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Effect of Salinity and Temperature on Seed Germination of *Atriplex halimus* L. (*halimus* and *schweinfurthii* subspecies) Harvested in Western Algerian Region

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ABSTRACT

Background: Temperature and salinity effects on the germination and seedling growth of two subspecies of *Atriplex halimus* were studied in order to select the best one for the rehabilitation of degraded lands at risk of desertification.

Methods: Influence of salinity levels (0, 100, 200, 300 and 400 mM) and temperature (15, 20, 25, 30 and 35°C) on seed germination of two subspecies of *Atriplex halimus*, *halimus* and *Schwein furthii*, were assessed. Results showed optimal germination at NaCl concentration of 100 mM that decreases above 200 mM for both subspecies. In *sp Schwein furthii* the germination capacity is higher at NaCl concentration of 100 mM and temperatures varying between 20 and 30°C.

Result: High doses of salt (300 and 400 mM) strongly decrease the number of seeds germinated at all temperature regimes. In contrast, at temperatures between 30 and 35°C, seed germination appears to be strongly affected in *sphalimus*. Salinity levels of 300 and 400 mM with temperatures of 15, 20 and 35°C, reduce germination and increase mean germination time. The presence of 100 mM of NaCl in the culture medium sometimes appears to be beneficial for the growth of the seedlings. However, at high doses of salt (300 and 400 mM), length, fresh and the dry weight of the seedlings are reduced.

Key words: *Atriplex halimus subsp,* Germination, *Halimus*, Salinity, *Schwein furthii*, Seedling growth, Temperature.

INTRODUCTION

Arid and semi-arid ecosystems are vulnerable to abiotic stresses like temperature, frequently severe droughts and soil salinization, which cause serious damages and limit plant development (Krasensky and Jonak, 2012). In these ecosystems, excessive amounts of salts (Morgan *et al*., 2018) in soils or irrigation water are constraints for plant development (Higazy *et al*., 1995) being a major environmental concern and a serious problem for agriculture, reducing arable land and threatening food security (Kinet *et al*., 1998). This phenomenon still intensifying affects seeds germination, seedlings, vegetative growth, flowering and fruiting to varying degrees (Cordovilla *et al*., 1995) and limits agricultural production (Dantas *et al*., 2005).

Atriplex genus of the family *Amaranthaceae* (Kinet *et al*., 1998) are halophytes naturally tolerant to soluble salts and grow equally well in a saline environment as under normal conditions (Malcolm *et al*., 2003). *Atriplex halimus* L., typical Mediterranean species, is a particularly valuable species which can be ecologically and sustainably used for rehabilitation of degraded lands in coastal environment and arid regions (Walker and Lutts, 2014), as they can control erosion and desertification (Marcar *et al*., 1999). Although *Atriplex* species have very high salt concentration in their tissues during the adult stage, their seeds show highly variable and species-specific tolerance to salt at the germination and seedling stages (Ungar, 1991). Germination stage, crucial for the establishment of species that thrive in saline environments (Khan and Gulzar, 2003) is more sensitive to salinity especially when associated with elevated temperatures (Gardarin *et al*., 2010), by affecting imbibition and root elongation (Katembe *et al*., 1998). So far, comparative studies of the two subspecies with respect to salt and temperature stresses are scarce.

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MATERIALS AND METHODS

The experiment was conducted during seasons between 2018 and 2021 at the laboratory of plant physiology of the University of Tiaret. Seeds of two subspecies of *Atriplex halimus* were collected during the fruiting period from two

different stations (Fig 1). Sub species *halimus* seeds were collected from Kharrouba site in Mostaganem, located on the western coast of the country, characterized by a semi-arid climate with temporary flows and subspecies *Schwein furthii* seeds were collected from Tiout site in Ain Sefra (Naâma) located in the southern west highlands, where the climate is dry with cold desert. The seeds are shelled by hand and sterilized in 0.5% calcium hypochlorite solution for 20 min. They are then rinsed with distilled water and germinated in petri dishes lined with Wattman paper soaked with 2ml of sterile distilled water supplemented with different concentrations of NaCl (0, 100, 200, 300, 400 mM) and incubated in the dark in the oven at temperatures of 15, 20, 25, 30 and 35°C. Each treatment was replicated 5 times (25 seeds per treatment) and followed every 24 h for 20 days. Germination is detected by the exit of the radical from the seed coats (Bajji *et al*., 1998). Measurements related to morphological parameters and physiological ones were done for final seedling height (mm), fresh matter (MF) and dry matter (DM) of the whole seedling. Fresh weights (mg/plant) are performed using a precision balance just after plant has reached isothermal equilibrium. The dry weights (mg/plant) are obtained after passing the samples in an oven for 24 hours at 85°C. Measurements related to physiological parameters concerned percentage of germination were calculated according to Mazliak (1982) and average germination time in days (Tm) according to Czabator (1962) and then was defined the final percentage of germination, considering then number of germinations obtained at the end of the experiment, expressed as a percentage of the number of seeds tested. The means of 5 repetitions were used for the statistical analysis consisting of an anova/ manova carried out according to the Newman-keuls test at a risk of error of 0.05 using the Statbox 6.4 software.

RESULTS AND DISCUSSION

Effect of salinity and temperature on germination

The results are as follows:

Effect of salinity on germination rate (TG)

The concentration of 100 mM NaCl is tolerated by the two subspecies since it only causes a slight decrease in germination capacity (Fig 2 and 3). The germination sensitivity threshold is 200 mM, when the stress intensity is high (300 and 400 mM) the seeds are affected and show a different germination rate than the control. The presence of a high salt concentration (300 and 400 mM) is not tolerated regardless of the temperature studied (Fig 2).

The germination of seeds of *subsp. Schwein furthii* appears to have been severely limited to a temperature of 15 and 20°C under treatments of 200, 300 and 400 mM (Fig 3). Based on analysis of variance, salinity and temperature were found to have a highly significant effect ($P<0.05$) on the percent germination of seeds.

Rate (% Gf) and mean germination time

Fig 4 indicates that for *subsp. halimus* at temperatures of 20, 25 and 30°C, the control and the seeds subjected to a concentration of 100 mM show a germination rate which varies between 72 and 100% and an average time of 10 and 11 days. The temperatures of 15 and 35°C and the concentrations of 300 and 400 mM in the medium cause an increase in the mean germination time and a decrease in the final germination percentage.

Fig 5 shows that for *subsp. Schwein furthii*, temperatures of 15 and 20°C and a concentration of 300- and 400 mM lead to a reduction not only in the germination rate but also an increase in the mean germination time which is significantly affected. The temperatures of 25 and 30°C



Fig 1: Geographical location of seed collection sites.
Kharrouba Mostaganem: 35°56′57.72″N 0°05′18.48″E Elevation 26 m.
Tiout Ain Sefra: 32°46′18.17″N 0°24′16.72″O Elevation 1033 m.

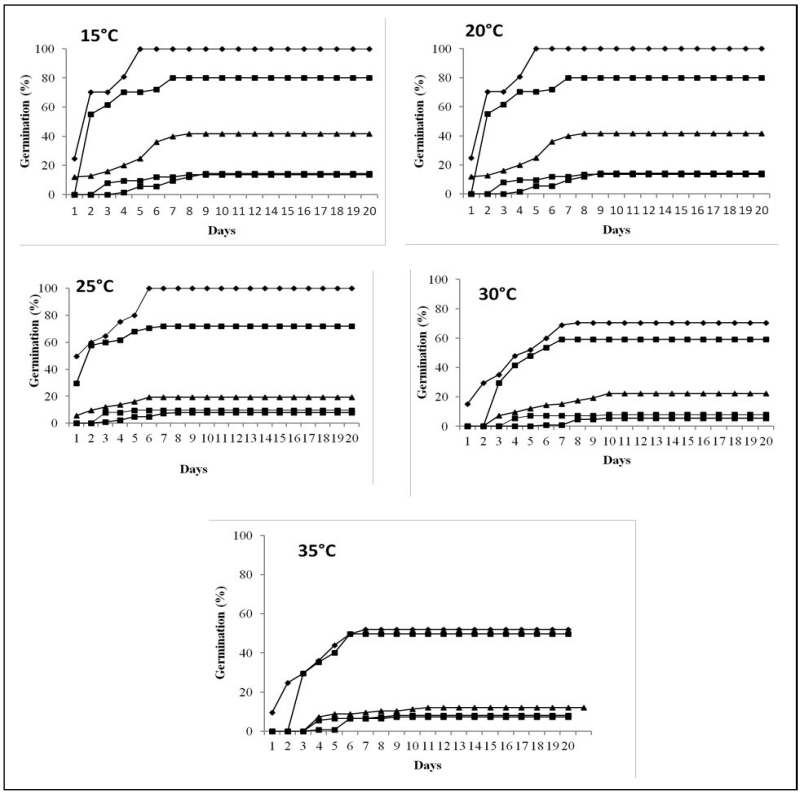

Fig 2: Percentage of germination of *Atriplex halimus sub sp. halimus* under the influence of salinity 0 (—◆—, 100 (—■—, 200 (—▲—), 300 (—■—) and 400 (—■—) mM and temperatures.

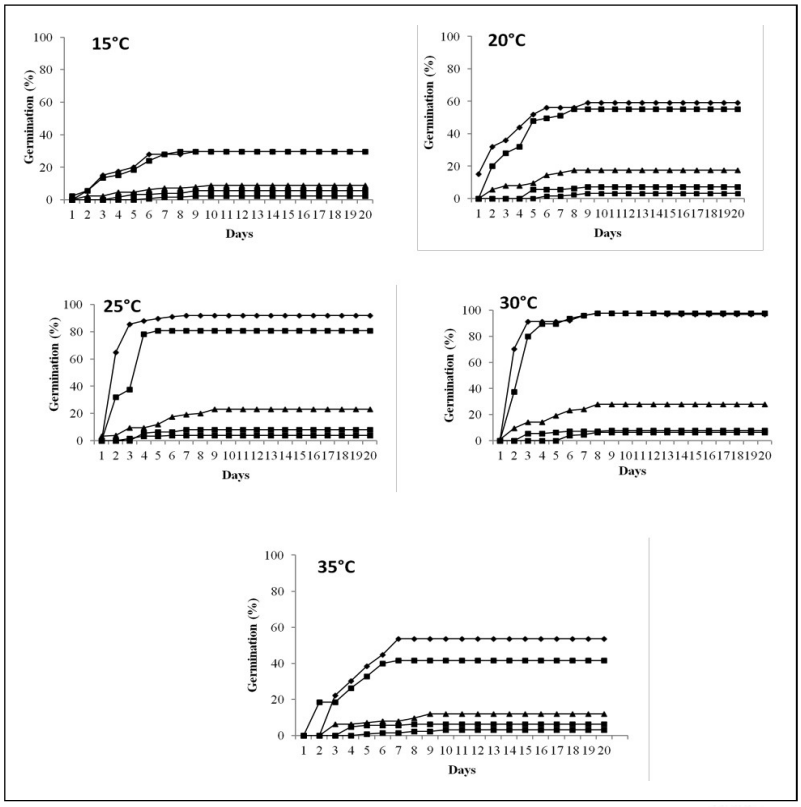

Fig 3: Percentage germination of *Atriplex halimus sub sp. Shwei furthii* under salinity 0 (—◆—, 100 (—■—), 200 (—▲—), 300 (—■—) and 400 (—■—) mM and temperatures.

seem favourable for the germination of the seeds of the control and the seeds stressed at 100 mM, the final germination percentages varied between 80.8 and 97.6% and the average time between 10 and 11 days. These results are confirmed by the analysis of variance which revealed a significant effect ($p<0.05$) on the final percentage of seed germination and an insignificant effect for the mean germination time.

Effect of salinity and temperature on the emergence of the vegetative system

At temperatures of 20, 25 and 30°C and under a salinity treatment of 100 and 200 mM the *subsp. halimus* shows the longest seedling length (Table 1), on the other hand, for *subsp. Schwein furthii* treatment of 200 mM and temperatures 25, 30 and 35°C are favourable over the length of seedlings (Table 2). Under treatment of 300 and 400 mM, the salinity exerts an inhibitory effect on the growth of seedlings which results in a reduction in their length and fresh weight.

The dry weight of seedlings of *subsp. halimus* increases with a treatment of 200 mM and at temperatures of 20, 25 and 30°C, on the other hand we notice that the dry weight of the seedlings of *subsp. Schwein furthii* increases with 200 mM treatment regardless of temperatures (Table 1 and 2). Analysis of variance shows a significant effect of temperature, salinity and their interaction for length and fresh and dry weight ($P<0.05$).

Results obtained in this work show that seeds of *Atriplex halimus subsp. halimus* and *Schwein furthii* are differently sensitive to temperature and salt stress depending on their provenance. Indeed, they are characterized by their low sensitivity to low salt concentrations and rapidity of germination which became significantly reduced at high concentrations of NaCl (300 and 400 mM). Temperature, salinity and their interaction affected the seed germination percentage of the two subspecies. These results are similar to those obtained by other researchers who note that the high concentrations of salts cause a total decrease in germination (Belkhodja and Bidai, 2004) and that the seeds of most species reach their maximum germination in distilled water (Naidoo and Keit, 2006).

Our results reveal significant reductions in the germination rates of seeds subjected to the higher salt

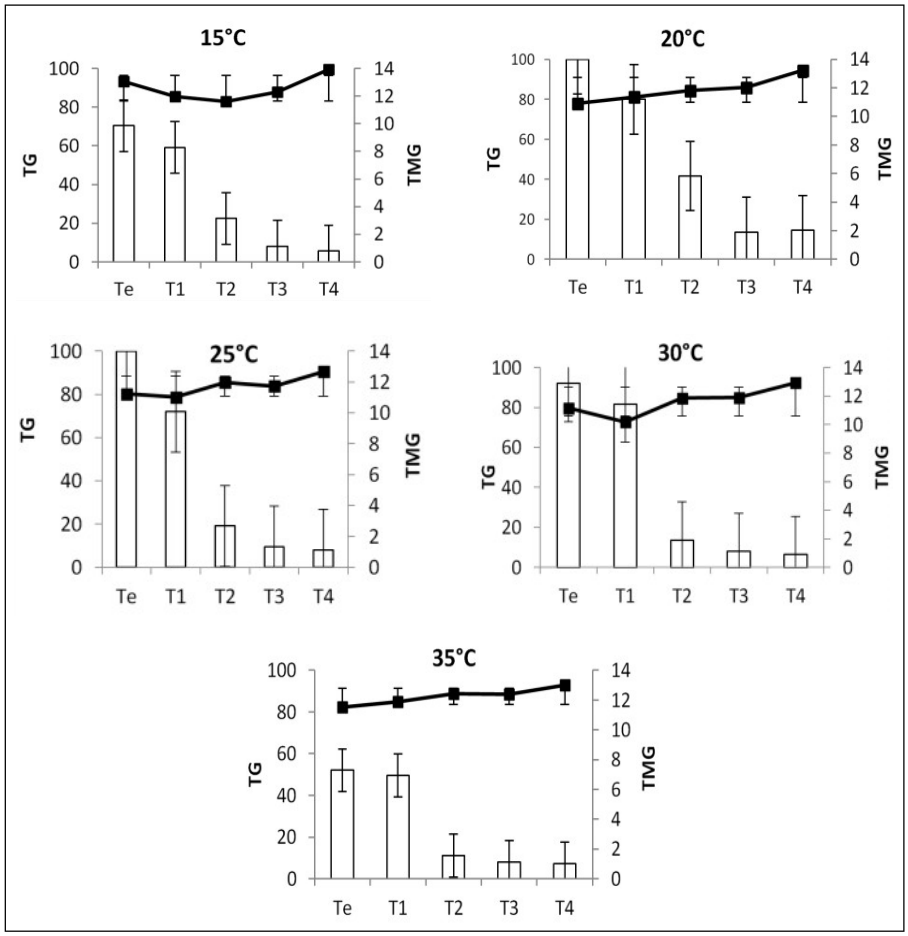

Fig 4: Final percentage of germination (%Gf) and mean germination time of *Atriplex halimus sub sp halimus* due to different temperatures and saline treatments.
Witness Te= 0mMT1= 100 mMT2= 200 mMT3= 300 mMT4= 400 Mm.

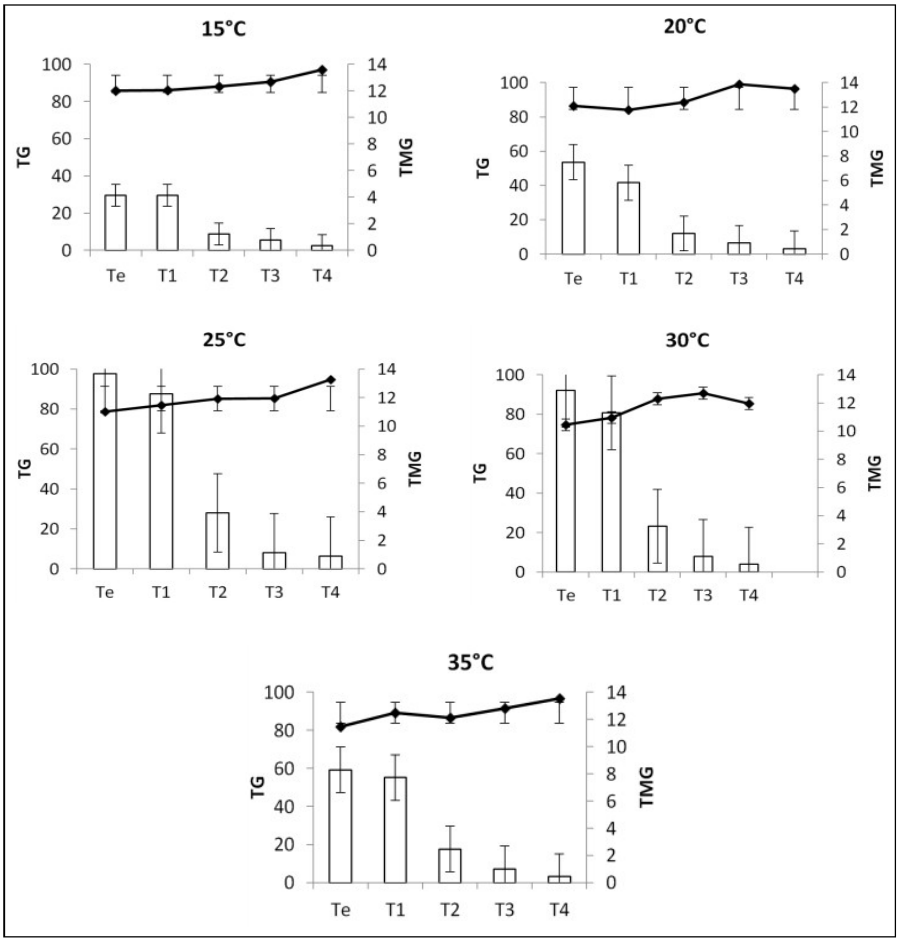

Fig 5: Final percentage of germination (%Gf) and mean germination time of *Atriplex halimus sub sp Shwei furthii* due to different temperatures and saline treatments.
Witness=0 mMT1= 100 mMT2= 200 mMT3= 300 mMT4= 400 mM.

Table 1: Effect of salinity and temperature on traits measured in seedlings of 20 days-old *Atriplex halimus sub sp. halimus*.

Parameters	Temperatures (°C)	Salinity (mM)				
		0	100	200	300	400
Length of seedlings (mm)	15	34.5±0.500a	37.3±1.745	30.5±0.432	16.8±1.851	10.6±0.247
	20	49.3±0.496	50.5±1.080	56.5±0.920	29.4±0.553	27.4±0.990
	25	48.3±0.920	45.4±0.673	26.1±0.828	20.7±0.496	15.2±1.006
	30	45.3±1.667	51.5±2.050	18.5±0.707	17.2±1.768	12.5±1.157
	35	25.6±0.294	31.3±1.232	15.2±0.424	17.1±0.355	13.7±0.355
Fresh weight (mg)	15	9.14±0.256	8.51±0.491	7.48±0.351	4.19±0.761	2.61±0.668
	20	12.0±0.298	12.3±0.353	13.8±0.285	7.21±0.361	6.72±0.267
	25	11.1±0.265	11.8±0.299	12.1±0.216	5.07±0.540	3.75±0.177
	30	11.1±0.725	11.6±0.949	12.0±0.408	4.21±0893	3.07±0.496
	35	7.67±0.335	6.27±0.329	3.75±0.285	4.00±0.081	3.36±0.333
Dry weight (mg)	15	0.68±0.021	0.63±0.029	0.56±0.085	0.31±0.016	0.19±0.014
	20	0.9±0.0748	0.92±0.139	0.95±0.063	0.54±0.299	0.50±0.071
	25	0.83±0.049	0.88±0.035	0.92±0.016	0.38±0.035	0.28±0.035
	30	0.84±0.089	0.87±0.014	0.90±0.120	0.33±0.028	0.23±0.028
	35	0.57±0.170	0.47±0.035	0.28±0.021	0.30±0.021	0.25±0.024

Medium±Type error. a,b,c, d and e: Homogeneous groups according to the Newman-Keuls test at the risk of error α=0.05.

Table 2: Effect of salinity and temperature on traits measured in 20-day-old *Atriplex halimus sub sp Shwein furthii* seedlings.

Parameters	Temperatures (°C)	Salinity (mM)				
		0	100	200	300	400
Length of seedlings (mm)	15	12.3±0.337	17.4±0.648	20.1±0.697	23.5±0.454	10.7±0.294
	20	22.3±1.358	24.5±0.408	27.3±0.509	26.9±0.216	14.1±0.697
	25	40.7±1.745	57.7±2.307	61.7±2.628	33.7±1.363	28.7±2.164
	30	38.3±1.275	47.7±1.023	52.7±0.697	31.7±3.302	17.9±0.080
	35	24.6±0.496	32.6±0.432	40.1±0.294	30.3±0.920	14.3±0.668
Fresh weight (mg)	15	3.36±0.835	4.77±0.362	5.46±0.458	6.40±0.294	2.93±0.174
	20	6.10±0.469	6.68±0.321	7.44±0.483	7.33±0.745	3.88±0.243
	25	11.1±0.535	15.7±0.310	17.3±0.574	9.12±0.914	7.82±0.327
	30	10.4±0.402	13.0±0.355	14.3±0.753	9.19±0.841	4.88±0.270
	35	7.25±0.735	8.89±0.094	10.9±0.058	8.25±0.735	3.90±0.294
Dry weight (mg)	15	0.22±0.021	0.31±0.017	0.36±0.043	0.42±0.024	0.19±0.083
	20	0.40±0.062	0.44±0.133	0.49±0.029	0.50±0.070	0.20±0.073
	25	0.74±0.299	0.96±0.108	0.99±0.069	0.61±0.069	0.52±0.058
	30	0.69±0.082	0.87±0.120	0.96±0.123	0.60±0.141	0.32±0.017
	35	0.44±0.099	0.59±0.029	0.73±0.049	0.55±0.040	0.26±0.043

Medium±Type error. a,b,c, d and e: Homogeneous groups according to the Newman-Keuls test at the risk of error α=0.05.

concentrations (300 and 400 mM) and a little effect of saline stress on the rate and speed of germination for moderate salinity levels 100 mM. In addition, a significant effect of temperature and salt stress on the germination rate of *Atriplex halimus subsp. halimus* was signalled. However, analysis of variance shows no significant effect on mean germination time. Reducing the germination rate and slowing the germination process under salinity conditions has been demonstrated by Murillo-Amador *et al*. (2002). Excessive salinity reduces the speed of germination as well as the germination capacity (Slama, 2004). According to Mâalem and Rahmoune (2009), the slowing of the speed of germination makes seeds more exposed to environmental risks.

Results show a reduction of the growth of *Atriplex halimus* subsp seedlings, which may reflect the expression of their halophilic character already reported by other authors (Haddioui and Baaziz, 2001). However, high doses of salt (300 and 400 mM) cause strong reduction in the length, fresh and dry weights of the seedlings. Most plants are more tolerant of salt at germination than at emergence and the first stage of growth (Maas and Grattan, 1999).

Indeed, several authors have reported that the general response of plants to salinity is reduced growth (Askari *et al*., 2017). These responses are attributed to reversible (osmotic stress) or irreversible (Na^+ and Cl^- toxicity) physiological changes in response to salinity depending on the temperature applied (Khan and Gulzar, 2003).

CONCLUSION

This work has allowed us to confirm the importance of the two subspecies given their low sensitivity to salt stress and their resistance to high temperatures during germination, this character being more visible for the subspecies *Schwein furthii*. The germination capacity of the two subspecies of *Atriplex halimus* and *Schwein furthii* under environmental constraints is certainly sufficient to consider their use against desertification and their exploitation for the production of fodder material in a program for the development of arid and semi-arid zones in Algeria.

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Conflict of interest: None.

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