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Algal extracts alleviates salinity stress on *Capsicum annuum*var. Baklouti

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ABSTRACT

This study was carried to evaluate the pretreatment of *Capsicum* seeds by algae aqueous extracts under NaCl stress. The thalli of *Padina pavonica* and *Jania rubens*, were socked in distilled water at five concentrations for 24 h. Primed and not primed seeds were sown in 0, 3 and 12 g/L NaCl. The germination was of 76% for the control to 93% and 85%, respectively for not dried seeds treated by *J. rubens* and *P. pavonica*, and an average of 84% for dried seeds. Salt stess induced a respective reduction of 27% and 100%, as compared to the control (67.66%) at 3 and 12 g/L NaCl, for untreated seeds. The priming by *J. rubens*, improved the not dried seeds rate germination by an average of 16%. For dried seeds, the most significant improvement (30 and 9%) was recorded with this extract at 20g/L, respectively, in the presence of 3 and 12g/L NaCl. The priming by *P. pavonica* extract, germination and increased the dried seeds rate of germination under 3 and 12g/L, with all concentrations of extract algal. However, an average improvement of 10% and 20%, respectively, for GNS and GS in all concentrations, in the presence of 3 g/L NaCl. At the highest concentration of salt, the improvement is an average of 3% in all casesThe priming reduced the time taken for 50% germination under salt and the mean germination time. Root growth of dried seeds was more improved than that of not dried seeds. Shoot growth of the two seeds sets was enhanced. Priming with extract *J .rubens* is more advantageous compared to that of *P. pavonica*. However, all pre-treatments have improved germination and growth in saline conditions.

Keywords: *Capsicum, algal extracts, salinity, tolerance.*

1. Introduction

Salinity stress is a major environmental constraint to crop productivity in the arid and semiarid regions of the world making large areas of lands substantially or partially unproductive. There is evidence that irrigation systems and type of irrigation water have contributed in a large extent in converting cultivated lands to saline lands (Ashraf and McNeilly2004). Today, about 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Roohi et al. 2011). The detrimental effects of high salinity on plants can be observed at the whole-plant level as the death of plants and/or decreases in productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within cells. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected. The earliest response is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies. Growth resumes when the stress is relieved. Carbohydrates, which among other substrates needed for cell growth, are supplied mainly through the process of photosynthesis, and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Mallek-Maalej et al.2004).

Seed germination takes the most important part in plants life cycle (Khan et al. 2000). Like many other cultivars, the seed's germination ability in different environmental conditions and fast and uniform germination are intended characteristics of spinach (Foolad et al. 2007). In many plant types, germination and seedling growing phases are very sensitive to salt stress. In general, the highest germination percentage occurs in non-salty conditions and it decreases depending on the ascending salt concentrations (Khan et al. 2000). Germination is described as the rootlet's coming out of the testa (Coopland and

McDonald 1995). Seeds' germination begins with water intake but it is decreased by the salt (Othman 2005). The decrease in water intake by the seed in salty conditions, osmotically and by the ion toxicity with accumulation of Na and Cl ions highly around the seed, prevents the seed germination (Murillo-Amodor et al. 2002). The increase of salt concentrations not only prevents the seed germination but also extends the germination time by delaying its starting (Rahman et al. 2008). Generally, low salt concentrations decrease the germination rate and high salt concentrations decrease germination percentage (Shannon and Grieve 1999). In addition, depending on plant species, salt stress not only affects germination percentage but also affects the germination rate and seedling growth in different ways (Zapata et al. 2004).

Seed priming is a pre-sowing treatment that involves exposure of seeds to low external water potential that limits hydration. This hydration is sufficient to permit pregerminative metabolic events but insufficient to allow radicle protrusion through the seed coat. This technique has become a common seed treatment that can increase rate, percentage and uniformity of germination or seedling emergence, mainly under unfavorable environmental conditions (Farhoudi et al. 2011).

Pepper is widely cultivated for its fruits which have a recognized nutritional value. They are an excellent source of various antioxidant compounds like flavonoids, carotenoids and vitamin C (Chuah et al. 2008). This later protects human body against oxidative damage and prevents various diseases such as cancer and cardiovascular diseases (Oboth and Rocha 2007). In Tunisia, pepper is the major cultivated plant and its fruits are mainly consumed either fresh or dry. It is cultivated on open air and under greenhouse. However, pepper is exposed to many biotic (virus, fungi) and abiotic stress, especially salinity, which has a negative effects on pepper growth and yield (Ibn Maaouia-Houimli et al. 2011).

This work aims to investigate the tolerance and the performance of pepper seeds, *Capsicum annuum* L. var. Baklouti, subjected to salt stress, after their priming with thalli aqueous extracts of two algae: *Jania rubens* and *Padina pavonica*.

2. Materials and methods

Biological material

Two species of algae were chosen: a red (*Jania rubens*) and a brown algae (*Padina pavonica*). The thalliwere collected from the Tunisian littoral (Monastir, Bekalta) in July 2012 at a depth of about 1 m. The identification was made based on color and morphological characters of the thallus (Taylor 1960). Their aqueous extracts were used for the priming of pepper seeds of the variety Baklouti (*Capsicum annuum* L. var. Baklouti).

Aqueous extracts

The algae thalli were washed thoroughly with tap water, to remove all epiphytes and attached debris, air dried for 15 daysat laboratory temperature and then ground into a fine powder using a mortar. The aqueous extracts were prepared at 20, 40, 60, 80 and 100g/L. Algal powders were soaked in distilled water for 24h at room temperature and darkness (Khanh et al. 2005). The mixture was filtered through a filter paper several times to obtain stock solution which stored at 4 °C in the dark for further use.

Seed priming and germination test

Seeds were soaked in aqueous extracts of algae (at 20, 40, 60, 80 and 100 g/L) with a ratio of seed mass weight to solution volume of 1:5 (g ml^{-1}) (Farooq et al. 2006), then washed thoroughly with distilled water. Untreated dry seeds were taken for comparison (Farooq et al. 2005). Two batches were considered i) seeds were dried (SD) closer to original moisture level in forced air at 28 ± 3°C, sealed in kraft bags, and stored at 4°C until use *ii)* seeds were placed in Petri dishes to germinate directly after pretreatment and washing, seeds not dried (SND).

Salt stress

Two NaCl concentrations (3 and 12 g/L) have been prepared to assess the tolerance of seeds to salt stress. The tolerance variation was evaluated by comparing the performance of treated and untreated Baklouti seeds. After pretreatment with aqueous extracts at different concentrations, the seeds were germinated in Petri dishes of 9 cm diameter (20 seeds), the bottom is lined with a double layer of filter paper soaked with NaCl solutions at 3 and 12 g/L. Untreated seeds have undergone salt stress and were used for comparison of salt tolerance. Germination of treated and untreated seeds in distilled water served as controls. Three replications were performed for each treatment.

The number of germinated seeds was counted every 24 hours for 20 days, and shoot and root length was measured at the end of the experience.

The inhibitory or stimulatory percent was calculated using the following equation given by Chung et al. (2001):

Inhibition/stimulation % = [(extract – control)/control] x100

With:

Extract: parameter measured in saline conditions

Control: parameter measured in control conditions

The time to 50% germination (T_{50}) was calculated according to the formula of Coolbear et al. (1984) modified by Farooq et al. (2005):

$$T_{50} = t_i + [(N/2-n_i) / (n_j-n_i)] (t_j-t_i)$$

Where N is the final number of germination and ni, nj are cumulative number of seeds germinated by adjacent counts at times ti and tj when ni < N/2 < nj.

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT= \sum Dn / \sum n$$

Where n is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination.

Germination index (GI) was calculated according to the AOSA (1983) using the following formula:

GI= No.of germinated seeds / Days of first count+……..+

No.of germinated seeds / Days of final count

Germination rate (GR) was calculated using formula below (Schelin and al. 2003):

% GR= (Number of germinated seeds / Total of number seeds)*100

Statistical analysis

The laboratory bioassays were conducted in a completely randomized design with three replications. Duncan, Student t and ANOVA tests were performed using PASW statistics 20.00, for Windows program, to analyze treatment differences. The means were separated on the basis of least significant differences at the 0.05 probability level.

3. Results

Effect of the priming of seeds on their germination

The time taken for 50% germination (T_{50}) was 8 days for control and increased to an average of 12.43 days in presence of 3g/L NaCl for not treated seeds (control). The application of allelochemicals of *J. rubens* and *P. pavonica* aqueous extracts reduced, more or less significantly, this parameter which varied with the extract concentration and the importance of the salt stress (Fig.1.). For seeds primed with *J. rubens* extracts, in non-saline conditions, T_{50} was reduced by an average of one day for not dried seeds (SND) and dried seeds (SD) at concentrations ranging from 40 to 100g/L. In presence of 3g/L NaCl T50 passed fron 10 to 8 days for SND, when *J. rubens* extract concetrations passed from 20 to 100g/L. In the same condition, T_{50} was reduced by an average of 4 days for SD, from the lowest salt concentration(Fig. 1.a). *P. pavonica* extracts were more efficient, allowing a gain oftwo and three days for SND primed at lower concentrations and equal to 80g/L, in the presence of 3g/L of salt. For SD, all concentrations have reduced T_{50} of 3 days, the lowest concentration of salt. At the highest concentrations of NaCl, germination is canceled for untreated seeds. Against by, for treated seeds, T_{50} is an average of 15 days for not dried seeds (SND) and dried seeds (SD) (of 20 to 80 g/L) (Fig.1.b).

NaCl has lengthened the mean germination time (GMT) which was 14 and 16 days in absence and under 3g/L NaCl, respectively. The priming with algal extracts has reduced this time under saline conditions (Fig. 2.a and b). Indeed, GMT was reduced by an average of 1.4 and 1.7 days, respectively, for the SND and SD in all cases to the lowest concentration of NaCl (Fig.2).

The germination index (IG), expressed in pourcent of control, reflects the germination speed which was more or less accelerated. In absence of salt stress, IG was increased by an average of 38% for SND primed with *J. rubens* extract at concentrations up to 80 g/L. At 100 g/L IG was similar to control. For SD and in absence of NaCl, IG has been improved by 11%, 62% and 5%, respectively, at 40, 60 and 80g/L. A significant improvement was noted under saline condition (3g/L), for both types of seeds (SD and SND). Indeed, IG varied between 63% and 195% for SND and between 121% and 130% for SD. The highest values were obtained with 60 and 100g/L for SND and with 60 and 20g/L for SD. At 12g/L NaCl, the germination was significantly affected for all primed seeds (Fig.3.a). For seeds primed with *P. pavonica* extract, at 0g/L NaCl, IG was increased to 156% for SND primed with 100 g/L and 64% for SD primed with 20 and 60g/L, otherwise values were near the control. In the presence of 3g/L NaCl, the germination speed, from SND, was increased by an average of 48% at concentrations less than and equal to 80g/L. The maximum was obtained at 40 g/L (77%). For SD, IG has been improved in all cases. The best stimulation was recorded at the lowest concentration of the extract (stimulation of 112%, compared to the control).The priming could not maintain the germination of SND and SD under 12g/L salt solution, in the all cases germination was completely stopped (Fig.3.b).

Regarding the germination rate (GR) and in absence of salt stress, the priming has ameliorate pepper germination by an average of 16% for SND and 9.34% for SD primed with all concentrations of *J. rubens* extract. At 3g/L NaCl, an average increase of 26% was recorded at all concentrations of the extract. At the highest concentration of NaCl, GR was highly increased (35%) following the priming with 20g/L for SND, otherwise the germination improvement was not significantly (Fig.4.a).

The priming with *P. pavonica* extract, at all concentrations, was beneficial for pepper seeds germinated in absence of salt, where an average enhancement of 8.33% and 6% was registered, respectively, for SND and SD. At 3g/L NaCl, the best result was observed at 20 g/L. the improvement was 39% and 36%%, respectively, for the SND and SD treated with *P. pavonica* extract, compared to untreated seeds (49.33%). None priming effect was registred at 12g/L NaCl (Fig.4.b).

Effect of the priming of seeds on the seedling growth

The seed priming was beneficial for seedling growth only under salt condition, since at 0g/L NaCl, root length was reduced by an average of 10% for seedling grown up from SND and SD primed with *J. rubens* extract at all concentrations, this reduction percentage was of 14% for shoot length. At moderate salinity (3g/L) root length was similar to the control, except for seedlings coming from SND primed with 60g/L and 100g/L of this extract, where a percentage stimulation of 30% and 20% was registred, respectively. These values were 40% and 50% for shoot of the same seedlings. For SD seedlings, a respective amelioration of 20% and 50% was recorded for root and shoot, with the priming at the hifhest concentration of *J. rubens* extrac. However, under strong salinity, the seed priming was very beneficial especialy with high algal concentration (100g/L) which inducing a percentage improvement of 44% and 42% for root length of SND and SD seedlings, respectively, these values were 84% and 85% for shoot length. Otherwise, root length was near the control except for SD primed with 80g/L (48% amelioration). Shoot length increase was ranged between 30% and 60% for SND seedlings at algal concentration up to 80g/L. For SD, the priming was benficial for shoot length, only since 60g/L (Fig. 5.a and b).

Likewise, the priming with *P. pavonica* extract was very beneficial, and more than *J. rubens* extract, for tolerance of strong salt stress (Fig.6). Indeed for SND seedlings, the lenght was similar to control at 0g/L NaCl, ameliorated by 40% for seeds primed with up to 40g/L concentration extract and similar to control above, in presence of 3g/L NaCl. For SD seedlings, the priming didn't ameliorate the growth up to 3g/LNaCl, and in all cases, values were near or below the control. However, at 12g/L NaCl, seedlings grwon up from SND, have their root length ameliorate by a percentage ranged between 10 and 25%, and between 59 and 114% for their shoot length. The percentages amelioration were better for SD seedlings, they were ranger, respectively, between 20 and 110% and between 25 and 198% (Fig. 6. a and b).

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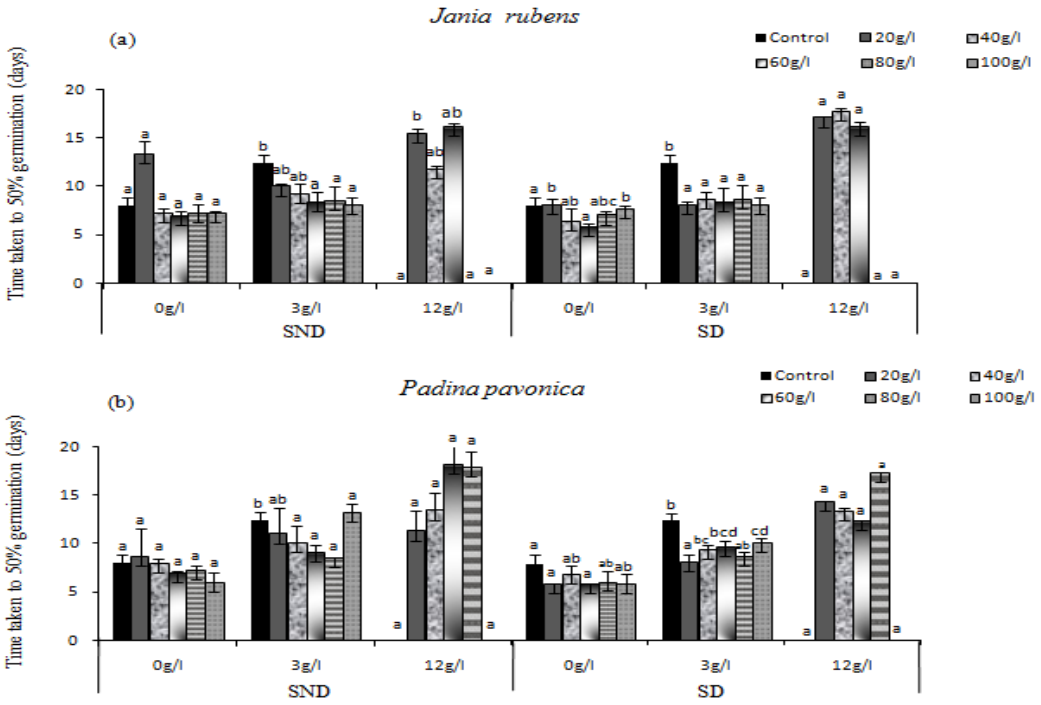

Fig.1. Variation of Time taken to 50% germination (T_{50}), of *C. annuum* var. Baklouti, untreated (control) and pretreated dried (SD) and not dried seeds (SND) by aqueous extracts (20, 40, 60, 80 and 100g/L) of *J. rubens* (a) and *P. pavonica* (b) thalli in the presence of NaCl at different concentrations. The bars on each column show standard error. Values (N=3±S.E.).Different letters on columns indicate significant differences among treatments at P<0.05.

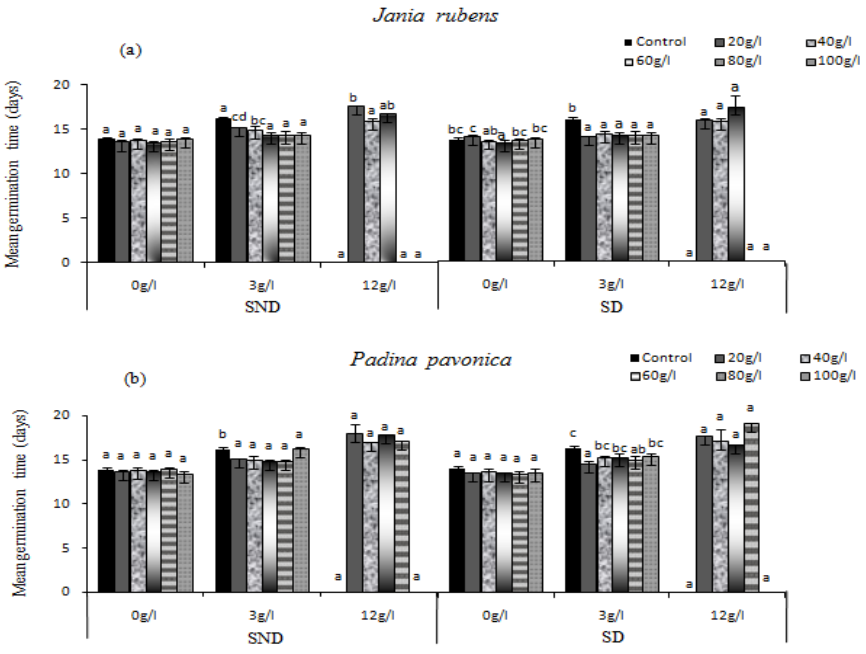

Fig.2. Variation of Mean germination time (MGT), of *C. annuum* var. Baklouti, untreated (control) and pretreated dried (SD) and not dried seeds (SND) by aqueous extracts (20, 40, 60, 80 and 100g/L) of *J. rubens* (a) and *P. pavonica* (b) thalli in the presence of NaCl at different concentrations. The bars on each column show standard error. Values (N=3±S.E.).Different letters on columns indicate significant differences among treatments at P<0.05.

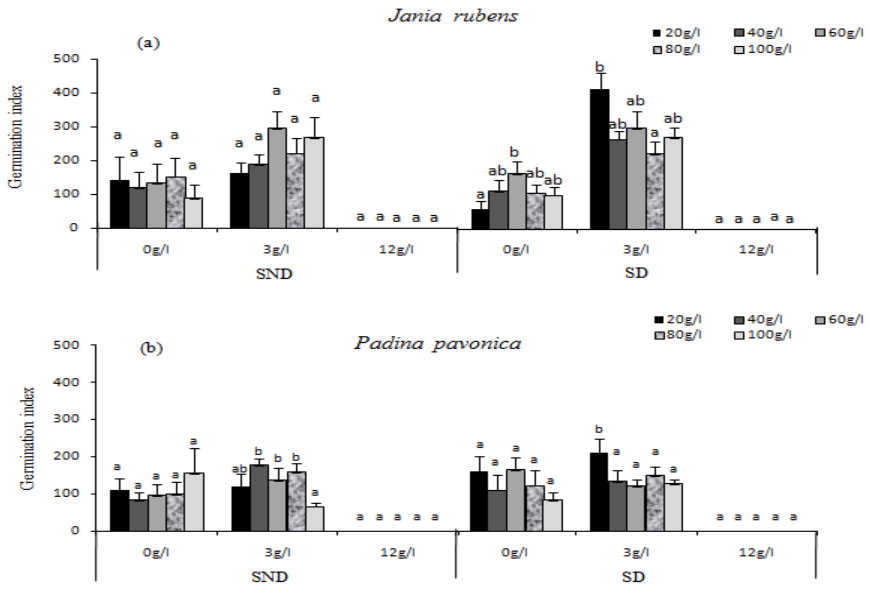

Fig.3. Variation of germination index (IG), of *C. annuum* var. Baklouti, untreated (control) and pretreated dried (SD) and not dried seeds (SND) by aqueous extracts (20, 40, 60, 80 and 100g/L) of *J. rubens* (a) and *P. pavonica* (b) thalliin the presence of NaCl at different concentrations. The bars on each column show standard error. Values (N=3±S.E.).Different letters on columns indicate significant differences among treatments at P<0.05.

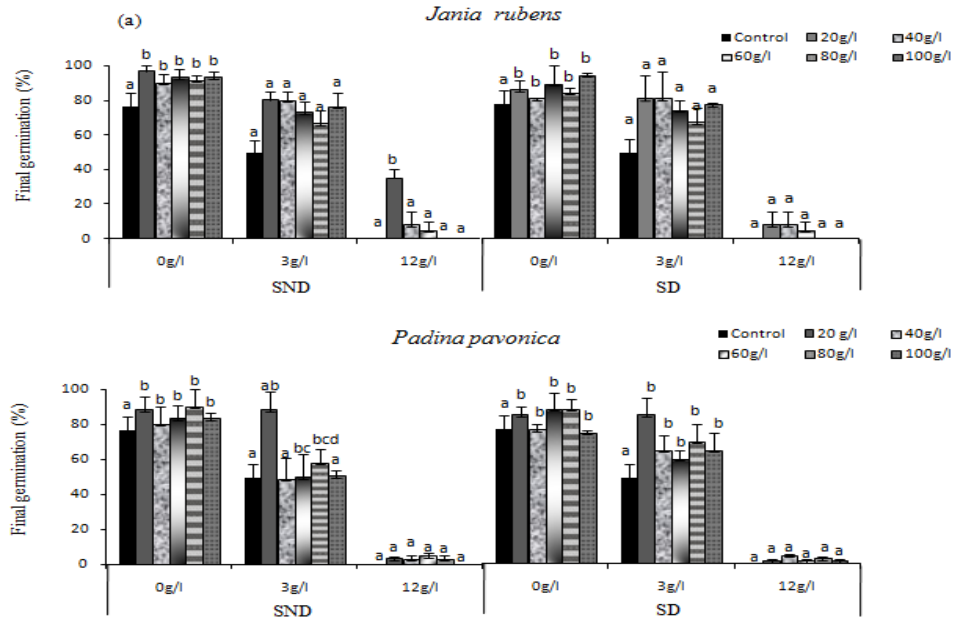

Fig.4. Variation of final germination, of *C. annuum* var. Baklouti, untreated (control) and pretreated dried (SD) and not dried seeds (SND) by aqueous extracts (20, 40, 60, 80 and 100g/L) of *J. rubens* (a) and *P. pavonica* (b) thalliin the presence of NaCl at different concentrations. The bars on each column show standard error. Values (N=3±S.E.).Different letters on columns indicate significant differences among treatments at P<0.05.

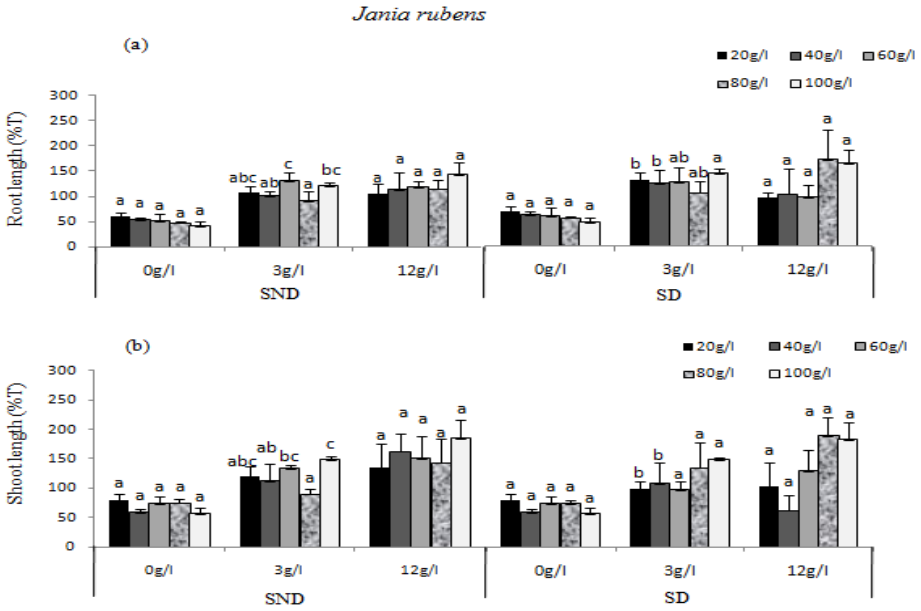

Fig.5. Root length (a) and shoot length (b), of *C. annuum* var. Baklouti, pretreated dried (SD) and unknot dried seeds (SND) by aqueous extracts (20, 40, 60, 80 and 100g/L) of *J. rubens* thalli in the presence of NaCl at different concentrations. The bars on each column show standard error. Values (N=3±S.E.).Different letters on columns indicate significant differences among treatments at P<0.05.

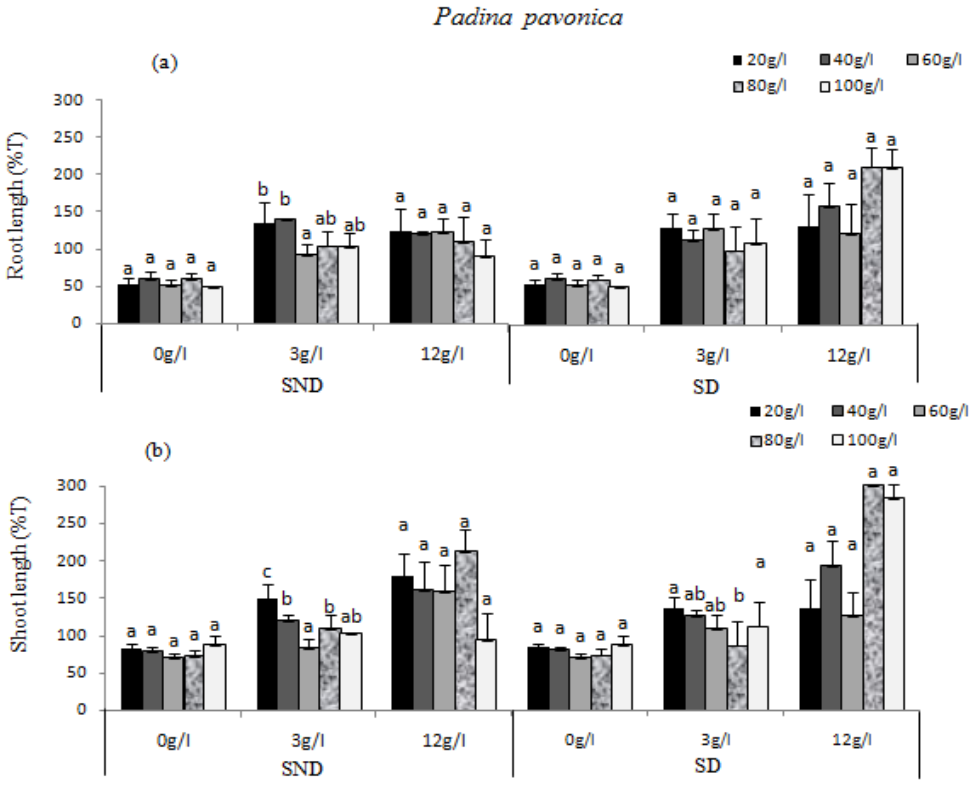

Fig.6. Root length (a) and shoot length (b), of *C. annuum* var. Baklouti, pretreated dried (SD) and not dried seeds (SND) by aqueous extracts (20, 40, 60, 80 and 100g/L) of *P.pavonica* thalli in the presence of NaCl at different concentrations. The bars on each column show standard error. Values (N=3±S.E.).Different letters on columns indicate significant differences among treatments at P<0.05.

4. Discussion

The present study showed that *C. annuum* L. var. Baklouti seed treatment with algal extracts may perform better results both under saline and non-saline conditions and the effectiveness of surface drying and re-drying strategies was different. In absence of NaCl, generally all germination parameters were improved, especially the germination index (IG) and the germination rate (GR). Overall, the better results were obtained with seeds primed with the lowest algal extracts concentrations. The improvement of germination by the priming is reported in the literature, by H_2O (Rinez et al. 2012, Shim et al. 2008) KNO_3 (Shim et al. 2008), H_2SO_4 (Rinez et al. 2012) and by plant extracts (Farooq et al. 2011). This is consistent with Afkari, (2009 in Mc Donald 2000) for the pretreatment of sunflower seeds in saline conditions with KNO_3 which have improved the germination of seeds treated compared to untreated.

The improvement of germination was attributed to certain secondary metabolites having a significant role in the important metabolism within the plant (Chon and Kim 2002). NaCl led a considerable reduction of pepper seeds germination, which rate reduction reached to 100 % with 12 g/L. NaCl affects all developmental stages particularly and it is fatal to the first of them (germination, seedling development). The most observable effect of the salt stress is the decrease in the germination percentage (Botia et al. 1998, Cuartero, Fernandez-Munoz 1999), explained by the reserves mobilization alteration (Hajlaoui et al. 2007). The NaCl affects this phase by its osmotic effects and/or by its toxic effects (Huang and Redmann 1995). Indeed, Katsuhara et al. (2011) showed that the accumulation of toxic ions Na^+ and Cl^- leads to the water absorption reduction during imbibitions, because it affects the expression of aqueous channels, responsible for regulating the transport of water molecules and also it causes K^+ and Ca^{2+} deficiency which makes osmotic adjustment difficult (Haddas 1977). As far as, NaCl exerts a depressant action on pepper seedlings growth.The effect of salt was similar on roots and shoots, while it is often reported that roots are more sensitive to salt compared to the aerial part (Jamil et al. 2006). Moreover, the response to salinity varies according to the amount, thus the sensitivity of Baklouti was noted since 3g/L with 50% reduction, compared to the control. The growth is the result of division and multiplication cell, that, several work showed their sensitivity to the hydrous deficit generated by salt (Acevedo et al. 1971).The depressive effect of salinity on plant growth is explained mainly by two factors: difficulties of water supply and nutrients caused by the alteration of the mobilization of reserves caused by NaCl, and by the toxicity of accumulated ions excess in the plant (Xiong et al. 2002).

The seed priming by algal extracts was beneficial with better results with *J. rubens* extracts, compared to that of *P. pavonica*, in control and saline stress conditions. The improving behavior of germination may be explained by a metabolism change of the seed during germination. Indeed, it is reported that certain proteins are synthesized only during the priming, such as the globuline (Job et al. 2000), and the expression of certain genes encoding these proteins responsible for the energy metabolisms and defense mechanisms are activated by the priming (Groot et al. 2003).The activation of the expression of the aquaporins that ensure transport of water and then induces the acceleration and the improvement of the imbibition is also registred following the priming (Gao et al. 1999). The activation of the aquaporins expression induces an activation of the degradation enzymes of the reserves such as lipase which ensures then a better mobilization of the reserves and consequently a better germination (Sung and Chang 1993).

Regarding growth, results showed an improvement in the growth of plants resulting from seeds pretreated by both algae extracts at higher doses. By comparing our results with those obtained consequently with pretreatments of seeds by vegetable steroid hormones (Houimli et al. 2008) or those by *Crithum maritimum* (Atia et al. 2006) we note that the pretreatments of pepper seeds by the aqueous extracts of algae gave more significant results. The priming induced a reduction of NaCl inhibitory effects. This improved tolerance may be attributed to the fact that priming allows enzymatic activation level of the seeds which stimulates the plant growth (Houimli et al. 2008). Also, priming allows the reserves mobilization which induces the nutrients availability and consequently a growth acceleration (Atia et al. 2006). Likewise, it allows the plant to develop defense systems in both chloroplast (Asada 2006), peroxisomes and mithochondries in response to stress in order to reduce cell damage (Del Rio et al. 2006).

5. Conclusion

The results of this work have shown that the biological priming with the aqueous extracts of the two algal allowed having seeds and seedlings having acquired significant salt tolerance compared to control. Improved seed germination following priming, under saline condition, was confirmed by a number of germination parameters. Thus the T_{50}, the MGT were reduced and the IG has been improved compared to

the control in the majority of cases. Examination of the results shows that pretreatment with the extract of *J. rubens* was more beneficial compared to that of *P. pavonica*. This technique of biological pretreatment may submit with agriculture an interesting tool to increase plant tolerance to salt stress and to find an alternative to any chemical use.

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