

Effect of *Natrum muriaticum* on photosynthesis and biomass of basil plants grown under salt stress (NaCl)

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Abstract

Highly diluted bioactive compounds have been successfully used in the agricultural sector to stimulate plant growth and development even in unfavorable crop conditions. For this reason, the feasibility of using *Natrum muriaticum* (NaM), a homeopathic ultradilution, as an elicitor agent against the negative effects of salt stress by NaCl, was studied in two varieties of basil (*Ocimum basilicum* L) grown in a saline medium, at CIBNOR, SC.), in February-March 2019. A completely randomized design with a factorial arrangement (2A×2B×3C) was applied, considering the Napoletano and Emily varieties as factor, the concentration of NaCl (0 and 75 mM) as factor B, and the homeopathic treatments NaM (7CH, 13CH and distilled water as control) as factor C, adding a total of 12 treatments with four repetitions each, and photosynthetic-rate and production of fresh and dry biomass of aerial part as response variables. The analysis of variance and multiple comparisons of means (Tukey HSD $p \leq 0.05$) revealed that both dynamizations of NaM (7CH and 13CH) favored an increase in the response variables evaluated in plants subjected to salt stress. The highest increase in photosynthetic rate (75%) and fresh biomass of the aerial part (40%) with respect to the control treatment was obtained with NaM 7CH in the Napoletano variety. In conclusion, the application of NaM attenuates salt stress in *Ocimum basilicum* L, confirming that agricultural homeopathy is an eco-friendly alternative that can increase basil production under salt stress conditions.

Keywords: agricultural homeopathy, aromatic species, arid zones, salinity stress.

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Introduction

Basil is a millennial aromatic plant from the tropical and subtropical regions of the Asian continent but cultivated around the world (Ono *et al.*, 2011). Because of its pleasant smell and flavour, the species has a great commercial importance and is widely used in the food industry, either for consumption fresh or dry and as flavouring for sauce and vinegar (Ojeda-Silvera *et al.*, 2015). It is used in cosmetics industry because of its essential oil content (Nieto *et al.*, 2016; Mazón-Suástegui *et al.*, 2018a; Batista-Sánchez *et al.*, 2022) and in the pharmaceutical industry because of its stimulating, diuretic, antispasmodic and anti-alopecic properties, among others (Batista-Sánchez *et al.*, 2015).

Basil is cultivated in the Mexican states of Guerrero, Nayarit, Morelos, Puebla, Baja California and Baja California Sur (BCS), of which this last one is the state with the greatest production (SIAP, 2017). Nonetheless, basil productivity in BCS is at risk because agricultural areas with tendency to salinity have increased due to its semiarid conditions (Batista-Sánchez *et al.*, 2017). In the last years, abiotic stress in plants associated to salinity has been an underlying effect for the international scientific community because of its negative consequences, causing a decrease in biomass production that reduces yield of cultivated species and agriculture sustainability in affected areas (Kandil *et al.*, 2017; Mazón-Suástegui *et al.*, 2020a; Rodríguez-Alvarez *et al.*, 2020).

According to Khalig *et al.* (2014), global losses in agriculture due to salinity, which has affected one-fifth of cultivated land, go beyond 12 thousand million USD yearly. Among the main harmful effects of salinity stress include decrease of water absorption, incoming ions that could cause toxicity, nutritional imbalance and physiological changes, such as photosynthetic rate due to reduced foliar area and less gas exchange, which isolated or synergic reduce cultivation viability (Abbas *et al.*, 2015; Hessini *et al.*, 2015).

At present, many research studies have been developed to confront the problem associated to water and salinity. These studies have focused on the selection of tolerant varieties to abiotic stress (Ojeda-Silvera *et al.*, 2013), assessment of tolerance transcription factors by molecular biology techniques (García *et al.*, 2013) and application of growth promoting substances (Calvo *et al.*, 2014). In this same line of research, agriculture homeopathy with ultra-diluted bioactive substances or nanomedicines is a novel topic for global agriculture (Mazón-Suástegui *et al.*, 2018a).

Recent studies have shown the beneficial action of some ultra-diluted homeopathic medicines in the plants, either for reducing the presence or harmful effects of pest organisms (Martínez *et al.*, 2014; Meneses, 2017) or stimulating plant growth and vigour in their presence (Modolon *et al.*, 2016; Mazón-Suástegui *et al.*, 2020b; Abasolo-Pacheco *et al.*, 2020; García-Bernal *et al.*, 2020). The application of nanomedicines as elicitor substances of salinity stress negative effects is a topic that has been little explored.

Therefore, the objective of this study was to assess the elicitor effect of *Natrum muriaticum* facing NaCl induced stress in two basil (*Ocimum basilicum* L.) varieties cultivated in hydroponic system, taking into account response variables of photosynthetic activity and biomass production.

Materials and methods

Study site: the experiment was performed at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), located at 24° 08' 10.03" N and 110° 25' 35.31" W at 7 masl north of the city of La Paz, Baja California Sur, Mexico (Ojeda-Silvera *et al.*, 2015). A structure built with galvanised steel tubes covered with a 1610 PME CR (threads of 16*10 cm⁻², crystal colour mesh vent of 0.4*0.8 mm), equivalent to 40% shade and medium, maximum and minimum temperature of 26.84 ±5.21, 44.17 ±4.92, 13.4 ±5.83 °C, respectively, and relative humidity of 52.8 ±14.95%. These climatological data were recorded with a portable climatologic station (Vantage Pro2[®] Davis Instruments, USA) inside a mesh structure.

Genetic material: plantlets were obtained from certified organic seeds of two basil varieties (Emily and Napoletano), from the Seed Company (San Diego, CA, USA) with differential response to NaCl stress.

Experimental design and treatments: seeds were sowed in 200-well polystyrene trays with Sogemix PM[®] (Canada) inert commercial substrate. To maintain humidity and achieve a homogeneous emergence, daily irrigation was applied until substrate saturation was achieved. When plants reached an average height of 10 cm, they were transplanted in 150 ml pots with vermiculite as support substrate and a fibre fraction of absorbent cotton, which was performed during the first post-transplant days to guarantee contact of the seedlings with water at the moment of placing them in a hydroponic system.

The system was conditioned with expanded polyurethane boxes of 69 × 38.5 × 25 cm and 38 L capacity, gauged with water from the purification plant at CIBNOR with an electric conductivity of 0.22 dS m⁻¹. The pots were placed on the polyurethane box cover in (with a diameter of 4 cm) wells (six per box) to guarantee contact of the radicle system with water. For plant nutrition, a nutritional solution (1 L 100 L⁻¹ water) was used adapted for basil according to Samperio (1997), adjusting pH to guarantee an optimum range of 6.5 ±0.2 (Mazón-Suástegui *et al.*, 2018a).

The NaM-7CH and NaM-13CH treatments were prepared at CIBNOR from *Natrum muriaticum* 6CH and *Natrum muriaticum* 12CH (Similia[®]-México), which has official register in Secretaría de Salud de México (Mexican federal health ministry) as medicine for human use (bought from Farmacia Homeopática Nacional, CDMX, México). Its initial active ingredient is sea salt or Guérande salt, whose main component is NaCl, but it also contains trace elements, such as bioavailable magnesium, potassium chloride, iron and calcium. Briefly, to prepare NaM 7CH and NaM 13CH dynamisations, centesimal dilutions (1:99) in distilled and deionised water were vigorously stirred in vortex equipment (BenchMixer[®], Edison, NJ, USA) for two min, applying basic homeopathy procedures to obtain ultra-diluted substances (Mazón-Suástegui *et al.*, 2017; Ortiz *et al.*, 2017).

A completely randomised experiment with factorial arrangement (2A × 2B × 3C) was applied, considering basil varieties (Napoletano and Emily) as factor A, at NaCl levels (0 and 75 mM) as factor B and homeopathic dynamisations the *Natrum muriaticum* (NM 7CH, 13CH and distilled water without treatment control) Factor C, with a total of 12 treatments with four repetitions each treatment.

Application of NaM treatments started at day seven subsequent to transplant and after an initial acclimation period, spraying the aerial part of the plants with 150 ml per plant every other day and with distilled water only those in the control treatment. Once the plants were fully established (15 days), application of the stressing salinity treatments started gradually to avoid osmotic shock (Murillo-Amador *et al.*, 2007). The application started with a concentration of 25 mM of NaCl and increased until reaching the expected concentration (75 mM).

Variables assessed: the photosynthetic rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured with (IRGA) LCpro-SD Portable Photosynthesis System that included a broad-leaf chamber (ADC, Hoddesdon, Herts, UK) in a completely turgid and healthy leaf, on completely sunny days (three measurements) during the previous week to cutting and at the time of greater solar radiation. At 45 days of transplant, we proceeded to cut and evaluate biomass by determining fresh weight of the aerial part (FWAP) in an analytical balance (Mettler Toledo[®], model AG204, USA).

To obtain dry weight of the aerial part (DWAP), all fresh material of every plant was placed separately in paper bags, which were introduced in a drying stove (Shel-Lab[®], model FX-5, series-1000203, USA) at a temperature of 70 °C for 72 h for total dehydration and then weighed in analytical balance (Mettler Toledo[®], AG204, USA) expressing dry plant material value in grams. Statistical analyses: Analyses of variance and multiple comparisons (Tukey's HSD, $p \leq 0.05$) were performed with Statistica v. 10.0 program for Windows (StatSoft, Inc, 2011).

Results and discussion

The results revealed significant differences ($p = 0.005$) for photosynthetic rate in varieties x salinity (NaCl) concentration interaction, corroborating the negative and stressful effect of NaCl in this response variable. A decrease greater than 25% was recorded for both basil varieties in this variable when plants were subjected to a greater salinity test of 75 mM (Table 1).

Table 1. Effect of varieties × NaCl interaction in plant photosynthetic rate and biomass production of two basil varieties subjected to salinity stress.

Varieties	NaCl (mM)	A ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	FBAP (g)	DBAP (g)
Napoletano	0	17.9 a	176.5 a	32.3 a
Napoletano	75	12.4 c	104.4 c	12.8 c
Emily	0	13.5 b	145.3 b	27.3 b
Emily	75	9.5 d	86.3 d	12.4 c
Significance level		**	*	*

A= photosynthetic rate; FBAP= fresh biomass aerial part; DBAP= dry biomass aerial part. Average values with different letters in the same column differ statistically (Tukey HSD, $p = 0.05$). Level of significance of Anova * = $p \leq 0.05$; ** = $p \leq 0.001$.

Reyes *et al.* (2017) obtained similar results when rice (*Oryza sativa*) plants were subjected to 100 mM of NaCl stress. Other authors found a decrease in mustard (*Sinapis alba*) photosynthetic activity during cultivation under salinity stress (Jamil *et al.*, 2013). Both studies agreed that the significant decrease of photosynthetic activity in plants subjected to salinity stress could be explained in function of the osmotic effect associated to stomatal closing, which as a consequence,

showed a decrease in incoming CO₂ to the cells; nonetheless, while analysing the effect of varieties × NaM interaction, a differential response was observed with a tendency to increasing photosynthetic rate (A) when basil plants were treated with the elicitor *Natrum muriaticum* homeopathic medicine.

This result was obtained independently of the applied dynamisation (NaM 7CH or NaM 13CH), but the Napoletano variety treated with NaM 7CH recorded the greatest significant increase ($p=0.008$) in photosynthetic rate (Table 2). This result could be explained by the presence of molecular nanoparticles or nanostructures of the active ingredient in NaM 7CH treatment in ultra-dilutions (Mazón-Suástegui *et al.*, 2018a).

Table 2. Effect of varieties × NaM interaction in plant photosynthetic rate and biomass production of two basil varieties.

Varieties	NaM	A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	FBAP (g)	DBAP (g)
Napoletano	0	11.3 c	118.1 d	22 b
Napoletano	7CH	19.8 a	165.8 a	24.5 a
Napoletano	13CH	14.4 b	137.3 b	21.1 b
Emily	0	8.6 d	100.2 e	17.8 c
Emily	7CH	14.4 b	129.2 c	24.9 a
Emily	13CH	11.6 c	117.9 d	16.8 c
Significance level		**	*	*

A= photosynthetic rate; FBAP= fresh biomass aerial part; DBAP= dry biomass aerial part. Average values with different letters in the same column differ statistically (Tukey's HSD, $p=0.05$). Level of significance of Anova * = $p \leq 0.05$; ** = $p \leq 0.001$.

The results obtained confirmed a basic homeopathy principle 'The Law of Similars' cited by Mazón-Suástegui *et al.* (2018b and 2018c), because the same homeopathic ultra-diluted (NaCl) substance had a favourable elicitor and attenuating effect of salinity stress, same which was experimentally induced with a massive dose of sea salt. Sea salt is mainly constituted by NaCl, but it also contains tracing elements, such as magnesium (Mg) that play a crucial role in forming the chlorophyll molecule, which is the centre of direct action of the luminous reaction during photosynthesis (Mazón-Suástegui *et al.*, 2018a).

For the FBAP variable, the results showed significant differences while analysing varieties × NaCl ($p=0.05$) interaction, observing a decrease in both of more than 35% when 75 mM of NaCl were applied (Table 1). This response was determined by the inhibition effect of NaCl in plants at cellular level that causes toxicity, which limits the correct metabolic development, also affecting cell division and elongation (Asghari and Ahmadvand, 2018). Another applicable explanation could be the photosynthetic rate decrease observed under salinity (75 mM) since plants have less biomass accumulation and less organic production when they experiment a decrease in photosynthesis (Reyes *et al.*, 2017; Sandoval *et al.*, 2010).

While analysing the triple interaction of variety factors × NaCl × NaM for photosynthetic rate, significant differences ($p=0.01$) were found, observing an increase in variable (A) in plants treated with both dynamisations of *Natrum muriaticum*, ultra-diluted from sea salt (NaM 7CH and NaM 13CH) even when they were simultaneously subjected to stress by adding NaCl (Figure 1).

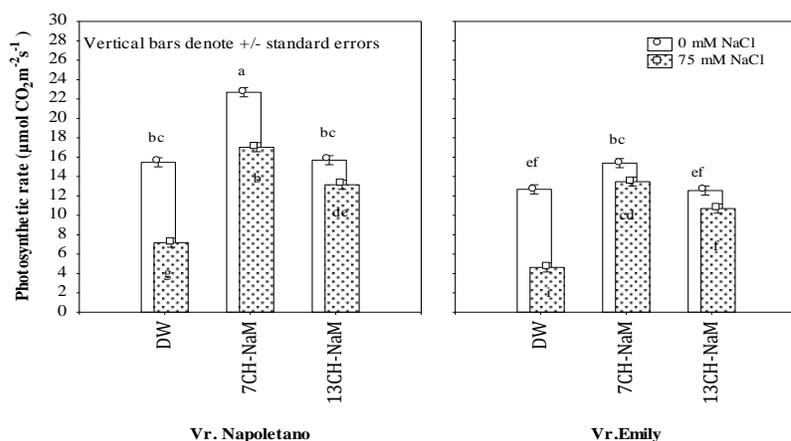


Figure 1. Effect of varieties × NaCl × NaM interaction in photosynthetic rate of two basil varieties cultivated in salinity stress conditions. Different letters show significant statistical ($p= 0.05$).

This result confirmed the hypothesis of NaM eliciting and attenuating effect of salinity stress on basil plants as previously described. These results could be determined by activation of the plant intrinsic physiological mechanisms for self-defence when facing stressful salinity conditions either as a direct response to sea salt nanoparticles or indirectly before the presence of molecular nanoaggregates that unchained a series of signalling mechanisms in cascade that propitiate a positive response in the treated organisms, cited by Chikramane *et al.* (2010); García (2018); Mazón-Suástegui *et al.* (2018a); Mazón-Suástegui *et al.* (2020a and 2020b); Rodríguez-Alvarez *et al.* (2020) and whose transduction allows activating mechanisms that still require more studies, such as osmoprotectant substances in plants (Batista-Sánchez *et al.*, 2017).

While analysing the interaction varieties × NaM, significant differences ($p= 0.048$) were observed for FBAP, independently of the homeopathic dynamisation applied. Both basil varieties recorded an increase in this variable in plants treated with NaM 7CH and NaM 13CH although the best response corresponded to Napoletano with application of NaM 7CH (Table 2).

This result could be explained because this variety has the characteristic of greater tolerance to salinity caused by NaCl since previous studies on Batista-Sánchez *et al.* (2017) reported similar results when studying the interaction of natural bio-stimulants (FitoMas-E) in initial plant germination and growth of basil subjected to different NaCl concentrations. This study confirms that the Napoletano variety responds more easily to the negative effects of NaCl saline stress, which may be determined by its genetic information, which guarantees a faster growth of its different organs, even under unfavourable conditions.

The triple interaction varieties × NaCl × NaM showed significant differences ($p= 0.05$) for the FBAP response variable, which increased in both basil varieties with NaM application even when they were found simultaneously under salinity stress conditions (Figure 2). This result confirmed the hypothesis that the NaM treatment had properties to mitigate salinity stress in plants already stressed by the experimental addition of NaCl, which was already discussed previously.

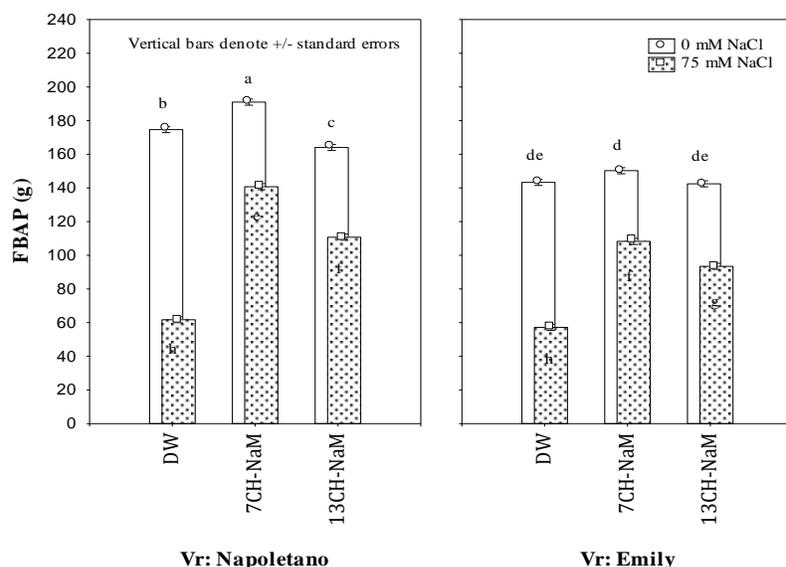


Figure 2. Effect of variety interaction \times NaCl \times NaM in fresh biomass of the aerial part of the two basil varieties cultivated in salinity stress conditions. Different letters show significant statistical differences ($p=0.05$).

The results of this study agree with those found by Giardini *et al.* (2012) while studying the attenuating salinity stress effect of *Natrum muriaticum* (NaM) in tomato (*Solanum lycopersicom*), which could also be justified by the presence of tracing elements, such as bioavailable magnesium, potassium chloride, iron and calcium in tracing amounts, likely as molecular nanoparticles or nanoaggregates cited by Mazón-Suástegui *et al.* (2018a) of these chemical elements and compounds that constitute the original active ingredient of homeopathic medicine officinales *Natrum muriaticum* 6CH and 12 CH (Similia[®] CDMX, México).

Finally, it is convenient to highlight that these tracing elements, even at minimum dose although measurable ponderable, play an important role in plant tissue development, growth and productivity (Jeyasubramanian *et al.*, 2016). The DBAP was significant ($p=0.04$) for varieties \times NaCl interactions, with a decrease of this variable in both basil plants stressed with 75 mM of NaCl (Table 1). Salinity stress is one of the essential factors that affect cell division and differentiation in plants, which have less production and biomass accumulation as a consequence, product of Na toxicity that favours a nutritional unbalance and generates direct affectations to the photosynthetic process (Rahneshan *et al.*, 2018).

According to the result produced by the analysis of the varieties \times NaM interaction, significant differences were observed ($p=0.05$), noticing a DBAP increase with respect to control when NaM 7CH was applied (Table 2). On the contrary, no significant differences were found in this variable for treatment NaM 13CH with respect to the control treatment. In this case, it could be argued that dynamisation 7CH contained measurable quantities of the original active ingredient (sea salt) because the dilution factor applied (seven serial dilutions 1:99) was found under the limit established by the Avogadro number while dynamisation 13 CH was found just above that limit. Nevertheless, it has been proven that marine species, such as molluscs, shrimp and fish obtain measurable benefits with the application of ultra-diluted homeopathic medicine beyond Avogadro

limit (serial dilution equivalent to 1×10^{-23}). It can be explained in function of the molecular nanoaggregates that activate response mechanisms in the individuals treated (Mazón-Suástegui *et al.*, 2017; 2018a, 2018b and 2018c; Ortiz *et al.*, 2017; Rosero, 2017; García, 2018; Mazón-Suástegui *et al.*, 2020a and 2020b).

While analysing the triple interaction varieties \times NaCl \times NaM for DBAP, the results showed significant differences ($p= 0.041$), of which one that stood out was an increase of this response variable in plants cultivated under salinity conditions and treated with NaM (Figure 3). This finding demonstrated a positive effect of the ultra-diluted substances of NaM to activate defence mechanisms of basil plants facing early NaCl stress due to the presence of molecular nanoparticles or nanoaggregates contained in the homeopathic dynamisations; these particles were capable of activating signals at cellular level that identified stress conditions and activated defence and/or adaptation mechanisms that still need to be studied further.

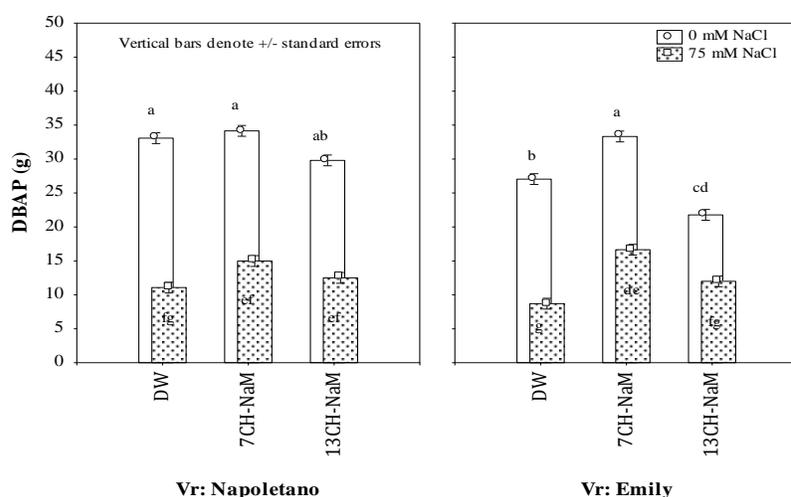


Figure 3. Effect of variety interactions \times NaCl \times NaM in dry biomass of the aerial part of two basil varieties cultivated in salinity stress. Different letters show significant statistical differences ($p= 0.05$).

In this sense, authors such as Zhu (2002) suggested that plants could identify and perceive signals referring to stress, same which were transduced in cells and transmitted, unchaining a cascade of biochemical, physiological and genetic responses that allowed the plant to adapt to unfavourable condition or clearly stressing in the surrounding environment. This adaptive response is possible when change in such conditions is gradual, which depends on the species and agent those modules the signal but always focused on maintaining an equilibrium or internal homeostasis of the individual with respect to environmental conditions.

Conclusions

Basil (*Ocimum basilicum* L varieties Napoletano and Emily) in hydroponic cultivation subjected to salinity stress by adding sodium chloride (NaCl), showed a differential physiological response for the different response variables assessed, such as photosynthetic rate and productivity in terms of fresh and dry plant biomass of the aerial part. This favourable response was also differential with respect to the application of centesimal dynamisations (7 CH and 13 CH) of the homeopathic

medicine *Natrum muriaticum* (NaM) for human use. In general, the application of NaM 7 CH favoured the best results and the highest values in the response variables under high salinity conditions (75 mM). This homeopathic nanomedicine mitigated the negative effects of salinity stress induced with NaCl in both basil (*Ocimum basilicum* L.) varieties (Napoletano and Emily), but the highest response with respect to the control treatment was obtained with NaM 7CH in the Napoletano variety.

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