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RESPONSE OF SESAME SEEDLINGS TO DIFFERENT CONCENTRATIONS OF HUMIC ACIDS OR CALCIUM NITRATE AT GERMINATION AND EARLY GROWTH

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ABSTRACT. This study was conducted in order to evaluate the response of sesame seeds (*Sesamum indicum* L.) to organic or mineral fertilization. The effects of two fertilizers, which were calcium nitrate and humic acids were studied separately at germination and early seedlings growth. Different concentrations of humic acids (HA_0: 0, HA_1: 500, HA_2: 1000 and HA_3: 2000 mg L^{-1}) or calcium nitrate (CaN_0: 0, CaN_1: 50, CaN_2: 100 and CaN_3: 200 mg L^{-1}) were applied distinctly to the growth media. The experimental design was accomplished in a completely randomized block design with three replications. Germination measurements, seedlings length, fresh seedling weight, total chlorophylls and carotenoids, total soluble proteins and sugars were determined. Results showed that humic acids applied at 1000 mg L^{-1} or calcium nitrate applied at 100 mg L^{-1} reduced the mean germination time (3.5 and 3.61 days) and had the highest germination index (GI) and the highest coefficient of velocity (CV). The longest seedling was obtained at the concentrations HA_2 and CaN_2 (+22%). Total chlorophyll and carotenoids were significantly higher in seedlings receiving the HA_2 and CaN_2 and these treatments increased total soluble proteins content by 32%. Higher concentrations of humic acids or calcium nitrate HA_3 and CaN_3 delayed germination and enhanced proline and total soluble sugars, respectively, by 42% and 46%, compared to control. These preliminary results indicated that the use of fertilizers should be optimized and they can be transformed at high level to an abiotic stress menacing plant productivity. On the other hand, suitable concentrations of fertilizers can be used in the future as a remedy to improve growth under abiotic stress.

Keywords: humic acids; calcium nitrate; proline; soluble sugars; soluble proteins.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an important oil crop, rich in marketable products, such as seeds and seed oil. This plant has been

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grown in tropical regions throughout the world since prehistoric times. The world production of sesame seed progressively increased due to an increasing demand for sesame oil worldwide. In fact, sesame oil is highly stable and rich in unsaturated fatty acids (Carvalho *et al.,* 2012). Consequently, sesame oil ranks sixth in the world among vegetable oils and Myanmar becomes the first seed producer of sesame in the world, followed by India. Sesame is considered as an important cash crop. So, it is cultivated in many small and marginal farmers in developing countries, due to its quite adaptability to different agro-environments and its great diversity. Although having an economic importance, the productivity of sesame is still low, due to poor soil fertility and imbalanced nutrition (Engoru and Bashaasha, 2001).

Soil quality depends on organic and mineral fertilization, which improve its chemical, physiological, and biological properties (Khaled and Fawy, 2011). Humic substances, as humin, humic and fulvic acids, are the principal organic soil compounds. These substances are used as stimulators to compensate the deficiency of organic matter in the soil and can build complex with various micronutrients to form chelates (Turkmen *et al.,* 2004). Indeed, humic acids make complex with sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), calcium (Ca), iron (Fe) and copper (Cu) to overcome ions deficiency in the soil (Aiken *et al.,* 1985). These complex forms inhibit the crystallization of mineral elements, which could be converted, in the absence of humic substances, to insoluble precipitates such as metal carbonates, oxides, sulfides and hydroxides. Also, humic acids promote the biological activity of microorganisms in the soil and serve as catalysts (Sharif *et al.,* 2002). An accurate measurement of the organic matter content of the soil would be helpful in controlling soil fertility. So, different doses were investigated to increase yields of corn, oats, soybeans and tropical crops (Hayes and Wilson, 1997).

Furthermore, calcium nitrate is one of the most preferred inorganic fertilizer sources especially when both calcium and nitrogen are required to be supplied at the same time. Calcium nitrate is highly soluble and more effective than calcium chloride, calcium sulphate and lime in increasing calcium concentration in the soil (Ahmad *et al.,* 2009). Compared to other fertilizers, calcium nitrate releases less N_2O, as compared to ammonium sulphate or urea, for the same amount of nitrogen applied. This is an important point to avoid nitrogen loss (Breitenbeck *et al.,* 1980). Calcium is always considered as one of the most important elements in soil and as a determinant of plant growth and production. It contributes to the maintenance of cell membrane stability and structure (Ozgen *et al.,* 2003). On the other hand, nitrate is often considered as the major source of nitrogen (N), which is available to higher plants. Nitrogen nutrient is still one of the major factors limiting crop yield (Marschner, 1995). Therefore, the efficient use of nitrogen as a fertilizer is an essential management to limit the increasing cost of N fertilizer and to minimize the environmental problems, which are relied to its uses, especially the loss of water quality (Jaynes *et al.,* 2001). The aim of this research is to investigate the effect of two fertilizers (organic and inorganic) in sesame seedling's growth. We investigate the morphological and biochemical responses of seeds to different calcium nitrate or humic acids concentrations during germination and early growth stage.

MATERIAL AND METHODS

Four solutions containing humic acids (HA_0: 0, HA_1: 500, HA_2: 1000 and HA_3: 2000 mg L^{-1}) were prepared by adding a commercial HA substance, 100 % water soluble, Powhumus WSG 85 (Humintech Co., Germany.) to distilled water. Four solutions of calcium nitrate (CaN_0: 0, CaN_1: 50, CaN_2: 100 and CaN_3: 200 mg L^{-1}) were prepared by dissolving Ca $(NO_3)_2$ in distilled water. HA_0 and CaN_0 were used as control. 30 seeds of sesame (*Sesamum indicum* L.) from Tunisian cultivar were sown in 9 cm Petri dishes containing two layers of filter paper and imbibed separately with 5 mL of every prepared solution for 7 days. Only seeds without visible defects were selected. Treatments, including control tests, involved three replications. Each Petri dish was considered as a replicate. Seeds were kept to germinate in laboratory under normal light intensity and controlled temperature (25°C) and the appearance of 2 mm or more of radicle was considered as a criterion for germination.

Parameters measured in this experiment were: total germination (TG) measured on the seventh day using the formula TG (%) = total number of germinated seeds/ total seed x 100; mean germination time (MGT, days), calculated according the formula of Ellis and Roberts (1981); germination index (GI) and coefficient of velocity (CV), calculated according to the equation of Kader and Jutzi (2004); seedling length (SL, cm), measured with a graduated ruler; seedling fresh weight (SFW) and seedling dry weight (SDW). Seedling dry weight was obtained after placing seedlings, after the 7th day, in an oven to dry at 70°C for 48 h.

Total chlorophylls and carotenoids content were calculated from cotyledons, according to Shabala *et al.* (1998) and results were expressed as µg g^{-1} FW. Proline was extracted with toluene, according to Bates *et al.* (1973), and absorbance was read at 520 nm by a spectrophotometer (T60 UV/VIS, Oasis Scientific Inc., Taylors, SC). Results were expressed as µg proline g^{-1} FW.

Total soluble proteins in cotyledons were estimated according to Bradford (1976), by measuring the absorbance at 595 nm and results were expressed as µg g^{-1} FW. Total soluble sugars were determined by the phenol sulfuric acid method (Dubois *et al.,* 1956), using glucose as a standard, and the obtained results were expressed as µg g^{-1} FW. Every experiment was carried out in triplicate.

The experimental design was accomplished in a completely randomized block design, with three replications. All results contained the mean values of duplicate analyses from at least three individual seedlings. All data were subjected to a one-way analysis of variance (ANOVA), using SPSS program (ver. 11.0, SPSS, Chicago, IL) and the differences between the means were compared using Fisher's Least Significant Difference (LSD) test. Comparisons with $P< 0.05$ were considered significantly different. In all the figures the spread of values is shown as error bars representing standard errors of the means.

RESULTS

The results reported in *Table 1* showed that humic acids (HA) or calcium nitrate (CaN) concentrations reduced significantly seed germination, respectively at 2000 and 200 mg L^{-1}. In fact, the lowest germination percentages resulted from HA_3 and CaN_3, respectively, were 75 and 62%. Concentrations less than 1000 mg L^{-1} and less than 100 mg L^{-1} for humic acids and calcium nitrate, respectively, had no significant effect in mean germination time (MGT) (*Table 1*). The shortest MGT (3.5 and 3.61 days) were obtained, respectively, for HA_2 and CaN_2. Data in *Table 1* indicated that increasing concentrations to 2000 and 200 mg L^{-1} significantly delayed germination speed, for both humic acids and calcium nitrate. Also, the highest germination index (GI) and the highest coefficient of velocity (CV) were obtained for HA_2 and CaN_2. The longest seedling was obtained at the concentrations HA_2 and CaN_2, which stimulated length by 22%, comparatively to HA_0 and CaN_0 (*Table* 1). Although there was no statistically significant difference between HA_0 and HA_3 for humic acids levels, calcium nitrate concentration CaN_3 reduced seedling length by 24%, comparatively to CaN_0. Humic acids and calcium nitrate concentrations significantly affected seedling fresh weight (SFW) and seedling dry weight (SDW). The highest fresh and dry weights were achieved at HA_2 and CaN_2. The lowest fresh and dry weights resulted from the HA_3 and CaN_3 levels (*Table 1*).

Total chlorophylls, carotenoids and proline content of seedlings were significantly influenced by the concentrations of humic acids or calcium nitrate (*Table 2*). Total chlorophylls and carotenoids were significantly higher in seedlings receiving the HA_2 and CaN_2 concentrations. The application of HA_3 and CaN_3 reduced photosynthetic pigments by 10%, comparatively to HA_0 and CaN_0. Reduction in carotenoids was more important and it was, respectively, 28% and 41% for HA_3 and CaN_3. The proline contents were 2-fold higher for HA_3 and CaN_3 (*Table 2*).

Humic acids and calcium nitrate application significantly increased total proteins content for HA_2 and CaN_2 by 32%, comparatively to HA_0 and CaN_0 (*Fig. 1*). While the highest proteins content (20, 5 and 19.5 μg g^{-1} FW) were determined in HA_2 and CaN_2 concentrations, the lowest protein content (15 and 10 μg g^{-1} FW) were found in HA_3 and CaN_3. Similarly, HA_3 and CaN_3 significantly increased total soluble sugars (*Fig. 2*), respectively by 42 % and 46%, comparatively with HA_0 and CaN_0. Soluble sugars content was stable for HA_2 and CaN_2 concentrations (120 and 125 µg g^{-1} FW), comparatively to HA_0 and CaN_0 (120 and 123 µg g^{-1} FW).

Table 1 - Effect of different concentrations of humic acids and calcium nitrate on germination parameters of sesame seeds

Seedling parameters	Humic acids (mg L^{-1})				Calcium nitrate (mg L^{-1})			
	0	500	1000	2000	0	50	100	200
TG, %	98 ±3.7a	99 ±3.5a	99 ±2a	75 ±3b	98 ±3.5a	98 ±3.1a	99 ±2.5a	62 ±2b
MGT, days	4.2 ±0.16b	4.1 ±0.14b	3.5 ±0.17c	5.3 ±0.11a	4.05 ±0.15b	4.11 ±0.1b	3.61 ±0.18c	5.7 ±0.1a
GI	18.5 ±0.3b	18.3 ±0.45b	20.5 ±0.5a	15 ±0.3c	18.7 ±0.45b	18.5 ±0.25b	19.7 ±0.35a	15.5 ±0.3c
CV	24.5 ±0.63b	24.3 ±0.9b	26 ±0.75a	18.5 ±0.66c	24.6 ±0.5b	24.5 ±0.9b	27.2 ±0.85a	18 ±0.8c
SL, cm	4.7 ±0.5b	4.8 ±0.7b	5.7 ±0.3 a	4.7 ±0.2b	4.6 ±0.4b	4.8 ±0.2b	5.6 ±0.2a	3.5 ±0.3c
SFW, mg	280 ±1.2c	285 ±1.1b	300 ±1a	250 ±1.3d	283 ±1.4b	282 ±1.2b	307 ±1.5a	242 ±1.6c
SDW, mg	92 ±1b	93 ±1.5b	97 ±1.2a	90 ±1.4c	93 ±1.2b	93.5 ±1.3b	101 ±1.2a	87.6 ±1.3c

Means (± SE) within a column, followed by the same letter are not significantly different at the 0.05 level of probability (LSD test).

Table 2 - Effect of different concentrations of humic acids and calcium nitrate on total chlorophyll, carotenoids and proline content of sesame seedlings

Seedling parameters (µg g^{-1} FW)	Humic acids (mg L^{-1})				Calcium nitrate (mg L^{-1})			
	0	500	1000	2000	0	50	100	200
Total chlorophyll	308.5 ±5.4b	306 ± 4.5b	325 ± 3.2a	275 ± 4.6c	298.4 ±3.5b	297 ±4.1b	317 ±6.3a	271 ±5.3c
Carotenoids	34.2 ±3.5a	34 ± 4.5a	33.7 ± 2.5a	24.6 ± 4.1b	24.5 ±5a	24.78 ±2.5a	23.61 ±2 a	14.4 ±2 b
Proline	5.8 ±2.3c	8.3 ±1.75b	10.2 ±1.5a	10.4 ±2.2a	8.6 ±1.5b	8.4 ±1.25b	15.7 ±2. 5a	16.5 ±2.6a

Means (± SE) within a column, followed by the same letter are not significantly different at the 0.05 level of probability (LSD test).

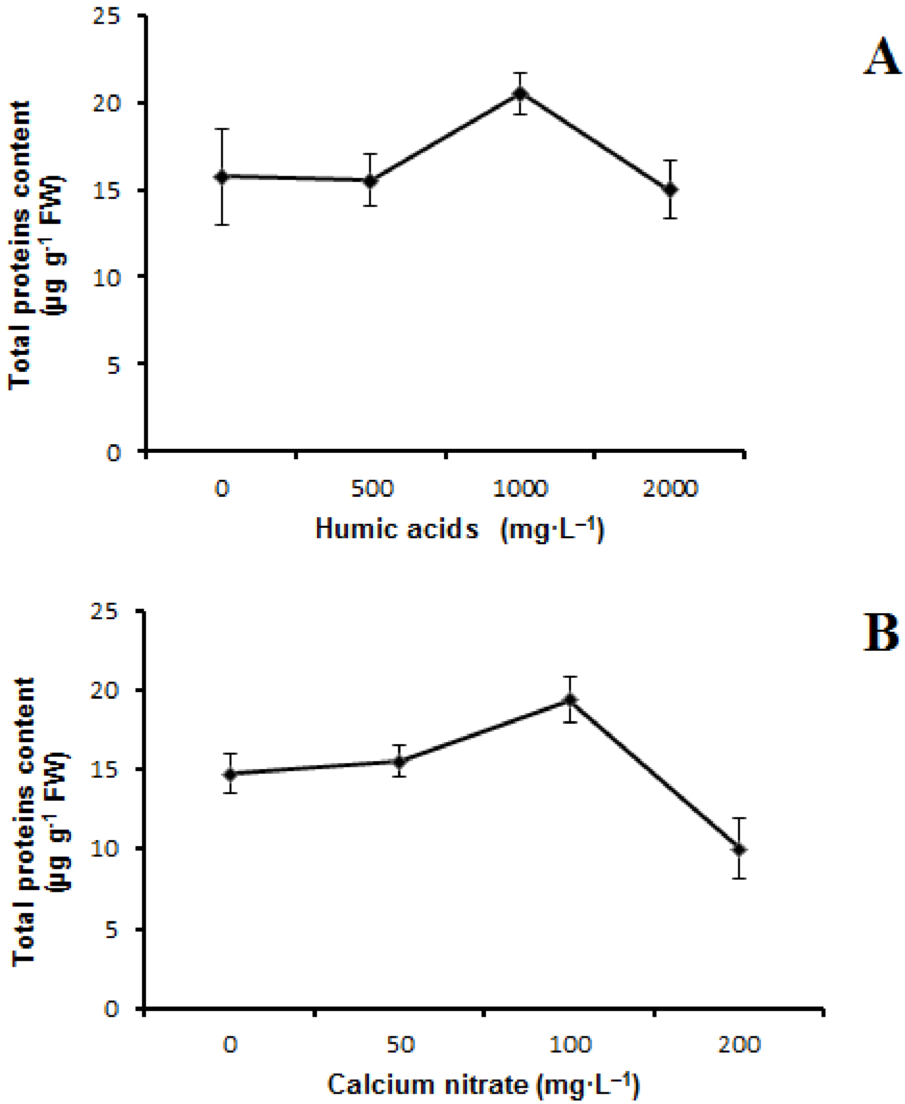
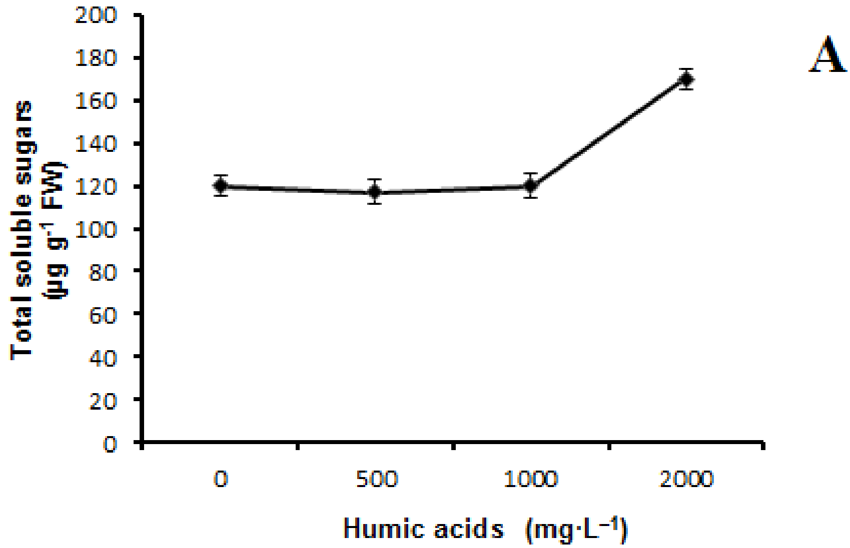


Figure 1 - Effect of different concentrations of humic acids (A) and calcium nitrate (B) on total soluble proteins content of sesame seedlings



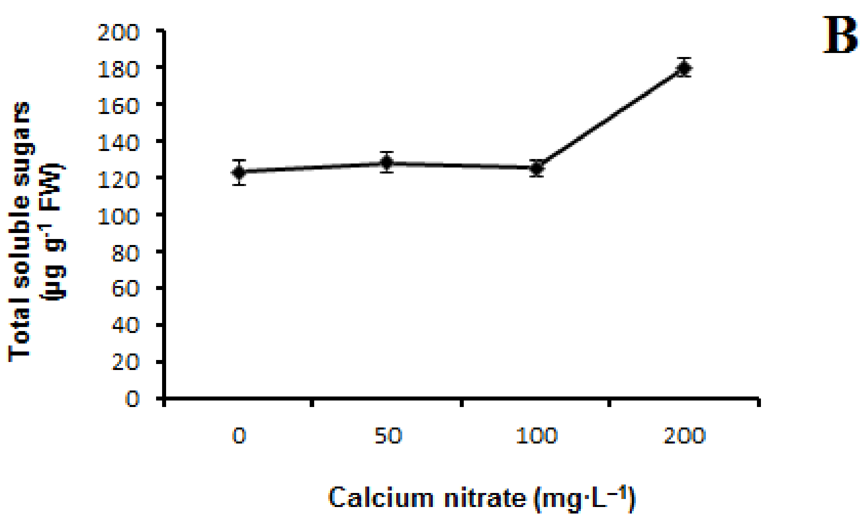


Figure 2 - Effect of different concentrations of humic acids (A) and calcium nitrate (B) on total soluble sugars content of sesame seedlings

DISCUSSION

The effects of humic acids and calcium nitrate on germination and seedlings growth were variables and depends on the concentration of the medium. The application of high concentrations of humic acids or calcium nitrate was less effective. In fact, positive effects of HA_2 (1000 mg L^{-1}) or CaN_2 (100 mg L^{-1}) were significant and ameliorated germination and early seedling growth parameters. On the other hand, the concentration HA_3 (2000 mg L^{-1}) of humic acids or CaN_3 (200 mg L^{-1}) of calcium nitrate had negative effects on seedling growth criteria of sesame seedling.

Many previous studies showed that humic acids stimulates germination parameters, such as germination percentage and mean germination time of various crop species (Piccolo *et al.,* 1993). Besides, calcium nitrate ameliorates germination indirectly by the action of Ca^{2+} ions. Low concentrations of Ca^{2+} are known as inorganic stimulators to seed germination. It is reported that Ca^{2+} ions stimulate water uptake and alleviate internal water conductance in membrane. As a consequence, these Ca^{2+} ions enhance cotyledon expansion (Leopold *et al.,* 1974) and increase seedling growth (Cramer, 1992). Fertilizers which contain calcium are required also to maintain the integrity of the cell membrane because calcium is important for the selectivity and the transport of K^+ ions across the cells (Tian *et al.,* 2015).

However, high concentrations of $Ca(NO_3)_2$ decreased seedling growth criteria. This can be the result of a salt stress. In fact, calcium nitrate is considered as an inorganic salt fertilizer and high concentrations can affect seedlings growth and delayed germination, especially with salt sensitive species. Similarly, it was reported that high concentrations of humic acids can damage membrane permeability and leads to water stress and, eventually, to the inhibition of some enzyme activities (Eyheraguibel *et al.,* 2008).

The longest seedlings were obtained at the concentrations HA_2 (1000 mg L^{-1}) or CaN_2 (100 mg L^{-1}), which were the optimum concentrations. Results from previous research demonstrated that humic acids increases length in maize seedlings (Eyheraguibel *et al.,* 2008). Also, Kauser and Azam (1985) indicated that the application of 54 mg L^{-1} of humic acids increased radicle length by 50% and stimulated dry matter with 22% in wheat. The increase in seedling length can be attributed to an increased cell division and to cell elongation. In fact, Zhang and Ervin (2004) showed that humic acids have cytokinin activity and Pizzeghello *et al.* (2001) found auxin-like and gibberellin-like activity in the plants treated with humic acids.

The increase in fresh and dry weights of sesame seedlings can be due to indirect effects of humic acids on seed reserve mobilization. In many studies, humic acids were reported to increase the uptake of mineral elements and to increase the fresh and dry weights of crop plants (Canellas *et al.,* 2002; Chen *et al.,* 2004). On the other hand, Gulser *et al.* (2010) demonstrated that calcium nitrate ameliorates shoot fresh and dry weights at 50 mg kg^{-1} in pepper seedlings.

Humic acids were reported to increase water uptake through plant membranes by the intervention in the cycle of cellular respiration or by stimulating some regulators proteins and enzymes activities (Nardi *et al.,* 2002; Piccolo *et al.,* 1992; Pizzeghello *et al.,* 2002). The chlorophylls and carotenoids content decreased at a significant high level of HA_3 and CaN_3. Probably, this decline is the result of a higher concentration of toxic ions and a reduction of water uptake by seeds. In fact, alteration in permeability of biological membranes under elevated humic acids concentrations were indicated by Samson and Visser (1989). Beneficial effects of Ca^{2+} on chlorophyll and carotenoids synthesis has been reported by many researches. In fact, it has been reported that the application of a moderate concentration of exogenous Ca^{2+} controls stomatal conductance, improves seedlings photosynthesis and stimulates Ca^{2+} dependent protein kinases (Naeem *et al.,* 2009; Zhang *et al.,* 2014). But some researches indicate that these beneficial effects disappear if Ca^{2+} supply exceeds critical concentration (Vaghela *et al.,* 2010).

The application of humic acids or calcium nitrate at concentrations 1000 and 100 mg L^{-1} stimulated total proteins content. These results are in accordance with previous studies, which concluded that humic acids increases nucleic acid and protein synthesis, stimulates mitotic activity of embryo and provides cell membrane stabilization (Travisan *et al.,* 2010; Garcia *et al.,* 2012). Calcium had an ameliorative effect on proteins synthesis by modulating overall metabolism and by controlling protein transport. Also, Ca^{2+} is required for the activation of Calmodulin (CaM). Once bound to Ca^{2+}, Calmodulin acts as a messenger protein, which interacts with various target enzymes, such as kinases or phosphatases. Previous works demonstrated that Ca^{2+} has an important role in regulating phosphorylation at threonine and serine residues in germination (St. Leger *et al.,* 1989). Higher concentration of humic acids or calcium nitrate at HA_3 and CaN_3 decreased total content of soluble proteins. This can be the result of an inhibition of their synthesis. Also, it was reported that a pronounced inhibition of the activity of photosystem II (PSII) may affect gene transcription in protein synthesis (Allakhverdiev and Murata, 2004).

Humic acids increased total soluble sugars and proline content at the highest concentration HA_3 and CaN_3 (2000 mg L^{-1}). These results indicate that at HA_3 and CaN_3 concentrations, sesame seedlings procured a water stress. In fact, osmotic adjustment through the accumulation of cellular solutes, such as proline and soluble sugars, has been suggested as one of the possible mechanisms for overcoming osmotic stress caused by water deficit (Caballero *et al.,* 2005). Therefore, sesame seedlings must synthesize soluble sugars and proline to survive and to make its osmoregulation.

Results showed that higher concentrations than 1000 mg L^{-1} for humic acids are not suitable for sesame seedlings growth. The same results were obtained by Lulakis and Petsas (1995) working with humic acids. Some previous work used different humic acids concentrations, which were up to 500 mg L^{-1} (Ferrara *et al.,* 2001), up to 2 000 mg L^{-1} (Turkmen *et al.,* 2004), or even up to 5 000 mg L^{-1} (Hartwigsen and Evans, 2000). The choice of the concentration plays a crucial role in controlling growth. In other research, whereas humic substances extracted from compost ameliorated tomato seedling growth at concentrations of 100-300 mg L^{-1}, it had an inhibitory effect at all concentrations, which were higher than 1000 mg L^{-1}. In our work, the application of the calcium nitrate as a fertilizer ameliorated the seedling parameters until the concentration of 100 mg L^{-1}. Higher concentrations of calcium nitrate affected seedling growth. The application of this fertilizer to the soil probably needs to be restricted. Similar observations have been reported previously by Garcia *et al.* (1999) and Maust *et al.* (1994). These authors suggested that high N concentration in the root atmosphere may reduce growth.

CONCLUSIONS

Preliminary results from the present research indicate that the application of humic acids or calcium nitrate, at suitable concentrations, may promote germination and seedlings growth of sesame, compared to control. However, the stimulation of growth depends on the applied concentration of humic acids or calcium nitrate. Ideal concentrations can be used in the future as a remedy to correct salinity-induced nutritional disorders. The addition of supplemental nitrate calcium or humic acids concentrations during germination can be a strategy for overcoming the negative impact of high salinity in the soil. Future research will study the effect of humic acids and calcium nitrate in alleviating the damages induced by salt stress (NaCl).

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