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Microalgae polysaccharides a promising plant growth biostimulant

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Abstract

The present study investigates the possibility to use microalgae polysaccharides as biostimulant of plant growth. The total polysaccharides extract (TPE) from *Spirulina platensis*was applied to *Solanum lycopersium* and *Capsicum annum* plants at different growth stages by spraying. The treatment with TPE solution (3 $g.L^{-1}$) increased the plants size of tomato and pepper by 20% and 30%, respectively. While the effect of treatment with TPE on roots weight was more pronounced in tomato plants (improvement of 230%) than pepper plants (improvement of 67%)Moreover, the size and number of nodes per plantwerealso increased after the TPE treatment by 57%-100% (size - number of nodes) and 33%-50% in tomato and pepper respectively. Thereby, this is the very promising evidence of the use microalgaepolysaccharides as plant growth biostimulant.

Keywords: Microalgae, polysaccharides extract, biostimulant, plant growth

Abbreviations:
TPE: Total polysaccharides extract; MAScIR: Moroccan Foundation for Advanced Science, Innovation and Reseach; LSD: Least Significance Difference.; TLR: toll-like receptors; LRR: Leucine Rich Repeat; BKI: Brassinoid Kinase Inhibitor; BKI: Brassinoid Kinase Inhibitor

Introduction

In agriculture systems, producers are increasingly encouraged to opt for sustainable production practices. This consists to reduce or substitute the use of chemical inputs speciallyfertilizers and pesticides with natural or biological substances. For this reason,development ofnatural substancesable to promote plant growth named “plant biostimulants” is receiving increased attention. Biostimulants includecompounds, other than fertilizers, capable to promote plant growth when applied at low doses (Zhang and Schmidt., 1997) likephytohormones, vitamins, amino acids, orother substances with a similar effect.In this context, polysaccharides, specially derived from seaweeds, are among the most interesting substances due to their efficiency. Due to the great similarity in biochemical composition of micro- and macroalgae, it is interesting to investigate the possibility to use microalgae as feedstock to develop new products for plant growth improvement.The application of microalgal biomass or extracts in agriculture as biofertilizers is limited to the use of some extracts as biofertilizers by nitrogen fixation ability, oligoelementapport or increase of soil fertility (Benemann et al., 1979; Faheed et al., 2008; Priyadarshani et al., 2012).

Thanks totheir enormousbiodiversity, easeof culture andability of metabolism control, microalgae areincreasinglyusedto produce active moleculeswith different industrial applications. Severalmicroalgae strains produce active compounds such as antibiotics, algicides,toxins, pharmaceutically active compounds (Metting et al., 1986; Borowitzka et al., 1992).*Spirulina*, *Chlorella*, *Dunaliella*, *Nostoc*and *Aphanizomenon* are among the most produced microalgae worldwide for different purposes.

Algal polysaccharides are biologically active compoundswith several potential applications. Polysaccharides extracted from *Spirulina* for medical application have shown biological activities(Kulshreshthaet al., 2008; McCartyet al., 2007).Sulfated polysaccharides from *Spirulina* inhibit the proliferation of tumor cells *in vitro* (Jia et al., 2008) and *in vivo* (Akaoet al., 2009). Moreover, active polysaccharides and oligosaccharides derived from seaweeds, showed a wide range of applications in term ofgrowth stimulation and plant defense (Vera et al., 2011; Gonzalez et al., 2013).Therefore, the study of the microalgal polysaccharides potentials in agriculture seems to be promising for the development of new products for plant growth stimulation.

The aim of this study is to investigatethe plant growth promotion ability of polysaccharides extracted from microalgae.

Material and methods

Microalgae culture

The blue green algae, *Spirulinaplatensis* was isolated from Lake FoumElouad,Laayoune (South ofMorocco) and maintained in MAScIR's(Moroccan Foundation for Advanced Science, Innovation and Reseach)Microalgae Collection.*S. platensis*was cultured in 2 L photo-bioreactor containing Zarrouk's medium (Belay, 2008)and agitated by air bubbling under continuous illumination (150 ± 10 µmol m^{-2} s^{-1}) and temperature of 30°C.After 14 days total biomass was harvested by centrifugation at 5000rpm during 10 min,rinsedwith distillated water and dried.

Polysaccharides extraction

Modified method of Chaiklahan et al., 2013 was used for polysaccharides extraction. One g of *S. platensis* driedbiomass was added in 50 ml of distilled water and mixture was incubated at room temperature for 30 min before being refluxed at 90°C with stirred at 400rpm for 1h, 2h, 3h or 4h or another extraction method by autoclave (121°C, 30min).After cooling, supernatant was recuperated by centrifugation and pellet refluxed twice to increase extraction efficiency. Extracts were then combined, concentrated and cooled to 4°C.Three volumes of ethanol were added and the final resulting mixture was centrifuged and pellet (total polysaccharides extract) was lyophilized and stored at -80 ° C for future use.

Plant material and culture conditions

Two plant specieswere used in the current study,*Capsicum annuum. var. andalus*(pepper) and *Solanumlycopersicum L. Var. metro*(tomato). Seeds (purchased from "Syngenta Maroc")were surface-disinfected during 20 min in a sodium hypochlorite solution (1%)containing 10µl of Tween-20 andrinsed with sterile water. Sterile seeds were kept at dark during7 daysand seedlings were transplanted into 12 cm diameter pots containing mixture of sandy soil and peat (60:40).Cultures were carried out in glass houseat 26 °C, photoperiod of 16h/8h and illumination intensity of 240µmol m^{-2} s^{-1}. Plants were sprayed with total polysaccharides extract (TPE) preparation (3 $g.L^{-1}$of TPE dissolved in distilled water, pH 6.0) twice with 3 days intervaland non-treated plants served as control.

After 30 days,plant growth was evaluated by measuring: plant size (shoot size), plant weight (shoot and roots dry weight: 70°C at 72h), foliar area as well as number of nodes per plant. Every experience was repeated twice with 15 independent replicates/treatment.

Solanum lycopersium Leaf area is calculated using the formula de Blanco et al., 2003. LA= 0.347.(Length × Width) – 10.7. *Capsicum annum* leaf area is calculated by De swart et al., 2004. LA= 0,690. (Length × Width).

Statistical analysis

Statistical analysis was performed using SPSS 13.0 for windows. Results were statistically analyzed with a quantity called the Least Significance Difference (LSD).

Results and Discussion

Spirulina platensis ***biomass production***

Evolution of biomass accumulation in controlled conditions is represented in Fig. 1. After inoculation, a log phase was followed by an accelerated growth during the next 8 days to reach1.2$g.L^{-1}$at end of exponential growth. Several studies have shown that the concentration of biomass varied depending on the size and shape of photo-bioreactor. Oncelet al.,(2008) showed that *Spirulinaplatensis*biomass can reach 2.21 $g.L^{-1}$ on day 10 in airlift photo-bioreactor. The same evaluation was made for the bubble column PBR culture, it was observed that the maximum dry biomass 1.87 $g.L^{-1}$ on day 7 (Oncelet al., 2008).

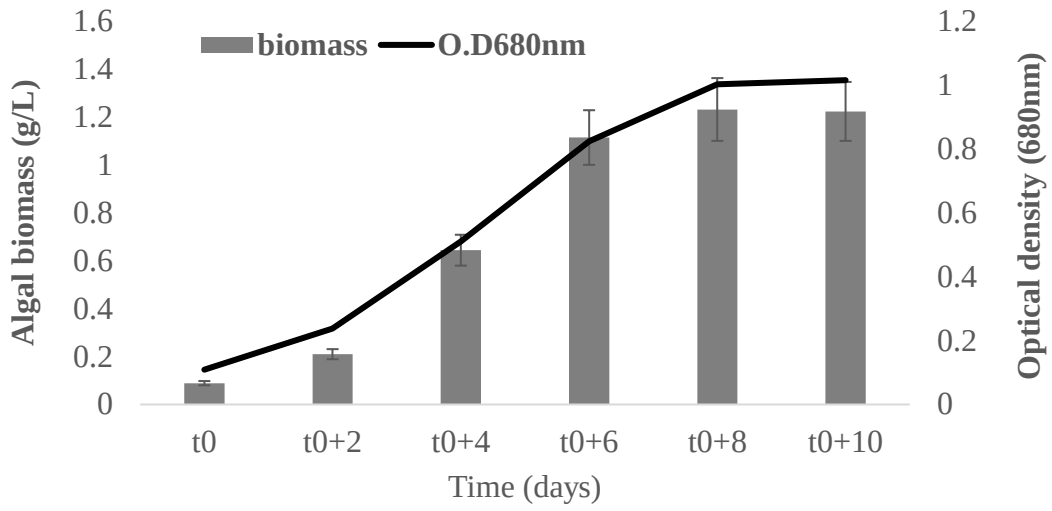

Fig.1 Growth and biomass of *SpirulinaPlatensis* grown in photobioreactor. Cultures were carried out in 2L of Zarrouk medium and optical density measured at 680 nm every two days.

Polysaccharides extraction

Extraction time and temperature plays an important role in the efficiency of polysaccharides extraction.Fig. 2 shows that, when biomass is heated at 90°C and stirred at 400 rpm,polysaccharides yield increases with increasing time of extraction.The best performance of polysaccharides extraction (5.43%/DW)was obtained after 4 h treatment.

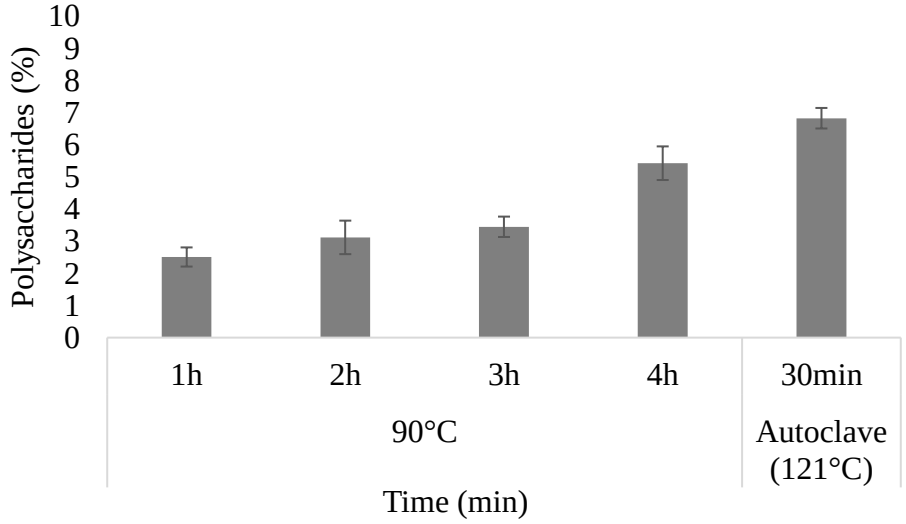

Fig. 2 Extraction methods of microalgae polysaccharides: hot-water extractionat 90°C stirred in 400rpm for 1h, 2h, 3h and 4h.and extraction assisted by autoclave *121°C 30min*

Compared to first extraction method,polysaccharides extraction assisted by autoclaving (121°C 20min) revealed a higher efficiency (6.83%/DW)and shortest time running (fig. 2).Others studies showed that the yield of the

polysaccharides was close to the peak value when extraction time was 4 h. After this point, the yield of the polysaccharides started to decrease with increasing the extraction time (Weirong et al., 2008).The temperature had a significant impact on polysaccharides rate derived from solid-liquid extraction of S*pirulina*. The polysaccharide content was significantly higher when the extraction was performed at 90°C than that performed at 80, 70, and 50°C (Chaiklahan et al., 2013).

Microalgae polysaccharides as biostimulant of plant growth

We investigated the effect of total *S. platensis* polysaccharides extract (TPE) on plant growth. Results presented in Fig.3 shows that the use of *S. platensis* TPE improved significantly the growth of tomato and pepper plants. Indeed, in both genres, TPE treatment increased plant size by 20 to 30% in comparison with non-treated plants. The effect of TPE treatment on plant weight was more pronounced since it increased shoot dry weight by 140% in both genres (Fig.4). *S. platensis* TPE have also a positive effect on shoot and root dry weight. Tomato plants were more responsive to TPE treatment than pepper plants (230% vs 67% respectively).Leaves traits (number, size, aspect, etc.) are important indicators of plant health. The treatment with TPE increased leaf size measured as foliar area (cm^2) by 57 and 100% in pepper and tomato plants respectively in comparison with non-treated plants (Fig.5). The same result was obtained for leaves number that was also improved by 33 and 50% for pepper and tomato respectively (Fig.6).

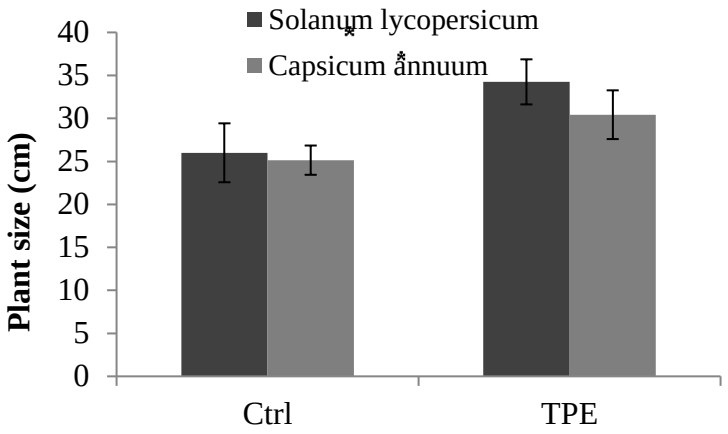

Fig. 3 Effect of *S. Platensis* polysaccharides on the plant size of *Solanumlycopersium* and *Capsicum annum* plants. Plant treatment with 0.5 g.L^{-1}of total algal polysaccharides showed a significant effect on the size of the two plants according to the statistical study based on SPSS 13.Ofor windows. *significant difference P≤0, 05

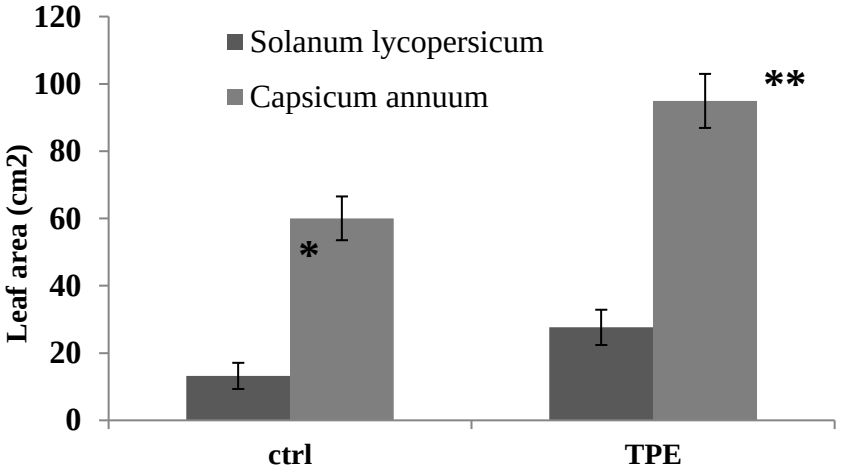

Fig. 5 Effect of A*. Platensis* polysaccharides in leaf area of *Solanumlycopersium* and *Capsicum annum* plants. Plant treatment with 0.5 g.L^{-1}of total algal polysaccharides showed a highly significant effect on leaf area of *Capsicum annum* and significant effect of *Solanumlycopersium*. According to the statistical study based on SPSS 13 data. ***** Significant difference P≤0, 05, **** ,**Highly significant difference P≤0, 01.

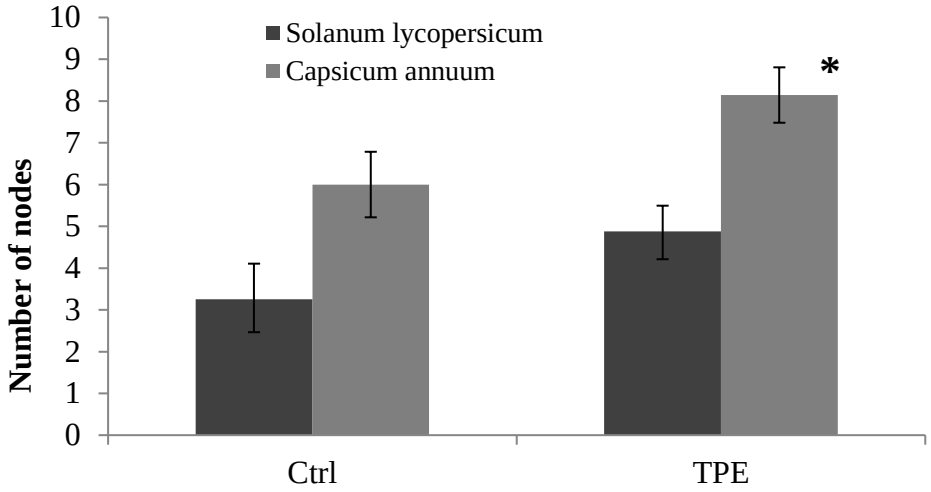

Fig. 6 Effect of A*. Platensis* polysaccharides in number of nodes of *Solanumlycopersium* and *Capsicum annum* plants. Plant treatment with 0.5 $g.L^{-1}$of total algal polysaccharides by spraying showed a significant effect on leaf area of *Capsicum annum* according to the statistical study based on SPSS 13.0 for windows. ***** Significant difference P≤0, 05

The growth stimulation effect of polysaccharides extracted from seaweeds has been previously demonstrated(Chojnacka et al., 2012).For microalgaeprevious works report generally the use of whole biomass for fertilization and plant growth stimulation. *S. platensis* is among the most studied and cultivated microalgae and the use of biomass as biofertilizeror plant growth stimulator was reported in some studies (Aly and Esawy 2008; Bhowmik et al., 2010). Generally,the ability of *S. platensis*total biomass to stimulate plant growth could be explained by the nitrogen fixation ability of this microalgae, beside its elevated vitamins and hormones content (Priyadarshani and Rath., 2012), and according to our results, it could be also explained by their polysaccharides activity.

In current study, an interesting effect of polysaccharide extracted from S. *platensis*on plant growth promotion was clearly demonstrated in term of plant weight, plant size, and leaves number and size. Application of the polysaccharide extract increased the plant biomass of *Capsicum annuum*and *Solanumlycopersicum*plants with 142% and 139% respectively. Otherwise, the morphological aspect of treated plants was also improved as shown in the (Fig.7). Bi et al., (2011) have reported the same effect of seaweed polysaccharide *k*-carrageenan on various growth characters of chickpea and maize plants. In fact, the application of k-carrageenan around the seeds or by foliar spraying increased significantly the plant height, number of leaves, number of pods and branches of chickpea. This treatment increased also the plant height and the stem diameters for maize plants (Bi et al., 2011).

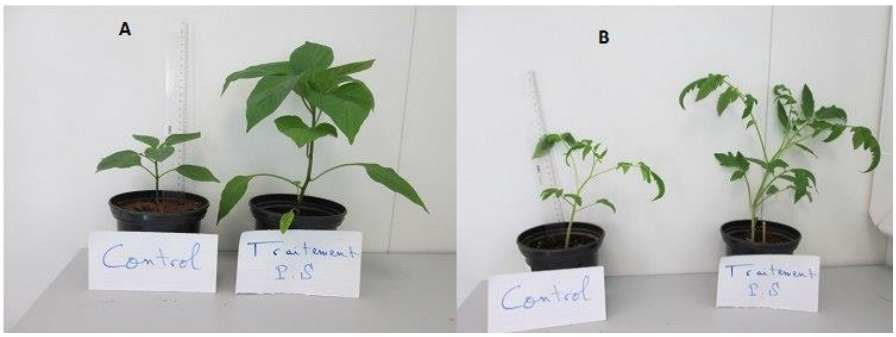

Fig. 7 Effect of treatment with 0.5 g / L of total algal polysaccharides by spraying on growth during 7 days post-treatments of *Capsicum annum* (A) and *Solanum lycopersicum* (B) plants.

The mechanism by which microalgal polysaccharides improve plant growth remains unclear and merits further investigation. It has been shown that in animal cells,toll-like receptors TLR2 and TLR4 areactivated by alginate treatment. In fact, the interaction between oligosaccharides and plants TLR induced cytokine productionwhich is responsible for several cell signaling pathways and cellular communication(Flo et al., 2002; Iwamoto et al., 2005). In plant plasma membrane there isa wide range of LRR receptors (Leucine Rich Repeat) that have similar interaction with polysaccharides. Among the most studied receptors in relation withplant growth regulationand containing LRR,BRI1-associated receptor kinase (BAK1) activates signal transduction on plant growth regulation (Gonzalez et al, 2013, Yin et al, 2002). Treatment of seedlings with Brassinosteroid, BRI1 phosphorylates the negative regulator Brassinoid Kinase Inhibitor 1 (BKI1) , which causes its displacement from the membrane to the cytosol where it is inactive.The LRR-RK co-receptor (BAK1) can then associate with BRI1 to form a complex that elevates the signaling output of the pathway phosphorylation/ dephosphorylation cascade leading to the transcriptional regulation of hundreds of genes and, ultimately, coordinated cell expansion (Belkhadiret al.,2014).Stimulation of plant growth by polysaccharides investigated in this study could be explained by the involvement of such receptor. We have no evidence; but light should be shed on these mechanisms in order to understand this regulation.

Conclusion

Microalgae polysaccharides showed a good capacity to improve plant growth and this have an interesting potential to be used as biostimulant. The present work offers new possible use of microalgae in agriculture. Further studies are needed to understand the mechanism by which plant growth improvement occurs and to optimize the culture conditions and maximize polysaccharide production. Other beneficial effects of microalgae polysaccharides might also be investigated especially in the field of agriculture.

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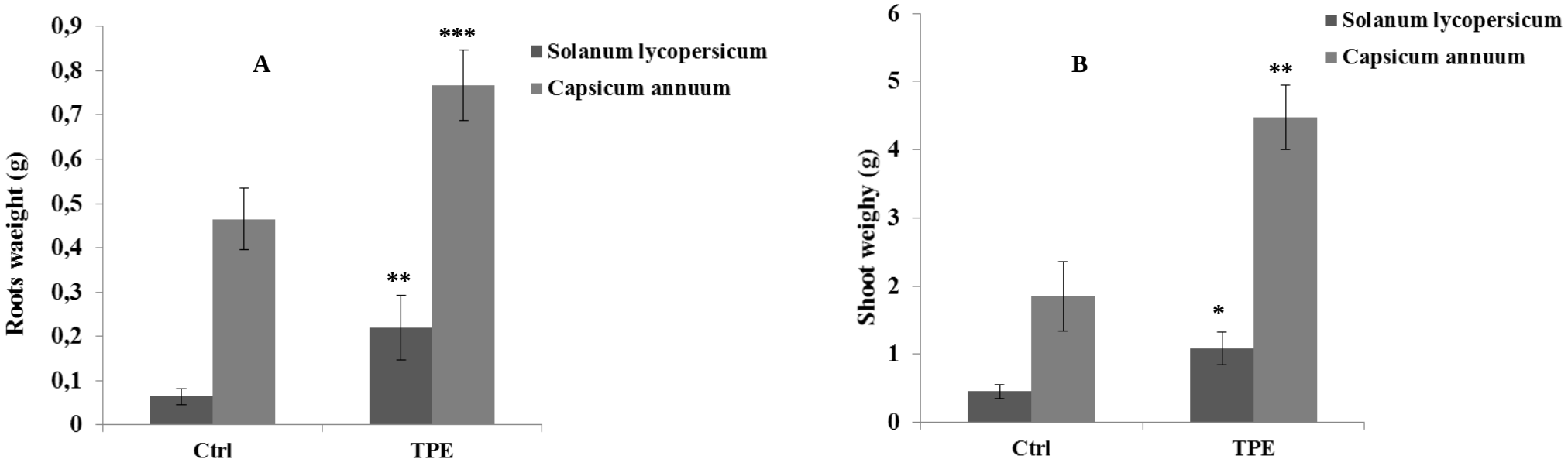

Fig. 4 Effect of A. *platensis* polysaccharides on the weight of *Solanumlycopersium* and *Capsicum annum* plants: (A) root weight: treatment of 3 $g.L^{-1}$of total algal polysaccharides showed a highly significant effect on the weight of the roots of *Capsicum annum* and a very highly significant effect on root weight of *Solanumlycopersium.* (B) Shoot weight: treatment of 0.5 $g.L^{-1}$of total algal polysaccharides showed a highly significant effect on the shoot weight of *Capsicum annum* and a significant effect on the shoot weight of *Solanumlycopersium*. Statistical study based on SPSS 13.0 for windows. ****** Highly significant difference P≤0, 01, ******* Very highly significant difference P≤0, 001