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Capsicum annuum L. Response to Highly Diluted Bioactive Compounds Application in Germination and Initial Growth Under Saline Conditions
Respuesta de *Capsicum annuum* L. a la Aplicación de Compuestos Bioactivos Altamente Diluidos en la Germinación y Crecimiento Inicial Bajo Condiciones Salinas

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SUMMARY

In line with the green economy concept, food using natural products is gaining importance worldwide. Thus, the present study aims to evaluate the effect of *Natrum muriaticum* (NaM) and *Silicea Terra* (SiT) as salt stress (NaCl) mitigators on *Capsicum annuum* L. germination and initial seedling growth of two chili varieties. The experiment was performed under controlled conditions, using a completely randomized design with a factorial arrangement of (2A × 3B × 5C), where factor A is the two varieties (Santa Fe and Jalapeño M); factor B is the three levels of NaCl (0, 50 and 100 mM) and factor C is the centesimal dynamizations (CH) of the highly diluted bioactive compounds (HDBC) *Natrum muriaticum* (NaM): NaM-7CH, NaM-13CH and *Silicea terra* (SiT): SiT-7CH, SiT-13CH. Distilled water (DW) was used as a control treatment for a total of 30 treatments with four replicates each. The response variables were the germination percentage (GP), germination rate (GR), and, after 14 days, the height of the seedlings (HS), radicle length (RL), fresh and dry radicle biomass, and aerial part. Analysis of variance and multiple comparisons of means were performed (Tukey HSD $P \leq 0.05$). The results show that NaM-7CH and SiT-7CH applications significantly increased GP, RL, and radicle and aerial fresh and dry biomass parts. The highest response was recorded in RL with NaM-7CH treatment, 57.8% in Jalapeño M variety, and 86.3% in Santa Fe variety with respect to the control treatment seedlings without HDBC (DW). In general, a NaM anti-stress effect was observed, demonstrating its effectiveness and great potential in sustainable organic agriculture as a low-cost alternative to mitigate the negative effects of abiotic stress.

Index words: *agricultural homeopathy, salt stress, vegetables.*

RESUMEN

En línea con el concepto de economía verde, la producción de alimentos está ganando importancia a nivel mundial utilizando productos naturales. El objetivo de esta investigación fue evaluar el efecto de *Natrum muriaticum* (NaM) *y Silicea Terra* (SiT) como mitigantes del estrés salino (NaCl) en la germinación y crecimiento inicial de plántulas de dos variedades de chile (*Capsicum annuum* L.). El experimento se realizó bajo condiciones controladas, utilizando un diseño completamente al azar con arreglo factorial de (2A × 3B × 5C), en el cual el factor A son las dos variedades (Santa Fe y Jalapeño M), el factor B los tres niveles de NaCl (0, 50 y 100 mM) y el

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factor C, las dinamizaciones centesimales (CH) de los compuestos bioactivos altamente diluidos (CBAD) *Natrum muriaticum* (NaM): NaM-7CH, NaM-13CH y *Silicea terra* (SiT): SiT-7CH, SiT-13CH; como tratamiento agua destilada (AD) como control para un total de 30 tratamientos con cuatro réplicas cada uno. Como variables de respuesta se registró el porcentaje de germinación (PG), tasa de germinación (TG) y a los 14 días la altura de las plántulas (AP), longitud de radícula (LR) y biomasa fresca y seca de radícula y parte aérea. Se realizaron análisis de varianza y comparaciones múltiples de medias (HSD de Tukey $P \leq 0.05$). Los resultados muestran que la aplicación de los CBAD NaM-7CH y SiT-7CH incrementaron significativamente el PG, AP y la biomasa fresca y seca de las radículas y parte del aérea. La mayor respuesta se registró en la LR con el tratamiento NaM-7CH, un 57.8% en la variedad Jalapeño M y 86.3% en Santa Fe con respecto a las plántulas del tratamiento control, sin CBAD (AD). En general se observó un efecto anti-estrés de NaM, lo que demuestra su eficiencia y gran potencial en agricultura orgánica sustentable como alternativa de bajo costo para atenuar los efectos negativos del estrés abiótico.

Palabras clave: *homeopatía agrícola, estrés salino, hortalizas.*

INTRODUCTION

The *Capsicum* genus of the *Solanaceae* family has great economic importance at national and global levels (Aktas, Abak, and Sensoy, 2009). In Mexico, chili (*Capsicum annuum* L.) is among the species with the greatest heterogeneity and biodiversity; due to its wide consumption in fresh, dried, or processed form, it is a species of great cultural and commercial relevance (Hermosillo-Cereceres *et al*., 2008). According to data recorded by FAOSTAT (2018), the world area planted with chili amounts to 1.7 million hectares, with a production of 25.1 million tons where Mexico is among the top three producing countries of this precious vegetable on the American continent. Despite having a wide distribution, production is still insufficient in semi-arid areas such as the state of Baja California Sur, where climatic conditions and irrigation water quality lead to a progressive increase in soil salinity (Batista-Sánchez *et al.,* 2022), an agricultural bottleneck that could face the application of the green economy concept as a fundamental strategy to face the environmental crisis by using natural ecosystem resources dynamically and inclusively, achieving healthier productions (Pineda, González, and Mora, 2017).

Excess salts have serious consequences for the agricultural sector since they can directly affect crop growth and development, causing a decrease in biomass production (Chao *et al*., 2013; Kandil, Shareif, and Gad, 2017; Ortega and López, 2024). Salinity affects plant metabolism through various effects associated with water stress and cytotoxicity derived from excessive absorption of some ions, such as sodium (Na^+) and chloride (Cl^-), which cause a nutritional imbalance in the plant. These harmful effects are often accompanied by oxidative stress due to the generation of reactive oxygen species (Hernández, Ferrer, Jiménez, Barceló, and Sevilla, 2001; Isayenkov, 2012; Batista-Sánchez *et al*., 2024). In the last decade, research has intensified to find viable alternatives to achieve an increase in agricultural production, even with the presence of abiotic stress, and it always aims to not damage the agroecosystem. The use of highly diluted bioactive compounds (HDBC) with plant growth stimulates properties and mitigates the negative effects of abiotic stress, which has been successfully used in several crops, such as *Ocimum basilicum* L. (Mazón-Suástegui *et al*., 2018), *Phaseolus vulgaris* L (Mazón-Suastegui *et al.*, 2020a; Mazón-Suástegui, García, Ojeda, Batista, and Ruiz, 2022), (*Capsicum annuum* L.) Rodríguez-Álvarez, Morales, Batista, and Mazón (2020), *Brassica oleracea* L. (Barbosa, Valério, Siqueira, Salgueiro, and das Neves, 2012), *Solanum lycopersicom* L. (Giardini-Bonfim, Dias, and Ronie, 2012) and other vegetables of commercial interest. The applications of these ultra-diluted substances of natural origin are compatible with the various forms of sustainable, organic, and conventional agricultural production since they contain nanoparticles that favor plant metabolic development. For this reason, the present research aims to evaluate the effect of *Natrum muriaticum* Similia® (NaM) and *Silicea Terra* Similia® (SiT) as salt stress (NaCl) mitigators in germination and initial seedling growth of two chili pepper (*Capsicum annuum* L.) varieties.

MATERIALS AND METHODS

Study Site and Genetic Material

The study was conducted under controlled laboratory conditions (Laboratorio de Fisiotecnología Vegetal, and Laboratorio de Homeopatía Acuícola-Agrícola) at Centro de Investigaciones Biológicas del Noroeste, S. C. (CIBNOR), located in the northwestern city of La Paz, B.C.S., México, at 24° 08′ 10.03″ N and 110° 25′ 35.31″ W, at 7 meters of altitude (Batista-Sánchez *et al*., 2017). Certified seeds of two *Capsicum annuum* varieties were used. Before the experiment, a germination test was performed using the International Seed Testing Association methodology (ISTA, 2010).

Experimental Design and Development

The experiment was conducted using a completely randomized design with a factorial arrangement of (2A × 3B × 5C), where factor A has the two varieties (Santa Fe and Jalapeño M); factor B has the three levels of NaCl (0, 50 and 100 mM); and factor C has the centesimal dynamizations (CH) of the HDBC *Natrum muriaticum* (NaM): NaM-7CH, NaM-13CH and *Silicea terra* (SiT): SiT-7CH, SiT-13CH; distilled water (DW) as control treatment, for a total of 30 treatments (Table 1) with four replicates each.

The treatments with HDBC (NaM-7CH; NaM-13CH; SiT-7CH, and SiT-13CH) were prepared in distilled water from the official medications (*Natrum muriaticum* 6CH, *Natrum muriaticum* 12 CH, *Silicea terra* 6CH and *Silicea terra* 12CH, respectively) of Similia® brand acquired from an authorized supplier (Farmacia Homeopática Nacional®, CDMX, México), which are registered with the Health Ministry of México and officially authorized for use in humans. During their preparation, the basic procedures established by the Mexican homeopathic pharmacopoeia (Secretaría de Salud, 2015) were applied, including centesimal serial dilution (1:99) and vigorous vortex agitation (BenchMixer®, Edison, NJ, USA), according to the technique described by Mazón-Suástegui, Ojeda, García, Batista, and Abasolo (2020a). Seeds were disinfected before sowing by immersion in a 1.5% sodium hypochlorite solution for 10 min, washed three times with deionized water to remove any disinfectant residue, placed on filter paper to dry, and then soaked for 60 min with the corresponding HDBC treatment or with distilled water (DW) in the case of the control treatment. Thirty seeds were sown per Petri dishes (150 × 15 mm) previously autoclaved, covering the bottom with a sheet of sterilized filter paper used as substrate. The dishes were moistened with 30 mL of the saline solutions (50 and 100 mM NaCl) and distilled water (0 mM NaCl), as appropriate, then subsequently incubated in a germination chamber (Lumistell®, model IES-OS, series 1408-88-01, USA) at a temperature of 25±1 °C, 80% humidity and 12 h daily of continuous light for 14 days.

Germination Percentage and Rate

The germination percentage (GP) was recorded daily for 14 days, considering the seed germinated when the radicle was about 1 mm long, and the germination percentage was determined at the end of that period. The germination rate (GR) was calculated using the equation proposed by Maguire (1962): M = n1/t1 + n2/t2 +… n30/t14; where n1, n2,… n30 are the number of seeds germinated at times t1, t2,… t14 (up to 14 days).

Morphometric Variables

After 14 days, 10 seedlings were randomly selected per replicate (40 per treatment); the morphometric variable height seedling (HS) and radicle length (RL) were both determined using an image analyzer (WinRhizo® Regent Instruments Inc. USA), whose operating principle is through direct measurements of digital images obtained by scanning different organs of the seedlings. The fresh and dry biomass of the radicle and aerial part were determined using an analytical balance (Mettler Toledo®, model AG204, USA). The plant tissues were divided into aerial part and radicle, weighed and placed in paper bags, and introduced into a drying oven (Shel-Lab®, model FX-5, series-1000203, USA) at a temperature of 70 °C for 48 h until complete dehydration and weighed again to determine dry biomass.

Statistical Analysis

An analysis of variance (ANOVA) was performed. When a significant difference was found between treatments, the multiple comparison test of means (Tukey's honestly significant difference (HSD), $P \leq 0.05$) was used, using the statistical program Statistica v. 10.0 for Windows (StatSoft, Inc, 2011).

Table 1. Treatments used in the study.

Treatments	Varieties	NaCl	HDBC
		mM	
1	JM	0	DW
2	JM	0	SiT-7CH
3	JM	0	SiT-13CH
4	JM	0	NaM-7CH
5	JM	0	NaM-13CH
6	JM	50	DW
7	JM	50	SiT-7CH
8	JM	50	SiT-13CH
9	JM	50	NaM-7CH
10	JM	50	NaM-13CH
11	JM	100	DW
12	JM	100	SiT-7CH
13	JM	100	SiT-13CH
14	JM	100	NaM-7CH
15	JM	100	NaM-13CH
16	SF	0	DW
17	SF	0	SiT-7CH
18	SF	0	SiT-13CH
19	SF	0	NaM-7CH
20	SF	0	NaM-13CH
21	SF	50	DW
22	SF	50	SiT-7CH
23	SF	50	SiT-13CH
24	SF	50	NaM-7CH
25	SF	50	NaM-13CH
26	SF	100	DW
27	SF	100	SiT-7CH
28	SF	100	SiT-13CH
29	SF	100	NaM-7CH
30	SF	100	NaM-13CH

JM = jalapeño M; SF = santa fe; HDBC = highly diluted bioactive compounds.

RESULTS AND DISCUSSION

Germination Percentage (GP)

The GP analysis showed significant differences between varieties ($P = 0.0000$), NaCl levels ($P = 0.000010$), HDBC treatments ($P = 0.00001$) in the interaction varieties × NaCl ($P = 0.00001$), varieties × HDBC treatments ($P = 0.000083$) and in the triple interaction varieties × NaCl × HDBC treatments ($P = 0.000010$). No significant difference was found in the germination rate (GR). The germination percentage decreased when NaCl increased, with the JM variety being the least affected by this stress (Figure 1A).

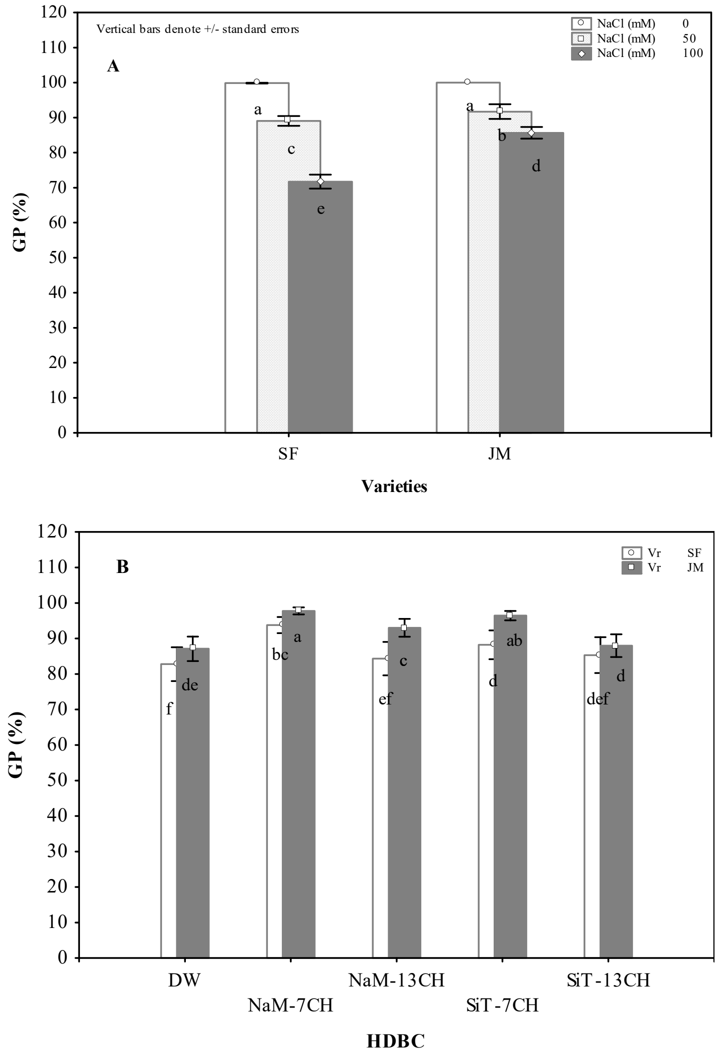

Figure 1. Effect of the interaction varieties × NaCl (A) and varieties × highly diluted bioactive compounds (HDBC); (B) on germination percentage of *Capsicum annuum* seeds. Different letters show statistical differences (Tukey's honestly significant difference (HSD), $P \leq 0.05$).

The results found in this variable (GP) are due to the osmotic effect caused by the presence of salts in the culture medium, making it difficult for the embryo to absorb water and consequently affecting the metabolic processes at the cellular level inherent to seed germination (Wang, Li, and White, 2020). This report coincides with that observed by Batista-Sánchez *et al*. (2017) when studying *Ocimum basilicum* L. germination and initial growth under saline conditions (0, 50, 100, and 150 mM NaCl), finding a decrease in GP as salt concentrations increased in the medium. Other authors, such as Goykovic-Cortés, Nina, and Calle (2014), also reported a decrease in tomato seed germination when subjected to different types of abiotic stress. When the seeds received HDBC treatments, an increase in GP was observed (Figure 1B, Figure 2A, and Figure 2B) with respect to the control treatment (DW). For the JM variety, the greatest response was with NaM-7CH treatment and for SF with NaM-7CH and SiT-7CH treatments. This finding may be determined by stimulation at the cellular level of the biological processes that lead to germination since the applied HDBC treatments contain nanoparticles in their active ingredient capable of inducing a biological response (Abasolo-Pacheco *et al*., 2020). Similar results were reported by Mazón-Suástegui *et al*. (2020b) when they applied NaM-7CH to *Salicornia bigelovii* (Torr) seeds and obtained a significant increase in GP with respect to the seedlings of the control treatment. The beneficial effects of HDBC treatments on the germination of *Crotalaria juncea* L. seeds have also been observed (Silveira, 2008[1]).

[1] Silveira, J. C. (2008). *Germinação de sementes de crotalária e de alface com o preparado homeopático de ácido giberélico. Departamento de Fitotecnia.* Dissertação de Mestrad. Universida de Federal de Viçosa, Brasil. Available on: https://locus.ufv.br/server/api/core/bitstreams/9a32edb5-97c5-437d-b0d4-b0f87274067d/content

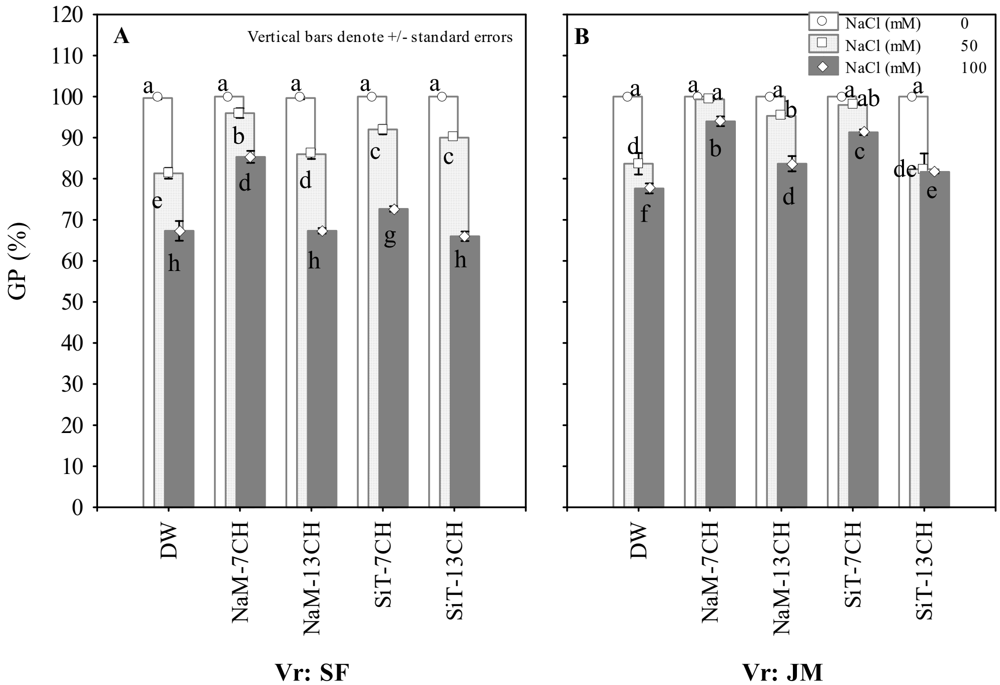

Figure 2. Effect of the interaction varieties × NaCl × highly diluted bioactive compounds (HDBC) on the germination percentage of *Capsicum annuum* seeds. Different letters show statistical differences (Tukey's honestly significant difference (HSD), $P \leq 0.05$).

Seedling Height of *Capsicum annuum*

For height seedlings (HS), significant differences were observed between varieties (*P = 0.001*), NaCl levels (*P = 0001*), HDBC treatments (*P = 0.0001*), in the interaction varieties × NaCl (*P = 0.0008*), varieties × HDBC treatments (*P = 0.00001*) and in the triple interaction varieties × NaCl × HDBC treatments (*P = 0.00001*). Through the interactive analysis, a significant decrease in this variable was found when NaCl presence increased from a moderate (50 mM) to a severe (100 mM) level, with greater emphasis on the SF variety (Figure 3A). The negative effect of salt presence on this variable is determined by Cl^- and Na^+ accumulation in the culture medium, causing a slowdown in metabolic processes, nucleic acid synthesis, enzymatic activity, and hormonal balance (Agüero-Fernández *et al*., 2019).

When both varieties received HDBC treatments, the greatest height was observed in JM variety seedlings treated with NaM-7CH (Figure 3B), with an increase of 64% with respect to the seedlings of the control treatment (DW). The seedlings of the SF variety also responded positively to the application of the HDBC treatments NaM-7CH, NaM-13CH, and SiT-7CH. In the analysis of the triple interaction varieties × NaCl × HDBC treatments, the results also revealed an increase in HS when the seedlings were treated with greater emphasis on the NaM-7CH treatment (Table 4), even when they were subjected to saline concentrations (NaCl) from moderate (50 mM) to severe (100 mM), a favorable response was recorded for this variable. The results observed in HS when they received HDBC dynamizations can be explained by the presence of trace elements in the active ingredient; one of these is magnesium (Mg), chemically present in NaM-7CH, essential for chlorophyll molecule formation, therefore, of vital importance in photosynthesis, which is the main process of plant biomass production from nutrients and light energy (Mazón-Suástegui *et al*., 2020b). Furthermore, Mg has a predominant role in the enzymatic activity associated with carbohydrate metabolism (Xiao, Hu, Chen, Yang, and Hua, 2014).

Radicle Length of *Capsicum annuum* Seedlings

In the variable radicle length (RL), the results of the analysis showed significant differences between varieties (*P = 0.00001*), NaCl levels (*P = 0.000001*), HDBC treatments (*P = 0.0001*), in the interaction´s varieties × NaCl (*P = 0.00001*), varieties × HDBC treatments (*P = 0.0002*) and in the triple interaction varieties × NaCl × HDBC treatments (*P = 0.00008*). A decrease in this variable was observed when salt levels (NaCl) were higher for both varieties under study (Figure 4A), which has the greatest impact on the SF variety with the application of 100 mM NaCl. This result can be explained by the phytotoxicity caused by NaCl, which inhibits water absorption and hinders the development of cell division and elongation processes (Lamz and González, 2015; Agüero-Fernández *et al*., 2019).

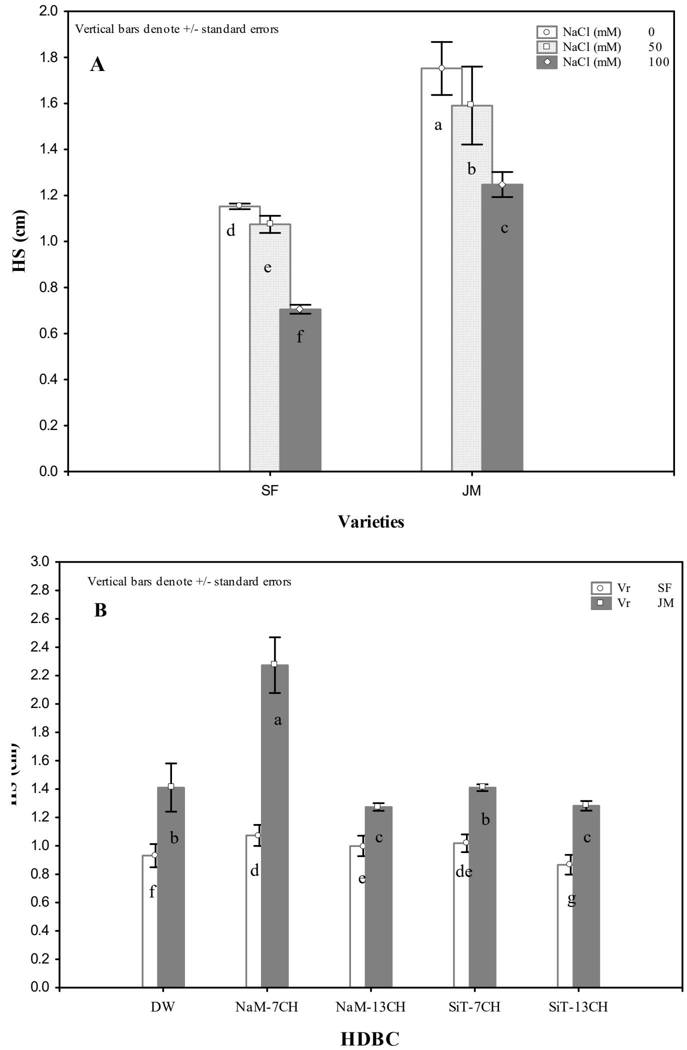

Figure 3. Effect of variety × NaCl (A) and variety × highly diluted bioactive compounds (HDBC); (B) interactions on average *Capsicum annuum* seedling height. Different letters show statistical differences (Tukey's honestly significant difference (HSD), $P \leq 0.05$).

When analyzing the data from the seedlings that received HDBC treatments, the results (RL) revealed a positive response to the NaM-7CH treatment in both varieties, with an increase of 57.80% in the JM variety and 86.30% in SF compared to the seedlings of the control treatment (DW) (Figure 4B). In the interactive analysis varieties × NaCl × HDBC treatments, an increase in RL was observed in the *Capsicum annuum* seedlings of the two varieties treated with NaM-7CH; for the three levels of NaCl (0, 50, 100 mM), this variable was higher than the control treatment (DW) (Table 4). This result confirms the anti-stress effect of *Natrum muriaticum* (NaM) previously reported by Mazón-Suástegui *et al*. (2018); when the interaction of two NaM dynamizations was studied at different saline levels in the cultivation of *Ocimum basilicum*, an increase in tolerance of the negative effects of abiotic stress was observed in the plants that received the NaM-7CH treatment.

Fresh and Dry biomass of *Capsicum annuum* Seedlings

No significant difference was found between varieties for fresh radicle biomass (FRB). However, a significant difference was observed between NaCl levels (P = *0.00024*), HDBC treatments (P = *0.0069*), in the variety × NaCl interaction (P = 00007), and in the interaction triple variety × NaCl × HDBC treatments (P = 0.007). The interactive study allowed observing the negative effect of NaCl on this variable, with an impact on both varieties when salt concentration was higher (Table 2), which may be related to salt phytotoxic characteristics when it is found in excess (Pan *et al.,* 2021). When the plants were treated with HDBC, a differential response was observed, where the JM variety recorded the highest FBR value when SiT-7CH was applied in a non-saline medium (0 mM NaCl). When the seedlings were subjected to saline conditions, the most effective treatment was NaM-7CH. For both varieties under study, a higher FRB was observed with this treatment, even when saline conditions were moderate to severe (Table 4).

In the dry radicle biomass (DRB), no significant difference between varieties was observed except for a difference between NaCl levels ($P = 0.00002$), HDBC treatments ($P = 0.006$) in the variety × NaCl interaction ($P = 0.0007$), and in the interaction of the triple variety × NaCl × HDBC treatments ($P = 0.00768$). The analysis showed a decrease in this variable when the seedlings were subjected to saline conditions (NaCl), with an impact of 8.60% in the JM variety and 44.10% in the SF variety, at a NaCl level of 100 mM (Table 2). *Capsicum annuum* seedlings treated with HDBC increased BSR even when subjected to moderate to severe saline stress (NaCl). A better response was noted with the treatment of SiT-7CH and NaM-7CH (Table 4). Similar results were obtained by Lensi, Siqueira, and Silva (2010), who demonstrated the effectiveness of dynamized NaM (NaM-6CH, and NaM-30CH) in common bean plants (*Phaseolus vulgaris* L.) since they did not show signs of toxicity during their growth stage. On the other hand, the mechanism of action of HDBCs may involve physiological changes that lead to the formation of secondary metabolic products related to the defense mechanism of the treated plants (Lensi *et al*., 2010; Sen, Chandra, Khatun, Chaterjee, and Das, 2018; Meneses, 2024; Damiani, Givacheski, Trzimajewski, and Deboni 2024).

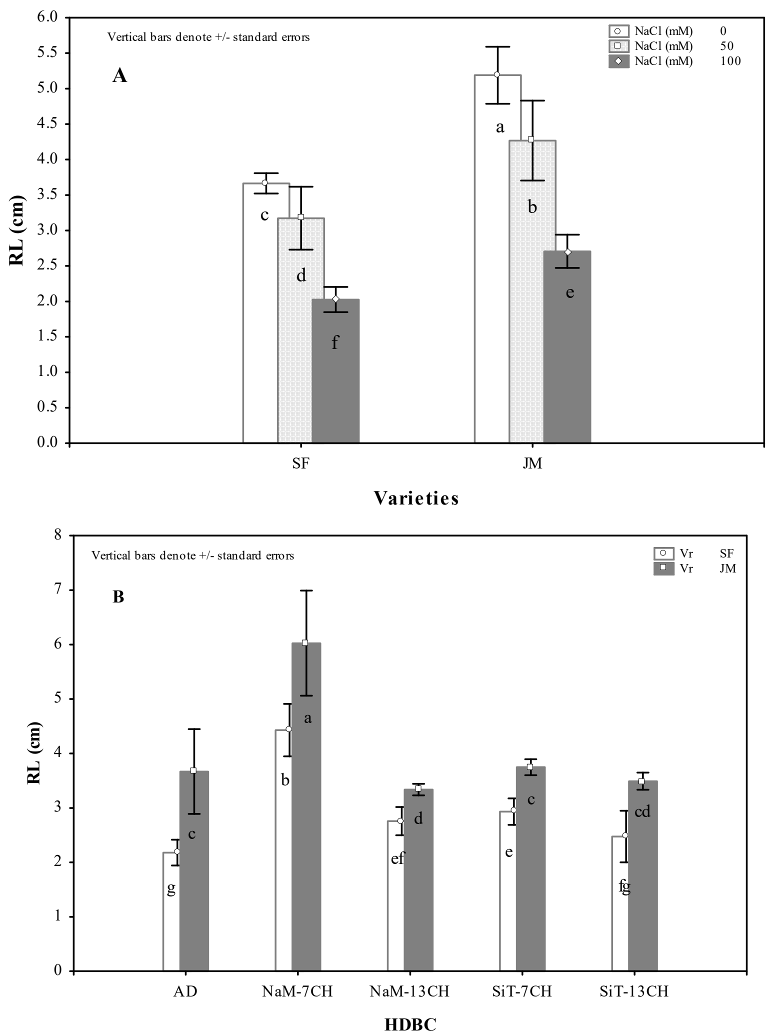

Figure 4. Effect of the interaction varieties × NaCl (A) and varieties × highly diluted bioactive compounds (HDBC) (B) on radicle length of *Capsicum annuum* seedlings. Different letters show statistical differences (Tukey's honestly significant differences (HSD), $P \leq 0.05$).

Table 2. Effect of variety × NaCl interaction on fresh and dry radicle biomass of *Capsicum annuum* seedlings.

Variety	NaCl	FRB	DRB
	mM	- - - - - - - - - - - - - - - - - mg	- - - - - - - - - - - - - - - - -
JM	0	168.8 ab	70.8 ab
JM	50	165.8 ab	69.7 ab
JM	100	153.7 b	64.5 b
SF	0	194.2 a	81.6 a
SF	50	151.4 b	63.5 b
SF	100	108.6 c	45.6 c

FRB = fresh radicle biomass; DRB = dry radicle biomass. Different letters in the same column show statistical differences (Tukey's highly significant difference (HSD), $P \leq 0.05$)

The results obtained in the present study may be possible due to the direct action of the nanoparticles contained in the applied dynamizations. Silica – or the raw material from which the SiT nanomedicine is produced – has a positive influence on nutrient balance (Tichavsky, 2007), increases synergies, reduces antagonisms, and also the absorption of elements that can become phytotoxic, such as excess NaCl. NaM also contains trace elements, such as magnesium (Mg), which are of great value in the synthesis of carbohydrates and important as sources of metabolic reserves and fuels (Mazón-Suástegui *et al*., 2020a).

In the fresh biomass of aerial part (FBA P), significant differences were found between varieties (*P = 0.00001*), NaCl levels (*P = 0.00001*), HDBC treatments (*P = 0.0001*), in the interaction´s varieties × HDBC treatments (*P = 0.00005*) and varieties × NaCl × HDBC treatments (*P = 0.00038*), no significant difference was observed in varieties × NaCl. When analyzing the result of the interactive analysis, an increase in FBAP was observed when the seedlings were treated with nanomedicines, with greater emphasis on the NaM-7CH treatment (Table 3). In the triple interaction varieties × NaCl × HDBC treatments, the greatest response of this variable (FBAP) was observed when the JM variety seedlings were subjected to saline stress of 50 mM NaCl and received the NaM-7CH treatment, the increase concerning the control treatment (DW) at this same stress level was 63.20% (Table 4).

Table 3. Effect of the variety × HDBC interaction on fresh and dry biomass of the aerial part of *Capsicum annuum* seedlings.

Variety	HDBC	FBAP	DBAP
		- - - - - - - - - - - - - - - - - mg	- - - - - - - - - - - - - - - - -
JM	DW	259.6 b	116.9 b
JM	NaM-7CH	325.5 a	144.8 a
JM	NaM-13CH	259.9 b	115.7 b
JM	SiT-7CH	311.6 ab	140.3 ab
JM	SiT-13CH	263.3 b	118.6 b
SF	AD	208.6 cd	93.9 c
SF	NaM-7CH	242.7 bc	109.2 bc
SF	NaM-13CH	230.6 cd	103.9 c
SF	SiT-7CH	230.8 c	103.9 cd
SF	SiT-13CH	208.9 cd	91.5 d

HDBC = highly diluted bioactive compound treatments; FBAP = fresh above-ground biomass; DBAP = dry above-ground biomass. Different letters in the same column show statistical differences (Tukey's highly significant difference (HSD), $P \leq 0.05$).

Table 4. Effect of the interaction varieties × NaCl × HDBC treatments on the morphometric variables of *Capsicum annuum* seedlings.

Vr	NaCl	HDBC	HS	RL	FRB	DRB	FBAP	DBAP
	mM		- - - - - - - cm	- - - - - - -	- - - - - - - - - - - - - - - - mg		- - - - - - - - - - - - - - - -	
JM	0	DW	2.07 c	6.56 c	229.6 abcd	46.4 bcd	316 abcd	142 abcd
JM	0	SiT-7CH	1.48 de	4.19 de	281.6 a	83.7 abc	311.6 bcde	140.2 bcde
JM	0	SiT-13CH	1.38 ef	4.11 de	210.6 bcd	76.3 abcd	249.3 cdef	112.2 cdefg
JM	0	NaM-7CH	2.44 ab	7.36 b	199.3 abc	96.4 a	322.6 abc	145.2 abc
JM	0	NaM-13CH	1.37 efg	3.71 efgh	108 cd	45.3 cd	251.3 cdef	113.1 cdef
JM	50	DW	1.22 hij	3.17 ghij	110 bcd	46.2 bcd	237.6 efg	106.9 efg
JM	50	SiT-7CH	1.34 fgh	3.25 fghi	175.6 abcd	73.7 abcd	345.6 ab	155.5 ab
JM	50	SiT-13CH	1.28 fghi	3.18 ghij	155.3 abcd	65.2 abcd	271.3 bcdef	122.1 bcdef
JM	50	NaM-7CH	2.85 a	8.47 a	187 abc	78.5 abc	388 a	174.6 a
JM	50	NaM-13CH	1.24 ghij	3.25 fghi	140 abcd	58.8 abcd	243.6 defg	109.6 defg
JM	100	DW	0.93 lm	1.27 n	112.6 bcd	47.3 bcd	225.3 fgh	101.4 fgh
JM	100	SiT-7CH	1.39 def	3.79 defg	175 abcd	73.6 abcd	277.6 bcde	124.9 bcdef
JM	100	SiT-13CH	1.17 ijk	3.18 ghij	173.3 abcd	71.4 abcd	269.3cdef	121.2 cdef
JM	100	NaM-7CH	1.52 d	2.32 klm	212.6 ab	89.3 ab	254 cdef	114.3 cdef
JM	100	NaM-13CH	1.20 ij	3.04 ij	173.3 abcd	72.8 abcd	276 bcde	124.2 bcdef
SF	0	DW	1.12 jk	2.85 ijk	198.6 abc	83.4 abc	241 defg	108.4 defg
SF	0	SiT-7CH	1.16 ijk	3.87 def	199.3 abc	83.7 abc	239.6 efg	107.8 efg
SF	0	SiT-13CH	1.12 jk	3.85 def	200 abc	84 abc	256.3 cdef	115.3 cdef
SF	0	NaM-7CH	1.20 ij	4.36 d	190 abc	85.8 abc	232 fg	104.4 fg
SF	0	NaM-13CH	1.16 ijk	3.38 fghi	183 abcd	76.8 abcd	225 fgh	101.2 fgh
SF	50	DW	1.06 kl	2.40 k	151 abcd	63.4 abcd	211 fgh	94.9 fgh
SF	50	SiT-7CH	1.12 jk	2.60 jk	131.3 abcd	55.1 abcd	217.3 fgh	97.8 fgh
SF	50	SiT-13CH	0.83 mn	1.42 n	131.3 abcd	55.1 abcd	230.3 fg	103.6 fg
SF	50	NaM-7CH	1.23 hij	6.29 c	211.6 abc	88.9 abc	253cdef	113.8 cdef
SF	50	NaM-13CH	1.11 jk	3.13 hij	131.3 abcd	55.1 abcd	219.3fgh	98.7 fgh
SF	100	DW	0.60 o	1.28 n	81.6 d	34.3 d	174gh	78.3 gh
SF	100	SiT-7CH	0.77 nñ	2.32 kl	81.6 d	34.3 d	152.3h	68.5 h
SF	100	SiT-13CH	0.65 ño	1.64 mn	111.6 bcd	46.9 bcde	206fgh	92.7 fgh
SF	100	NaM-7CH	0.78 n	3.14 hij	135.6 abcd	56.7 abcd	243.3defg	109.5 defg
SF	100	NaM-13CH	0.71 nño	1.75 lmn	132 abcd	55.7 abcd	247.6cdef	111.4 cdefg

Vr = varieties; HDBC = highly diluted bioactive compound treatments; HS = height seedling; RL = radicle length, FBAP = fresh shoot biomass, FRB = fresh radicle biomass, DBAP = dry shoot biomass, DRB = dry radicle biomass. Different letters in the same column show statistical differences (Tukey's highly significant difference (HSD), $P \leq 0.05$).

For the dry biomass of aerial part (DBAP), a significant difference was found between varieties ($P = 0.0001$), NaCl levels ($P = 0.00001$), HDBC treatments ($P = 0.00001$), in the interaction varieties × HDBC treatments ($P = 0.00005$) and in the triple interaction varieties × NaCl × HDBC treatments ($P = 0.0003$), no significant difference was found in varieties × NaCl. The results showed that the NaM-7CH treatment increased DBAP in both varieties under study (Table 3). These results coincide with those obtained by Rodríguez-Álvarez *et al.* (2020) when they applied NaM to *C. annuum* L. var. Glabriusculum plants that were subjected to abiotic stress by adding NaCl (200 mM) in a hydroponic system, observed an anti-stress effect of NaM.

When analyzing the effect of different salinity (NaCl) levels on *Capsicum annuum* L. seedlings and their interaction with HDBC treatments, the results revealed a differential response (Table 4). An increase in DBAP was observed when the seedlings of both varieties received the NaM-7CH treatment; even under saline conditions, the response was superior to the control treatment (DW), confirming that the highly diluted treatment used can activate defense mechanisms in the seedlings that have yet to be studied to attenuate the negative effects of saline stress (NaCl). In this same field of research, the anti-stress effectiveness of the 7CH dynamization of the NaM nanomedicine has already been proven in *Ocimum basilicum* and *Phaseolus vulgaris* (Mazón-Suástegui *et al*., 2018 and 2020a).

CONCLUSIONS

Treating *C. annuum* seedlings with highly diluted bioactive compounds NaM and SiT, the response variables increased, favoring the reduction of the negative effects of salt stress (NaCl), with a greater emphasis on seedling height and radicle length variables and NaM-7CH, which was the most effective treatment. Due to their high dilution, HDBC have the highest safety and compatibility with the green economy concept. In general, the results obtained reveal the anti-stress effect of this nanomedicine and its high potential to be used as a salt stress mitigator in sustainable, organic, or even conventional agriculture.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The data set in this experiment is available by the corresponding author if it is requested.

COMPETING INTERESTS

The authors declare no competing interests.

FINANCING

Not applicable.

AUTHORS' CONTRIBUTIONS

Original draf preparation: C.M.O.S., and J.M.M.S. Formal analysis: B.M.A. Review and editing: D.B.S., and B.M.A. Methodoly: D.B.S., and M.G.B. Visualization and supervision: J.M.M.S. and C.M.O.S.

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