus*, *A. canescens*, *A. leucoclada*, *A. nummularia*, *A. lentiformis*, *Salsola vermiculata* and *Haloxylon aphyllum* were collected in the autumn of 2011 from the ICARDA pastoretum (Table 1).

### Experimental procedure

The pots were arranged in a randomised complete block design with five replications. The treatments consisted of removal and non-removal of bracteoles. Bracteoles were manually removed on half of the seeds (termed seeds without bracteoles) of all species by scarification using sand paper 212, P80, and aluminum oxide. The other half of each seed sample retained its bracteoles (termed seeds with bracteoles) and were considered as the control treatments.

The recommended rate for direct seeding (drill-seeding) of most *Atriplex* species is between 25 and 35 kg ha<sup>-1</sup> (seeds with bracteoles) (Watson and O'Leary 1993; Booth 2002). The average weight of 1000 seeds without bracteoles was 0.50 g for *A. leucoclada*, 0.64 g for *A. lentiformis*, 0.93 g for *A. halimus*, 0.72 g for *A. nummularia*, 1.02 g for *S. vermiculata*, 1.81 g for *H. aphyllum* and 2.82 g for *A. canescens*. Three seeding rates consisting of 10 (low), 30 (medium), and 60 (high) seeds per pot for each species were compared. This resulted in 21 treatment combinations for seeds without bracteoles and seeds with bracteoles. These combinations resulted in 42 three-way treatments (species × bracteole removal × seeding rate).

Seeds without bracteoles and seeds with bracteoles were sown directly in the pots at a depth of 0.5 to 1 cm and patted down firmly on 27 January 2012. Five randomly selected pots were assigned for each treatment combination, for a total of 210 pots. The pots were kept under natural environmental conditions of rainfall, temperature, and light. During the course of this experiment (27 January to 10 March 2012), the site received 121.6 mm of rainfall and the average minimum and maximum temperature varied from 1.9°C to 12.5°C. The long average of rainfall and temperature is presented in Table 2.

**Table 1. Geographic origin of the seven evaluated halophytic rangeland shrub species. Three of the species (*A. halimus*, *A. leucoclada*, *S. vermiculata*) are indigenous to the study area**

Species	Origin (reference)
<i>Atriplex halimus</i> L.	Mediterranean Basin (Ortiz-Dorda <i>et al.</i> 2005)
<i>Atriplex canescens</i> (Pursh) Nutt.	Arid North American (Barrow <i>et al.</i> 1997)
<i>Atriplex leucoclada</i> Boiss.	Middle East (Sankary 1986)
<i>Atriplex nummularia</i> Lindl.	Australia (Le Houérou 2000)
<i>Atriplex lentiformis</i> (Torr.) S. Watson	North America (Le Houérou 2000)
<i>Salsola vermiculata</i> L.	Middle East (Osman and Ghassali 1997)
<i>Haloxylon aphyllum</i> L.	Central Asia and the Middle East (Pyankov <i>et al.</i> 1999)

**Table 2. Long-term monthly rainfall (mm), average daily air temperature, average maximum and minimum temperature from 1985 to 2012 in Tel Hadya, north-west Syria**

Month	Long term means (1985–2011)				During the experiment implementation			
	Precipitation (mm)	Daily air temperature (°C)			Precipitation (mm)	Daily air temperature (°C)		
		Mean	Max.	Min.		Mean	Max.	Min.
January	65.7	6.6	11.7	1.5	12.4	6.8	10.9	2.7
February	55.0	8.1	13.8	2.3	104.8	7.2	13.0	1.3
March	46.2	11.3	18.3	4.4	4.4	7.7	13.6	1.8
April	28.5	16.0	24.0	8.0				
May	15.1	21.1	30.1	12.1				
June	1.4	26.3	34.9	17.6				
July	0.0	29.7	37.7	21.7				
August	0.5	29.9	37.9	21.8				
September	4.6	26.0	34.5	17.5				
October	21.9	20.3	28.3	12.3				
November	43.1	12.7	19.4	5.9				
December	57.7	7.9	13.0	2.8				

Emerged seedlings were counted at 10, 15, 20 and 33 days after seeding. Seedling emergence percentages were calculated as the ratio of the number of seedlings that emerged during the monitoring period to the number of seeds sown, and then multiplying this value by 100. The speed of emergence was calculated according to Gairola *et al.* (2011), using the following formula;

$$\text{Speed of emergence} = \sum_{i=1}^j \frac{ni}{tj}$$

where,  $n$  = number of emerged seeds,  $t$  = time after seeding.

#### *Effect of machine removal of bracteoles on A. leucoclada*

Results from the manual bracteole removal performed on *A. leucoclada* revealed lower seedling emergence rate compared with seed with bracteoles intact. Therefore, to provide possible explanations, machine removal of bracteoles was performed on *A. leucoclada* seed using a Perten Laboratory disc Mill 3310 (calibrated from level one to level six). Calibration was set at level four (4), as levels between 1 and 3 resulted in unacceptable mechanical damage to the seed. The experimental design was a randomised complete block, with bracteole removal as the blocking factor, and was conducted in March 2017 at the ICARDA facility in Terbol experimental station, Lebanon.

There were four replicates of 50 seeds of *A. leucoclada*. Half of the seeds were machine scarified (machine removed bracteoles) whereas the remainder were not (seeds with intact bracteoles). Seeds were exposed to two media (paper and river sand) for evaluation of germination and emergence (International Seed Testing Association 2017). Laboratory conditions were maintained at  $20 \pm 2^\circ\text{C}$ , with a 16-h light at  $75\,347\text{ lm m}^{-2}$  and 8-h darkness regime, for 14 days.

#### *Statistical analyses*

Seedling emergence counts were taken on each of four different dates. In order to factor in the effect of time on seedling emergence, linear regressions passing through the initial first seedling emergence day were performed, and the slopes of the regression estimated using the last observation date.

It is possible that the time it takes a seedling to emerge from the soil might surpass the period of observation, for example, 90% absolute seedling emergence percentage may not be reached within a given time period. To account for this, we rather estimated the percentage of the observed maximum emergence which is interpreted as seedling emergence potential. The slope and time to reach 90% emergence are a measure of emergence strength of the species and treatment, which explains the dynamics of emergence. These two estimated parameters were analysed using ANOVA. The pair-wise comparisons of the species under each combination of treatment and time were carried out using Bonferroni test at  $\alpha=0.01$ . Data of the complimentary laboratory experiment were analysed using ANOVA for the two treatments (bracteole removal and growing media). All statistical analyses were carried out using GENSTAT software (GENSTAT 2009).

## **Results and discussion**

### *The effect of bracteole removal on seedling emergence percentage*

Three of the species (*A. halimus*, *A. leucoclada*, *S. vermiculata*) are indigenous to the study area (Table 1). There were significant differences in seedling emergence among the species in response to bracteole removal ( $P<0.01$ ) and there was an interaction between bracteole removal treatment and species for seedling emergence percentage ( $P<0.01$ ). For seeds without bracteoles and seeds with bracteoles, *A. halimus* and *S. vermiculata* showed the highest seedling emergence percentages (72.6%, 69.4% and 65.2, 64.5% respectively). Bracteole removal enhanced seedling emergence of *A. halimus*, *A. canescens*, *A. lentiformis*, *A. nummularia*, and *H. aphyllum*. There were no significant differences in seedling emergence between seeds without bracteoles and seeds with bracteoles in *S. vermiculata* (Table 3). These results confirm the common belief that native perennial species (*A. halimus* and *S. vermiculata*) are well adapted to the harsh arid environments (recurrent droughts, shallow soils, crusted soil surface, high summer temperature) and perform better than most introduced species (D'Antonio and Meyerson 2002; Niane 2013). These results are similar to the findings of DePuit *et al.* (1980) where

**Table 3.** Seedling emergence percentage (%) of seven halophytic species under two seed treatments (seeds without bracteoles and seeds with bracteoles), over four time-sampling periods (10, 15, 25 and 33 days)

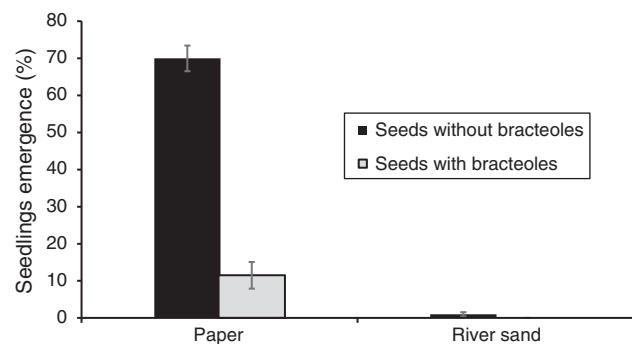
The means are represented along with standard errors. s.e.m., Standard error of the mean. Means within the same column with the same letter(s) are not significantly different at 5% level of significance, using Bonferroni test

Species	Seeds with bracteoles					Seeds without bracteoles				
	Number of days of counting				Mean	Number of days of counting				Mean
	10	15	25	33		10	15	25	33	
<i>Atriplex canescens</i>	47b	50.2b	51bc	50.3bc	49.6b	54.8b	58.1ab	59.2ab	57.8bc	57.5b
<i>Atriplex halimus</i>	66.2a	65.2a	66.1a	66.9a	66.1a	71.8a	72.1a	72.8a	73.7a	72.6a
<i>Atriplex lentiformis</i>	6.6d	34c	38.2cd	44.4c	30.8c	0.7d	37.7c	46.9b	53.4bc	34.7cd
<i>Atriplex leucoclada</i>	30.9c	33.8c	36.4d	37.2cd	34.6c	5.6d	22.3d	28.6c	31.8d	22.1d
<i>Atriplex nummularia</i>	22.9c	20.7c	21.7e	22.2e	21.9c	28.6c	46.8bc	48.6b	49.3c	43.3c
<i>Haloxylon aphyllum</i>	31.7c	31.4c	31.8de	29.9de	31.2c	32.6c	40.9c	46.7b	48.8c	42.2c
<i>Salsola vermiculata</i>	64.4a	63.6ab	63.1ab	63.8ab	63.7a	67.3ab	64.9a	64.4a	64.2ab	65.2ab
s.e.m.		± 3.28					± 3.28			
Mean	38.5	42.7	44.1	45.0	42.6	37.3	49.0	52.4	54.1	48.2
s.e.m.		± 0.54						± 0.54		

direct seeding of Gardner saltbush (*A. gardneri*) in disturbed lands experienced limited success due to the presence of seed bracteoles. Similarly, bracteoles of river saltbush (*A. amnicola*), and wavy-leaved saltbush (*A. undulata*) reduced final germination percentage and seedling emergence in Western Australia (Stevens *et al.* 2006).

Overall, the presence of bracteoles reduced seedling emergence except in *S. vermiculata*, where emergence was similar between seeds with bracteoles (63.7%) and seeds without bracteoles (65.2%). However, seedling emergence of *A. leucoclada* was significantly higher for non-scarified seeds (34.6%) compared with scarified seeds (22.1%) (Table 3). This could be attributed to mechanical damage caused by the bracteole removal method. It is likely that excessive bracteole removal damage may have occurred due to low precision of manual removal of bracteoles, owed to the fact that the bracteoles of *A. leucoclada* seeds are much more tightly attached to the endosperm than the rest of the studied species. To test this hypothesis, a precise and adjustable seed threshing machine was used. The difference in seedling emergence for the seed with machine removed bracteoles was significantly higher ( $P < 0.01$ ) for *A. leucoclada*, in both paper and river sand media (Fig. 1). Such differences in the response of seeds of different species to different bracteole removal techniques needs to be taken in considerations in rangeland rehabilitation using direct or indirect seeding.

In the present study, seeds with bracteoles of *S. vermiculata* showed similar seedling emergence percentage to seeds without bracteoles (Table 3). This response could be attributed to environmental conditions that contribute to seedling emergence potential, with or without bracteoles (Pearson *et al.* 2002). Therefore, despite the presence of bracteoles, seeds with bracteoles were still able to demonstrate comparable emergence levels suggesting that bracteoles in this species do not physically hinder germination and they may not harbor chemicals that interfere with germination as occurs with some other shrubs. Ungar and Khan (2001) studied the influence of bracteoles on the germination response of *Atriplex prostrata* and *A. griffithii*. They found that attached bracteoles did not inhibit germination of *A. prostrata* but completely inhibited germination of

**Fig. 1.** Seed germination (%) of *A. leucoclada* under laboratory conditions. The error bars represent the standard error.

*A. griffithii* seeds. Germination of seeds of *A. griffithii* was also inhibited in the presence of detached bracteoles, suggesting that the reduced germination of *A. griffithii* may be due to the presence of relatively high concentrations of dissolved salts in the bracteoles.

Although there were some inconsistencies, removing bracteoles from the seed improved seedling emergence for most species, possibly through breaking the seed coat, thus partially exposing the cotyledons to moisture (Table 3). Exposing the cotyledons can permit the process of hydrolysis and hormone activation, leading to an increase in nucleic acid transcription to promote protein synthesis for germination and early seedling growth (Müntz *et al.* 2001; Okunlola *et al.* 2011).

Bracteole removal substantially increased seedling emergence. Separating the seeds from the bracteoles could be beneficial for both physical and physiological reasons including (1) the inhibitory effects of the leachate from the bracteoles (many of them contain a high concentration of salt); (2) the physical barrier the bracteoles impose on the seed; (3) the presence of growth regulating substances or allelopathic compounds in the bracteoles that delay germination; (4) a reduction in oxygen diffusion due to bracteole presence; (5) the effect of the bracteoles on the quality of light falling on the seed (Koller 1957; Aizazzi and Argüello 1992; Schütz 2000;



Mandak and Pysek 2001; Li *et al.* 2008). Similarly, Stevens *et al.* (2006) reported that removing bracteoles increased germination and improved seedling emergence of *Atriplex amnicola* and *Atriplex undulata* by 50% and 150%, respectively. Webb *et al.* (2009) also found that the removal of seed husks and bracts of sawgrass (*Cladium jamaicense*) enhanced the percentage and success of germination. The inhibition of germination was attributed to the presence of abscisic acid and other compounds stored in the husks and bracts. Osman and Ghassali (1997) also found that seed germination of *S. vermiculata* and *A. halimus* was significantly improved by removing fruiting bracts from the fruits (utricles).

In line with the findings of the present study, mechanical bracteole removal also increased seedling emergence of the coated seed of *Tylosema esculentum* (Travlos *et al.* 2007). Our findings add to the consensus that removal of bracteoles stimulates germination and increases seedling emergence. The bracteole removal treatments applied in this study and more specifically the mechanical treatment used for *A. leucoclada* seems to be very effective in protecting the embryo and hence improving seedlings emergence. Therefore, they would be useful in rangeland revegetation by direct seeding.

*The effect of bracteole removal on seedling emergence dynamics, speed of emergence, and time taken to reach maximum seedling emergence*

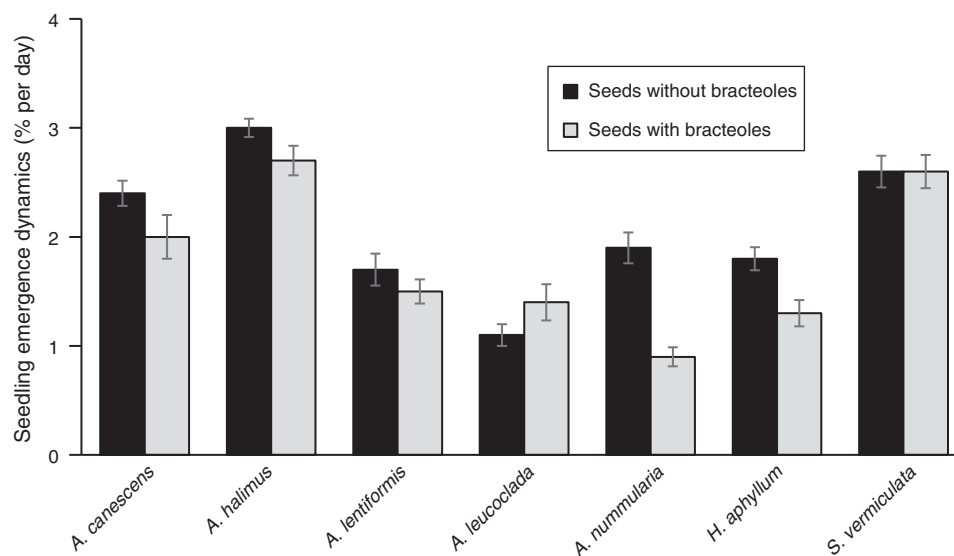
Since no species had reached 90% emergence during the experiment period, the time to 90% observed seedling emergence was estimated from the linear regression. This time and the speed of emergence, showed significant differences in relation to species, bracteole removal, and their interactions ( $P < 0.01$ ). When compared across all shrub species, bracteole removal resulted in higher seedling emergence percentage per day. The exception was the seeds with bracteole of *A. leucoclada*, where the opposite was noted. There was no response to bracteole

removal in *S. vermiculata* whereas *A. halimus* showed the highest seedling emergence percentages per day and together with *S. vermiculata* recorded the highest mean seedling emergence per day (Fig. 2).

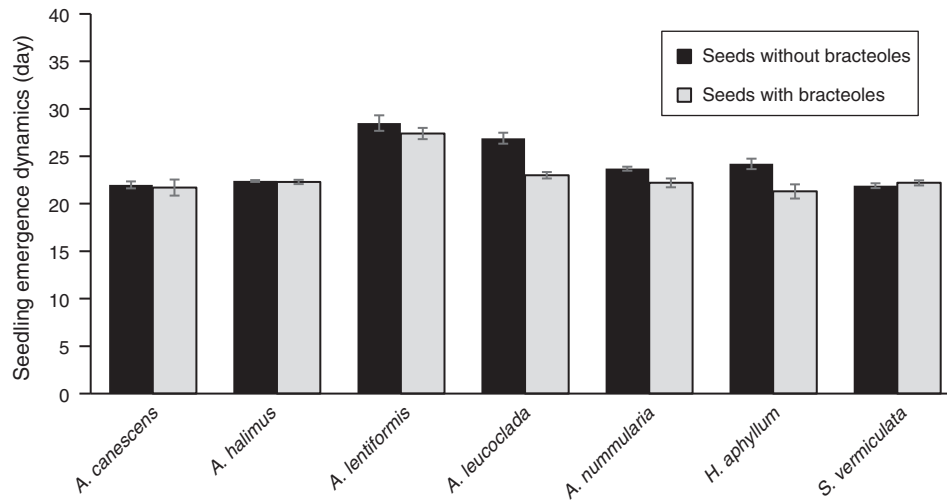
Bracteole removal treatments enhanced significantly ( $P < 0.01$ ) the dynamics of seedling emergence in terms of time to 90% of maximum seedling emergence (seedling emergence potential) compared with seeds with bracteoles, except for *Salsola vermiculata*. *A. lentiformis* showed the greatest seedling emergence potential and was the fastest species whereas *A. canescens* was the slowest (Fig. 3). Overall, all except one species have shown better seedling emergence in response to bracteole removal. Having bracteoles, native halophyte species seem to be more adapted to the arid harsh environmental conditions of the WANA region. Of particular interest is the native *S. vermiculata* that showed great potential with high seedling emergence even without bracteole removal. This shrub is known for its high palatability and ability to regenerate (Louhaichi *et al.* 2014) and should be recommended for large-scale restoration in the WANA region. The only problem is that it has a very short seed longevity and therefore should be collected and seeded in the same year (Niane *et al.* 2013).

The predicted seedling emergence rate on the last observation date was significantly higher for seeds without bracteoles compared with seeds with bracteoles for all the species except for *A. leucoclada* and *S. vermiculata*. The highest and lowest predicted rate of emergence were recorded for *A. halimus* and *A. leucoclada* respectively (Fig. 4).

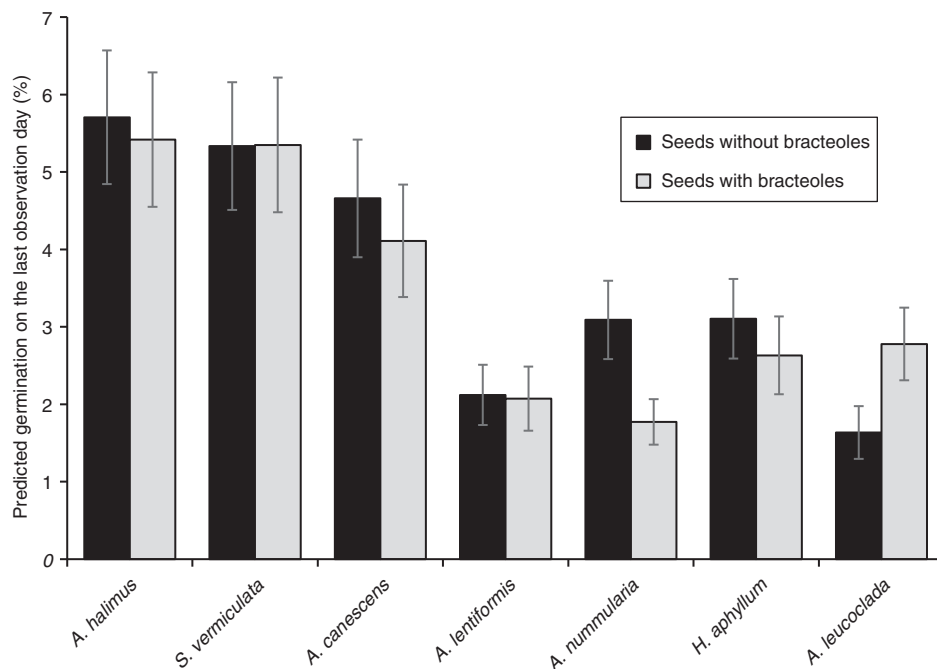
Seedling emergence includes two biological steps: seed germination, initiation followed by radicle and shoot elongation. Each step requires different environmental conditions (Roman *et al.* 2000). The relationship between environmental factors such as climatic conditions, soil, seed, and seedling emergence response, varies with species and the physiological status of the seed (Finch-Savage *et al.*



**Fig. 2.** Seedling emergence dynamics of the seven halophytic species under treatments (seeds without bracteoles and seeds with bracteoles) in terms of seedling emergence trend (% per day).



**Fig. 3.** Seedling emergence dynamics of seven halophytic species under treatments (seeds without bracteoles and seeds with bracteoles) in terms of time to 90% of maximum observed seedling emergence.



**Fig. 4.** Predicted germination on the last observation day of seedling emergence for seeds without bracteole and seeds with bracteole of seven shrubs under natural conditions.

2001). However, the interactions among these factors are complex (Vleeshouwers and Kropff 2000). The low percentage and speed of seedling emergence of arid and semi-arid species have been attributed to seed sensitivity to extreme temperatures and moisture availability (Roundy and Biedenbennder 1996; Chesson *et al.* 2004). Findings from this study suggest that the interaction of species and the presence of bracteoles may significantly contribute to the variability in seedling emergence responses. The initiation of seedling emergence is often more sensitive to water

availability (Alvarado and Bradford 2002). This might explain the positive effect of treatment on seedling emergence as the bracteole removal may increase the access to water by the seeds. However, the results did not show any significant benefits of higher seeding rates on seedling emergence, confirming the reports of Linhart (1976) and Florentine *et al.* (2013). Bracteole removal, in contrast, enhanced both the percentage and speed of seedling emergence of most of the studied species. This is a strong indication of successful establishment of shrub species (Raveneau 2012).

## Conclusion

In arid and semi-arid environments, seedling emergence of preferred species may be limited by several factors that regulate seed dormancy, germination and emergence. The success and efficiency of shrub seedling survival and growth under arid conditions depends on removing barriers that inhibit germination and stifle seedling emergence. This study found that bracteole removal from seeds of some shrub species increased seedling emergence percentage and speed. Therefore, seed bracteole removal of shrub species should be considered for large-scale rehabilitation of degraded arid rangelands under the ongoing climate change patterns. This is a crucial step, especially when direct seeding is the only viable option for reversing the trend of rangeland degradation.

Another key finding of this study is the fact that even with the presence of bracteoles, the native halophytic species *S. vermiculata* and to a lesser degree *A. halimus* have greater tolerance of extreme temperatures and moisture availability and exhibit high seedling emergence than the introduced species studied. Therefore, these native and well adapted species should be recommended for rehabilitating degraded WANA rangelands.

Nevertheless, to gain a greater understanding of the role of bracteoles in regulating emergence of these rangeland species, further physiological studies are needed to identify factors that minimise seed dormancy. Furthermore, seeding rate should be investigated thoroughly at a larger scale, in the field rather than in pots, and for a longer period to assess factors influencing seedling emergence and establishment.

## Conflicts of interest

The authors declare no conflicts of interest.

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