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INFLUENCE OF HUMATES TO MITIGATE NaCl-INDUCED ADVERSE EFFECTS ON *OCIMUM BASILICUM* L.: RELATIVE WATER CONTENT AND PHOTOSYNTHETIC PIGMENTS

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Abstract

Aqueous extracts of humic substances constitute one of the alternatives in the group of products used in sustainable agriculture. They are fundamentally obtained from recyclable organic sources, such as compost and vermicompost. The objectives of this study were 1) to define the salinity tolerance of two sweet basil varieties submitted to NaCl-stress; 2) to evaluate the effect of humates as mitigator of NaCl-induce adverse effects and 3) to test the criteria that leaf relative water content (LRWC) and photosynthetic pigments are accepted as salinity tolerance indicators. The plants were subjected to three NaCl concentrations (0, 50, 100 mM) and one dilution (1/60 v/v) of humates isolated from vermicompost and a control (distilled water) in a completely randomized design with factorial arrangement with six replications. The study was developed under shade-enclosure conditions. The results showed that there is a differential response among varieties with respect LRWC and chlorophyll content. Napoletano was most NaCl tolerant than Sweet Genovese. The LRWC and chlorophyll content perhaps used as tolerance indicators, while defining the NaCl tolerance of sweet basil varieties. The capacity of humates isolated from vermicompost to mitigate NaCl-induced adverse effects in basil development has been proved, when improve some physiological indicators like LRWC and chlorophyll. The discussion of the differential response among basil varieties subjected to different NaCl concentrations and humates isolated from vermicompost is addressed.

Key words: Bio-stimulant; NaCl; Stress; Physiological variables; Aromatic herbs.

Introduction

One of the main problems faced by agriculture worldwide is salinity (Chen *et al*., 2008) because it affects plant functions (Hoque *et al*., 2008). In arid and semi-arid areas, salinity is one of the main environmental issues reducing plant production (Tester & Davenport, 2003). Under soil salinity conditions, plant growth is affected as a result of abnormal biochemical and physiological processes of the plant, and water mobility decreases limiting its availability (González & Ramírez, 1999); therefore, the use efficiency of water results in a decrease of agricultural production. Hence, the study of variables that characterize water regime of plants is imperative since the total amount of water available and the efficient use determine crop yield. A plant able to acquire more water or with higher water use efficiency will be able to better withstand drought (Siddique *et al*., 2000). According to Shmatko *et al*., (2003) in studies related to plant stress, in particular drought stress, it is imperative to study variables related to water regime, but plant pigments should also be evaluated since are especially sensitive products formed by plants to cope with stressful environmental conditions. Ever more stressful climate conditions have motivated the search for alternatives to sustainable organic nutrition, not only for production but also for improving and preserving the environment. One of the most widespread alternatives is the use of bio-stimulants for plant growth (Stark, 1992). In the last two decades several bio-stimulants have been used in agriculture, which have helped to reduce plant stress to adverse environmental conditions, increasing plant growth and development and also increasing agricultural output (Velazco & Fernández, 2002). There are humic substances, which according to Aydin *et al*., (2012) influence salinity resistance in bean plants. Furthermore, Calderín-García *et al.,* (2012) reported that different doses of humates could ameliorate negative impacts in plants submitted to water stress conditions.

The positive effects of humic substances on plant development and growth highlight their assertive influence with ion transport, making absorption easier and improving membrane permeability. This effect improves metabolic processes such as respiration, protein synthesis and photosynthesis increasing or decreasing activity of various enzymes and hormones, reflected in growth indicators and some biochemical-physiological processes (Muscolo *et al*., 2007; Machado *et al*., 2009). Basil (*Ocimum basilicum* L.) it is especially valuable as aromatic herb species because of their use in the food industry as flavoring and/or seasoning; as a stimulant, antispasmodic, and/or as an anti-alopecic in pharmacy and for specific coloring, scents in cosmetics or in the perfume industry (Klimánková *et al*., 2008). In this study, basil was chosen from saline arid environments, such as those found in the Sonora desert, it is a promising crop for the agro industrial sector. However, crops are affected by salinity, thus, it is imperative to evaluate salinity tolerance varieties. Based on the aforementioned, it is needed to understand how this type of cultivation performs with bio-stimulants in these environments since they mitigate salinity effects in respect to the relative water content and photosynthetic pigments. On the other hand, has been definite that plants with higher chlorophyll submitted to NaCl-stress are most tolerant to NaCl, therefore, the accumulation of chlorophyll contents in sweet basil under NaCl-stress could be suggested as significant physiological indicators of NaCl tolerance. Leaf relative water content has been widely used as salinity tolerant indicator, because of tolerant genotypes improve their water use efficiency in stressful condition. The objectives of this study were 1) to define the salinity tolerance of two sweet basil varieties submitted to NaCl-stress; 2) to evaluate the effect of humates as mitigator NaCl-induce adverse effects and 3) to test the criteria that leaf relative water content and photosynthetic pigments are accepted as salinity tolerance indicators.

Materials and Methods

Study area: The experiment was carried out in La Paz, Baja California Sur, Mexico (24°08′ 09.73” N, 110°25′ 41.73” W) at 7 m.a.s.l., under a shade-enclosure made of monofilament stabilized polyethylene, a density of 160 filaments cm^{-2}, a square aperture of 0.4×0.8 mm (model 1610 PME CR).

Genetic material: Seeds of Napoletano and Sweet Genovese basil varieties are not considered an endangered species and were obtained from a seeds and fertilizers shop in La Paz, Baja California Sur, Mexico, which were imported from Vis® Seed Company (USA). These varieties were selected previously as salinity tolerant and sensitive, respectively, when were evaluated under NaCl-stress in three stages, germination, emergence, and early vegetative development (Reyes-Pérez *et al*., 2013a; Reyes-Pérez *et al*., 2013b; Reyes-Pérez *et al*., 2013c; Reyes-Pérez *et al*., 2014).

Experimental design and treatments: The experiment was set up as a completely randomized design with a trifactorial model of 2×3×2 with six replications. Factor one had two sweet basil varieties, tolerant and sensitive to salinity (Napoletano and Sweet Genovese, respectively), factor two had three NaCl concentrations (0, 50, and 100 mM) and factor three had the control (distilled water) and one dilution (1/60 v/v) of humates isolated from vermicompost.

Composition of humates: Reyes-Pérez *et al*., (2014) reported the composition of humates previously. Briefly, humates isolated from vermicompost have nutrients such as Ca, Mg, Na, P_2O_5, K, and N; contain free amino acids, polysaccharides, carbohydrates, inorganic elements, humic substances, beneficial microorganisms, plant hormones and soluble humus.

Experimental conditions: Reyes-Pérez *et al*., (2014) reported the experimental conditions previously. Briefly, the seeds were sown in 200 cavity polystyrene containers with Sogemix PM® and keeping moisture with daily irrigations. The transplant was performed in pots (1 kg) containing Sogemix PM® as substrate. The transplant was done when seedlings had an average height of 15 cm. Once transplanted, the seedlings were irrigated with drinking water with nutrient solution reported by Samperio (1997). On the second week, NaCl (0, 50, and 100 mM of NaCl) was applied gradually to elude sudden change in solute concentration and its adverse impacts according to Murillo-Amador *et al*., (2007). Five-hundred milliliters of NaCl were applied in all irrigation, achieving that solution applied drained through the pot holes in order to prevent accumulation of salts in the substrate. Foliar application of the corresponding dilution of humates isolated from vermicompost (1/60 v/v) was iniciated and distilled water as control. By adding H_2SO_4 or KOH, the pH was maintained at 6.5.

Leaf relative water content: Leaf relative water content (LRWC) was determined by Yamasaki & Dillenburg (1999) method. Briefly, the leaves were collected in the middle part of the plant to reduce the effect of age on the variability of results. Individual leaves were weighed to get fresh weight (FW). Complete leaves were settled in distilled water in a closed Petri-dish to determine turgid weight (TW). During imbibition process, leaves were weighed daily after removing water from the leaves surface. At the end of the imbibition period, leaves were placed in an oven at 80° C during 48 h to obtain dry weight (DW). All weights were registered using an analytical scale with a precision of 0.0001 g. Values of FW, TW and DW were used to calculate the LRWC by the equation:

$$\text{LRWC (\%)} = [(\text{FW-DW})/(\text{FW-TW})]\times 100.$$

Chlorophyll content (a, b and total): Chlorophyll a, b and total was determined at 58 and 65 days after emergence by the Arnon (1949) method and expressed on a leaf area basis (mg cm^{-2}). The procedure followed was described in detail by Ruiz-Espinoza *et al*., (2010), being concise, the method involved macerating leaves in aqueous acetone (80%), centrifuged (typically 2 to 3 min) and absorbance determined using a spectrophotometer (Spectronic Unicam®, Cambridge, UK) at 645 nm and 663 nm.

Statistical analysis: Kolmogorov-Smirnov ($p\leq0.05$) and Bartlett ($p\leq0.05$) tests were applied to determine normality and homogeneity of variance, respectively. The data were analyzed through a three way of ANOVA with basil varieties as factor one, NaCl as factor two and humates isolated from vermicompost as the factor three. Tukey HSD was used to test for mean differences at $p\leq0.05$. Values of leaf RWC that are expressed in percentage were arcsine transformed before ANOVA according to Sokal & Rohlf (1998).

Results

Leaf relative water content: Leaf relative water content showed differences among varieties ($p\leq0.0001$), NaCl ($p\leq0.0001$), humates ($p\leq0.0001$), varieties × NaCl ($p\leq0.0001$), varieties × humates ($p\leq0.0001$), varieties × humates ($p\leq0.0001$), NaCl×humates ($p\leq0.0001$) and varieties × NaCl×humates ($p\leq0.001$). Napoletano showed the higher LRWC than Sweet Genovese in all NaCl concentrations; however, LRWC decreased in both varieties as NaCl concentrations increased (Table 1). In both varieties, the LRWC increased when the humate was applied, being slightly higher in Napoletano in both, control and 1/60 dilution (Table 2). The analysis of varieties×NaCl×humates showed higher LRWC at 0 mM NaCl and 1/60 of humates and both varieties showed that LRWC increased in relation to control at 1/60 dilution of humates in all NaCl concentrations. The LRWC was lower for Sweet Genovese in 100 mM NaCl and zero of humates (Table 3). The results indicated an increase in the LRWC when humates isolated from vermicompost were applied, which promoted the LRWC (Table 2) and this dose counteracted NaCl-stress.

Chlorophyll *a*, *b* and total, 58 days after emergence: Chlorophyll *a* (Chl A) showed differences among varieties ($p \leq 0.0001$), NaCl ($p \leq 0.0001$), humates ($p \leq 0.0001$) and NaCl×humates ($p \leq 0.0001$). Napoletano showed higher Chl A than Sweet Genovese in all NaCl concentrations; however, Chl A decreased in both varieties as NaCl increased (Table 1). Napoletano showed higher Chl A in both, the control and 1/60 of humates, increasing in both varieties with humates (Table 2). The analysis of the three factors interaction revealed that Napoletano showed higher Chl A with respect to Sweet Genovese in all NaCl concentrations (Table 3).

Chlorophyll *b* (Chl B) showed differences among varieties ($p \leq 0.0001$), NaCl ($p \leq 0.0001$), humates ($p \leq 0.0001$), varieties×NaCl ($p \leq 0.0001$), NaCl×humates ($p \leq 0.001$) and varieties×NaCl×humates ($p \leq 0.001$). The Chl B in both varieties decreased as NaCl increased (Table 1). Napoletano showed higher Chl B in both, control and 1/60 of humates, noting that in both varieties increased Chl B with humatesin all NaCl concentrations (Table 2). The analysis of the main factor interactions revealed that Napoletano showed higher Chl B with respect to Sweet Genovese in all NaCl concentrations (Table 3).

Total chlorophyll (TChl) showed differences among varieties ($p \leq 0.0001$), NaCl ($p \leq 0.0001$), humates ($p \leq 0.0001$), varieties×NaCl ($p \leq 0.0001$) and NaCl×humates ($p \leq 0.0001$). Napoletano displayed higher TChl than Sweet Genovese in all NaCl concentrations; however, TChl decreased in both varieties as NaCl increased (Table 1). Napoletano showed higher TChl in both, control and 1/60 humate. In both varieties, TChl increased with humates (Table 2). Napoletano revealed the higher TChl in 100 mM NaCl and 1/60 humates; in both varieties TChl increased over control at 1/60 humate in all NaCl concentrations (Table 3).

Table 1. Analysis of the interaction varieties × NaCl on average leaf relative water content, and photosynthetic pigments of two sweet basil varieties under NaCl-stress.

Varieties	LRWC (%)			58 days after emergence								
				Chl *a* (μg cm^{-2})			Chl *b* (μg cm^{-2})			Total Chl (μg cm^{-2})		
	NaCl (mM)											
	0	50	100	0	50	100	0	50	100	0	50	100
Napoletano	93.21a	90.91a	85.00a	34.21a	31.20a	29.35a	10.18a	8.85a	9.27a	44.39a	40.05a	38.62a
Sweet genovese	87.70b	85.07b	76.77b	30.74a	25.76a	20.04a	9.28a	7.81a	6.09a	40.03a	33.57a	26.13a

	65 days after emergence								
	Chl *a* (μg cm^{-2})			Chl *b* (μg cm^{-2})			Total Chl (μg cm^{-2})		
	NaCl (mM)								
	0	50	100	0	50	100	0	50	100
Napoletano	36.23 a	38.48 a	32.36 a	11.91 a	11.75 a	10.45 a	48.15 a	50.24 a	42.82 a
Sweet genovese	28.97 a	24.29 b	18.37 b	8.96 b	7.55 b	5.33 a	37.93 a	31.84 b	23.70 a

LRWC= Leaf relative water content; Chl= Chlorophyll; NaCl= Sodium chloride. Values within the same column with same letters are not significantly different (Tukey´s HSD multiple range test p≤0.05)

Table 2. Analysis of the interaction varieties × humates isolated from vermicompost on average leaf relative water content and photosynthetic pigments of two basil varieties under NaCl-stress.

Varieties			58 days after emergence					
	LRWC (%)		Chl *a* (μg cm^{-2})		Chl *b* (μg cm^{-2})		total Chl (μg cm^{-2})	
	Humates isolated from vermicompost (v/v)							
	0	1/60	0	1/60	0	1/60	0	1/60
Napoletano	76.55a	82.04a	23.24a	39.92a	7.16a	11.71a	30.40 a	51.64 a
Sweet genovese	70.68a	73.69a	18.33a	32.70b	5.27b	10.18b	23.60 a	42.89 b

	65 days after emergence					
	Chl *a* (μg cm^{-2})		Chl *b* (μg cm^{-2})		Total Chl (μg cm^{-2})	
	Humates isolated from vermicompost (v/v)					
	0	1/60	0	1/60	0	1/60
Napoletano	31.03 a	40.35 a	10.20 a	12.54 a	41.24 a	52.90 a
Sweet genovese	11.74 b	36.01 b	3.84 b	10.72 b	15.58 b	46.73 b

LRWC= Leaf relative water content; Chl= Chlorophyll; NaCl= Sodium chloride. Values within the same column with same letters are not significantly different (Tukey´s HSD multiple range test p≤0.05)

Table 3. Analysis of the interaction varieties × NaCl × humates isolated from vermicompost on average leaf relative water content and photosynthetic pigments of two basil varieties under NaCl-stress.

Varieties	NaCl (mM)	HV (v/v)	LRWC (%)	58 days after emergence			65 days after emergence		
				Chlorophyll *a* (µg cm^{-2})	Chlorophyll *b* (µg cm^{-2})	Total chlorophyll (µg cm^{-2})	Chlorophyll *a* (µg cm^{-2})	Chlorophyll *b* (µg cm^{-2})	Total chlorophyll (µg cm^{-2})
Napoletano	0	0	90.91b	26.45a	8.19de	34.65a	30.65a	10.81a	41.47a
	0	1/60	95.51a	41.96a	12.16a	54.13a	41.80a	13.01a	54.82a
	50	0	82.88ef	23.57a	6.73de	30.31a	36.92a	11.44a	48.36a
	50	1/60	87.12cd	38.82a	10.98b	49.80a	40.05a	12.07a	52.12a
	100	0	72.33h	19.70a	6.55e	26.26a	25.53a	8.35a	33.88a
	100	1/60	80.78g	39.00a	11.98a	50.98a	39.20a	12.54a	51.75a
S. Genovese	0	0	85.07de	24.34a	7.11de	31.46a	18.48a	6.59a	25.07a
	0	1/60	90.33bc	37.15a	11.44ab	48.59a	39.45a	11.32a	50.78a
	50	0	72.21h	21.19a	6.46e	27.66a	12.95a	4.26a	17.22a
	50	1/60	81.33f	30.32a	9.15bcd	39.48a	35.62a	10.84a	46.46a
	100	0	63.79i	9.45a	2.22f	11.67a	22.78a	6.68a	34.46a
	100	1/60	77.58g	30.63a	9.95bc	40.59a	32.95a	9.99a	42.94a

HV= Humates isolated from vermicompost; LRWC= Leaf relative water content. Values within the same column with same letters are not significantly different (Tukey´s HSD multiple range test p≤0.05). S. Genovese= Sweet Genovese

Chlorophyll *a*, *b* and total, 65 days after emergence: Chlorophyll *a* (Chl A) showed differences among varieties ($p\leq0.0001$), NaCl ($p\leq0.0001$), humates ($p\leq0.0001$), varieties × NaCl ($p\leq0.0001$), varieties × humates ($p\leq0.0001$) and NaCl × humates ($p\leq0.0001$). Napoletano exhibited higher Chl A than Sweet Genovese in all NaCl concentrations; however, in both varieties Chl A decreased as NaCl increased (Table 1). Napoletano showed higher Chl A than Sweet Genovese in 1/60 of humates, noting that Chl A increased by adding humates in both varieties (Table 2). Napoletano reached higher Chl A with respect to Sweet Genovese in 100 mM NaCl and 1/60 humates; nevertheless, Chl A increased in both varieties with 1/60 of humates (Table 3).

Chlorophyll *b* (Chl B) showed differences among varieties ($p\leq0.0001$), NaCl ($p\leq0.0001$), humates ($p\leq0.0001$), varieties × humates ($p\leq0.0001$) and NaCl × humates ($p\leq0.0001$). Napoletano exhibited the higher Chl B than Sweet Genovese in all NaCl concentrations, but Chl B decreased in both varieties as NaCl increased (Table 1). Napoletano showed higher Chl B than Sweet Genovese, but Chl B increased in both varieties with 1/60 humate (Table 2). Napoletano showed higher Chl B than Sweet Genovese in 100 mM NaCl and 1/60 humates; in both varieties, Chl B increased with 1/60 humates (Table 3).

Total chlorophyll (TChl) showed differences among varieties ($p\leq0.0001$), NaCl ($p\leq0.0001$), humates ($p\leq0.0001$), varieties × NaCl ($p\leq0.0001$), varieties × humates ($p\leq0.0001$), NaCl×humates ($p\leq0.0001$). Table 1 shows that Napoletano had higher TChl than Sweet Genovese in all NaCl concentrations, but TChl decreased in both varieties as NaCl increased, except for Napoletano at 50 mM NaCl. Napoletano showed higher TChl in both control and 1/60 humates; in both varieties TChl increased with humates (Table 2). Napoletano showed the higher TChl than Sweet Genovese in 100 mM NaCl and 1/60 humates; however, in both varieties TChl increased over control in 1/60 humates in all NaCl concentrations (Table 3).

Discussion

The results show that leaf RWC decreased in both varieties as NaCl increased, because of NaCl causes a decrease in water osmotic potential. Leaf relative water content is expressed in the plant water status; that is, the plant trends to lose water so that it must retain a more negative water potential than the substrate to ensure water absorption (Buchanan *et al.* 2000). These results coincide with Srivasta *et al.*, (1998), Katerji *et al.*, (2003), Kaya *et al.*, (2003) who reported that LRWC decreases under salinity stress and has been recognized a criterion that is used to define the water content of plants (Schonfeld *et al.*, 1988). The present study showed an increase in the LRWC with humates, which promoted the LRWC and this dose counteracted NaCl-stress since 1/60 (v/v) humates mitigated this negative effect. Similar result reported Albuzio *et al.*, (1994) when LRWC increased with the application of humic substances. Humates isolated from vermicompost has hormones such as ABA and the increase of LRWC could be related with the well-established induction of ABA signal for the root, which goes to the leaves across transpiration to induce stomatal closure to reduce water loss (Buchanan *et al.*, 2000). Medrano *et al.*, (2002) confirmed that leaf water condition interacts with stomatal conductance and transpiration under water deficit, and an important relationship occurs among leaf water potential and stomatal conductance. In this study, the LRWC decreased in both varieties when plants were grown under different NaCl concentrations; it is likely that despite the LRWC difference in 50 and 100 mM NaCl was very light, it was enough to lose turgor in tissues. Different LRWC values observed in 100 mM NaCl in both varieties were related to the response mechanisms of each variety. As the plant water status was affected by exposure to high NaCl, changes occurred in the water flow in a way that cells and tissues can adapt. Although the variation of the LRWC in Napoletano and Sweet Genovese was slightly different when the plants were submitted to 100 mM NaCl, it is suggested that

Napoletano showed better respond to water deficit caused by the NaCl because it has been demonstrated that salinity tolerant genotypes increase their water use efficiency in adverse environment.

Chlorophyll *a*, *b* and *total* decreased in both varieties as NaCl increased in both stages, 58 and 65 days after emergence, results are in accordance with Akram and Ashraf (2011), Ashraf & Harris (2013), Siddiqui *et al*., (2014), Tayyab *et al.,* (2016) and Jan *et al.,* (2016). In the present study, the decrease of these pigments can have several factors, some of them could be that reduction in leaf water content affected the physiological processes and altered the metabolism, phenomena that was demonstrated by Iyengar & Reddy (1996) 22 years ago who concluded that lower water potential in leaves caused decline in photosynthesis. Other factor is related to the destruction of chloroplasts and to the increase of chlorophyllase enzyme activity affecting chlorophyll synthesis. Because of the decrease in chlorophyll, plant growth and development are reduced because solar light absorption and conversion, the first process of photosynthesis and therefore carbon fixation and carbohydrate synthesis, are affected (Spyropoulus & Mavrommatis, 1978). Ashraf & Harris (2013) argue that the chlorophyll biosynthesis is much more affected than the breakdown of chlorophyll contents. The most important point in the present study is related to the fact that both varieties increased Chl A, B and total in all NaCl concentrations when humate was apply. Napoletano was most NaCl tolerant and showed higher pigments in all NaCl concentrations, which are in accordance with those reported by Alamgir and Ali (1999) who concluded that salt tolerant species could store most chlorophyll than sensitive, then, the increase of chlorophyll is suggested as the important biochemical indicator of salt tolerance in some plants species. The increase of leaf pigments after applying humates isolated from vermicompost even under NaCl stress conditions could be related to increase of Mg in the leaf, since humates of vermicompost have positive effects in plants as bio-stimulator and/or nutrient carrier. This result agrees with those reported in rice by Calderín-García *et al*., (2012) who described that humic fractions stimulated growth, chlorophyll content, P, K, Ca, Fe, Mn, Zn, and Mg, and it was the total humic extract. Clapp *et al*., (2001) relate this increase to the capacity of complexation of humic substances with micronutrients such as Fe and Zn, which facilitate their absorption capacity and influence chlorophyll synthesis and therefore, will improve the photosynthetic efficiency and the biomass production (Canellas & Olivares, 2014). A recent study (Guridi-Izquierdo *et al*., 2017) demonstrated that previous treatment of humate improved the root membrane permeability, the net radical biomass production and the Chl A and concluded that this previous treatment protected the rice plants against a posterior hydric stress that was induced. Vermicompost humic acids also promoted chlorophyll content of banana under *In vitro* conditions (Moya-Fernández *et al.,* 2016). The present study shows that various chemical fractions of humates establish some stimulus, perhaps hormone-like, trigger a cascade of responses at membrane level, such as activation of enzyme systems that operate in chlorophyll synthesis or nutritional type. The presence of nutrients or possible complexation of humic-nutrients in the system of humates isolated from vermicompost can influence the synthesis of photosynthetic pigments. Pflugmacher *et al*., (2008) proved that the application of aqueous extracts of humic substances stimulates chlorophyll and photosynthetic pigments, and that it is not necessarily an indicative of an increase in the photosynthetic process. Analogous results were obtained by Calderín-García *et al*., (2012) who determined the content of pigments (carotenoids and chlorophylls) in *Oryza sativa* submitted to water stress treating leaves with vermicompost extract. Treated plants reached a significantly higher mean for chlorophyll and carotenoid contents, pointing out that the effect could be linked to the combination of humic substances and other components in the extract. The positive effect of humates in physiological process in plants has been demonstrated (Martínez, 2006; Nardi *et al*., 2007; Reyes-Pérez *et al*., 2011; Calderín-García *et al*., 2013). Some studies revealed that humates stimulate plants' growth because they have analogous effects with plant hormones like auxins. Other pointed out that are because of humates contain free amino acids, polysaccharides, carbohydrates, inorganic elements, humic substances, beneficial microorganisms, and soluble humus that act positively in the total metabolism of the plants. However, the use of these constituents induces an increase in photosynthetic pigments such as chlorophyll and other physiological and morphometric characteristics in plants, although the principal effect of humates in some of these processes is not clear yet. Nardi *et al*., (2002) pointed out that the effects of humates on plants could be discriminatory and flexible, depending on their concentration, pH of the culture medium, the physiological state of plants, the concentrations of the material and can induce an increase in chlorophyll although in some cases could be stimulated or inhibited depending of these factors.

Conclusions

There is a differential response among basil varieties with respect to LRWC, chlorophyll *a*, chlorophyll *b* and total chlorophyll under NaCl-stress and humates.

When the LRWC and chlorophyll content increase, Napoletano is most tolerant to NaCl-stress than Sweet Genovese.

In sweet basil, the LRWC and chlorophyll content could be used as tolerance indicators while defining the NaCl-tolerance of sweet basil genotypes.

The ability of humates isolated from vermicompost to enhance plant development has been demonstrated. This positive effect was revealed in an improvement of some physiological parameters like LRWC and photosynthetic pigments (Chlorophyll *a*, *b*, and total).

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