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Original Article

Morpho-physiological Characteristics of Basil *(Ocimum basilicum* L.) under NaCl-stress and *Rhizophagus fasciculatum* as NaCl-stress Mitigator

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Abstract

Salinity stress is one of the main problems limiting growth and development of cultivated species. The objective of this study was to assess NaCl-stress basil plants (*Ocimum basilicum* L.) cv. 'Nufar' and to determine whether the mitigating effect of an arbuscular mycorrhizal fungus strain (AMF). A completely randomized factorial design was used considering three NaCl concentration (0, 50 and 100 mM) as factor 1 and presence or absence of AMF (0 and 10 g of inoculum) as factor 2, with four replicates per treatment and four plants per repetition. The assessed response variables were, fresh and dry of aerial part and root, root length, leaf area, relative water content, water potential, plant height, number of spores and mycorrhizal colonization percentage after 20 and 50 days (T_{20}, T_{50}) of the experiment. The results showed greater values in all variables in the control group (0 mM NaCl) than in plants inoculated with *R. fasciculatum* with T_{20} and T_{50}; although values decreased as NaCl concentration increased; the tendency to increase was maintained even in at 50 and 100 mM of NaCl with AMF with respect to 50 and 100 mM NaCl without AMF. The AMF colonization percentage decreased as NaCl concentration increased. Nonetheless, the development and growth response for all variables in the inoculated plants with AMF was greater *vs* non-inoculated, which suggests that basil plant inoculation with AMF has a positive effect in mitigating NaCl stress.

Keywords: arbuscular mycorrhizal fungi (AMF); biomass; colonization; water potential

Introduction

Salinity is one of the main abiotic factors that worldwide negatively affect agriculture production (Iqbal *et al*., 2015). The increase of salt concentration in soil is an abiotic factor that stresses plants and decreases their capacity to absorb water, limiting important metabolic processes, affecting osmotic equilibrium, nutrient absorption, hydraulic and stomatal conductivities, raw photosynthesis rate and intracellular CO_2 concentration, which directly damages plant development (Batista-Sánchez *et al*., 2017). Salinity stress affects water absorption in plants in the radicle area because of a decrease of osmotic potential and consequently plant soil hydration uptake (Batista-Sánchez *et al*., 2015). It is estimated that more than 800 million hectares have been affected by salinity around the world, which represents a loss of more than 12 billion USD annually for the agricultural industry (Khalig *et al*., 2014). In México, the salinization of agricultural soil affects 3.2% of its territory (SEMARNAT, 2009), with salinity problems being more frequent in dry lands where water used for irrigation is rich in salts, thus consequently causing further and progressive soil deterioration (Zamudio-González *et al*., 2004).

Arbuscular mycorrhizal fungi (AMF) are plant root symbionts, considered to be the cornerstone of mutualism in terrestrial ecosystems (Wimmer *et al*., 2016). Studies have demonstrated that AMF are capable of promoting plant growth while providing increased tolerance to soil salinity in many plants species by a use of diverse mechanisms, such as favoring nutrient acquisition, plant growth hormone production, rhizosphere development as well as improving

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soil conditions (Elhindi *et al*., 2017). Research on AMF efficiency in increasing plant halotolerance has been performed with horticulture (Abdel-Latef and Chaoxing, 2011) and industrial (Yamato *et al*., 2008) cultivation, while scarce reports exist on aromatic species. From a scientific and technological point of view, it is important to study these microorganisms and their mitigating affects on salinity stress in aromatic species, such as basil and others of commercial importance.

Basil (*Ocimum basilicum* L.) is an aromatic plant of the family *Lamiaceae* that is native to southern Asia and is widely distributed in tropical and subtropical regions (Briseño *et al*., 2013). It is a plant species considered to have important applications in medicine and culinary areas (Makri and Kintzios, 2008). Basil has different therapeutic applications, such as the ability to reduce blood pressure and glucose and cholesterol levels in blood, also it has anti-inflammatory and anti-stress properties (Tomar *et al*., 2010). The cultivation of this species generates important economical income for farmers, but salinity in irrigation water and soil limit production, especially of the varieties sensitive to salinity. The objective of this study was to assess the mitigating effect of arbuscular mycorrhizal fungus (*Rhizophagus fasciculatum*) strain in 'Nufar' cv. in basil plants subjected to NaCl-stress during the initial growth stage.

Materials and Methods

Study area

This study was developed at the experimental field of Centro de Investigaciones Biológicas del Noroeste (CIBNOR) located 24° 08' 10.03 N and 110° 25' 35.31W northwest of La Paz, Baja California Sur, Mexico at 7 masl. The experiment was done under a white anti-aphid mesh-house with 30% shade (55 mesh) and cover with a second black mesh with 35% shade (20 mesh). The experimental site has a type Bw (h') hw (e) climate, characterized as semiarid with xerophilous vegetation (García, 2004). During the experimental time, the average, maximum and minimum temperatures were 29, 30 and 20 °C, respectively; average relative humidity was 69%, dewpoint 22 °C, showing a total precipitation of 14.6 mm and average solar radiation of 293.3 W m^2. These variables were recorded daily using a portable meteorological station (Vantage Pro2® Davis Instruments, U.S.A.) placed inside the mesh house.

Genetic material

The seeds used were from basil 'Nufar' cv. which were provided by the Vis Seed Company, Inc. (http://www.visseed.com/) (Arcadia, California, USA). The arbuscular mycorrhizal fungus (AMF) was a commercial strain of *Rhizophagus fasciculatum* which was provided by from Republic of Cuba. This strain contains about 50 to 70 spores per gram. The morphological strain description showed globose shape, hyaline colour to pale cream and could reach a run travel from 60 to 110 micrometers. The number of pairs constituted three layers (Rodríguez *et al*., 2004).

Experimental design

A completely randomized design with factorial arrangement was used, considering three NaCl concentrations (0, 50 and 100 mM) as factor one and the presence or absence of AMF *R. fasciculatum* (control and 10 g of inoculum) as factor two. The factorial arrangement was 3×2 treatments with four replicates per treatment and four plants per repetition.

Experimental conditions

Seeds were previously disinfected by 5-min immersion in a calcium hypochlorite solution containing 5% of active chlorine and subsequently washed with distilled and sterilized water. The seeds were seeded in polystyrene 200-well trays with the commercial substrate previously described. To maintain substrate humidity, subsequently after sowing, daily irrigation was applied to achieve a homogeneous emergence. When seedlings reached an average height of 15 cm, they were transplanted in 1-kg plastic pots with the same sterilized commercial substrate according to the label, placing one plant per pot with daily application of 250 mL of water with 0.35 dS m^{-1}, pH 6.7 and nutritional solution according to Samperio (1997) and modified in P content according to Swift (2002).

Spore content in the inoculum and mycorrhizal colonization percentage

Inoculation of *R. fasciculatum* was performed at the moment of transplanting, applying 10 g of AMF to each seedling, equivalent to an average content from 50-70 spores per gram of the product. The colonization percentage was assessed 50 days subsequent to NaCl treatment. Previous to inoculation, the spores were extracted following the wet sieving and decanting method described by Daniels and Skipper (1982) and modified by Utobo *et al*. (2011). Colonization percentage was calculated by the methodology described by Hashem et al. (2014) through the following formula:

Colonization percentage = Total colonized segments/Total studied segments × 100.

NaCl treatments

One week after transplanting, NaCl was gradually applied according to the proposed experimental design and the methodology of Murillo-Amador *et al*. (2007), applying daily 250 mL of the corresponding solution to each pot. This amount was enough to drain and avoid NaCl accumulation in the substrate. This was confirmed when electrical conductivity (CE) and pH of drained water were measured and then compared with CE and pH of the original NaCl concentration during the experimental period.

Morphometric variables

The first and second biomass production measurement was performed 20 and 50 days after applying the NaCl treatments. The samples collected were transferred to the plant physiology laboratory. The leaf area (cm^2) was determined by integrated leaf area (Li-Cor®, Model-LI-3000A, series PAM 1701, USA). Fresh and dry biomass (g)

were measured with an analytical balance (Mettler® Toledo, Model AG204, USA). Dry biomass was obtained by placing leaves and stems in paper bags that were introduced into an oven (Shel-Lab®, Model FX-5, series-1000203, USA) at a temperature of 70 °C until a constant weight (approximately 72 h). Total biomass crop was performed at day 50, which included the collection of the complete plant and separation of leaves, stems and roots. The plant height (cm), root length (cm), fresh and dry biomass (g) of the aerial part (leaves and stems), root and foliar area (cm^2) were measured.

Physiological variables

Relative water content (RWC) was assessed 20 and 50 days after applying NaCl treatments, following Yamasaki and Dillenburg (1999) method. Leaves were collected from the middle part of each plant to avoid decreasing age effect. The collected samples were transferred to the laboratory where three disks of 3.14 cm^2 of each leaf were cut with a hole puncher (Model DIN® 7200, México); then, the disks were subsequently weighed to obtain fresh (FW) and turgid (TW) weights. Turgid was obtained by placing the disks in distilled water inside a Petri dish and during this process (imbibition), the disks were weighed periodically after eliminating surface water with absorbent paper. At the end of the imbibition process, the disks were placed in an oven at 80 °C during 48 h to get the dry weight (DW). Fresh, turgid and dry weights were obtained using an analytical balance with 0.0001 g precision (Mettler® Toledo, Model AG204, USA). The relative water content (RWC %) was calculated using the values of FW, TW and DW using the following equation:

$$RWC\ (\%) = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW}} \times 100$$

Water potential (Ψw) was measured 20 and 50 days after applying the NaCl treatments. The leaves were collected at the most critical hour (12:00 h), considering the interval in which the highest temperatures were recorded during the day. The Ψw (MPa) was determined with a water potential meter (Dewpoint Water Potential Meter Model WP4-T, Decagon® Devices, USA) for soil and botanical samples (grains, seeds, leaves, plant tissues in general), which functioned according to the dew point principle with sensors, a condensation mirror an infrared light. Starting from the collected leaves, disks of 9.60 cm^2 were obtained by using a hole puncher (Model DIN® 7200, México), and subsequently each disk was placed inside the equipment bucket.

Statistical analysis

Analyses of variance (ANOVA) and multiple comparison of means (Tukey HSD p = 0.05) were performed using Statistica v. 10.0 for Windows (StatSoft®, 2011).

Results

Morpho-physiological variables assessed at day 20 after applying NaCl

The analysis of the interaction factors (NaCl×AMF) showed that fresh and dry biomass of the aerial part (FBAP and DBAP) and leaf area (LA) decreased as NaCl concentrations increased, showing highest values at 0 mM NaCl in plants inoculated with AMF and lowest in those subjected to 100 mM of NaCl without AMF; nonetheless, fresh and dry biomass and leaf area increased in 50 and 100 mM of NaCl with AMF compared to 50 and 100 without AMF (Table 1).

The factor interaction analysis showed that water potential (Ψw) values were less negative in plants subjected to 0 mM NaCl and inoculated with AMF and most negative values in those subjected to 100 mM NaCl without AMF. The Ψw showed lowest negative values in 50 and 100 mM of NaCl with AMF compared with 50 and 100 without AMF (Table 1). The effect of applying NaCl on relative water content (RWC) showed a greatest RWC at 0 mM NaCl with AMF but decreased at 100 mM NaCl without AMF (Table 1).

Morpho-physiological variables assessed on day 50 (crop) after applying NaCl

The interaction factors (NaCl×AMF) analysis showed that plant height (PH), root length (RL) fresh and dry biomass of aerial part (FBAP and DBAP), fresh and dry root biomass (FRB and DRB) and leaf area (LA) decreased as NaCl concentrations increased. These variables showed highest values in those plants treated with 0 mM NaCl with AMF and lowest in those plants subjected to 100 mM NaCl

Table 1. Effect of NaCl-stress on morpho-physiological characteristics of basil plants at 20-days treatment (T_{20}) and inoculated with an arbuscular mycorrhizal fungus (*Rhizophagus fasciculatum*) strain as NaCl-stress mitigator

NaCl (mM)	AMF (g)	FBAP (g)	DBAP (g)	LA (cm^2)	Ψw (MPa)	RWC (%)
0	AMF	97.05±2.47a	17.10±0.64a	1655.25±22.05a	-0.12±0.07a	97.11±2.37a
50		66.61±2.33b	9.20±0.16c	1220.21±15.94c	-1.35±0.25bc	86.71±1.69b
100		56.86±1.46c	7.65±0.51d	1005.36±32.53d	-1.75±0.15c	81.52±5.08b
0	Non-AMF	67.99±6.29b	11.20±1.26b	1413.54±49.73b	-0.76±0.32b	82.84±0.89b
50		53.00±1.46c	8.55±0.36d	1160.51±72.16d	-1.49±0.22c	81.75±1.64b
100		43.79±5.52d	7.20±0.14e	948.55±110.64e	-2.71±0.42d	66.47±0.64c
Significance level		***	***	***	*	**

NaCl= Sodium chloride (mM), AMF= arbuscular mycorrhizal fungus (AMF= with AMF, Non-AMF= without AMF), FBAP= fresh biomass of aerial part (g), DBAP= dry biomass of aerial part (g), FA= leaf area (cm^2), Ψw= water potential (MPa), RWC= relative water content (%). Average and standard deviation values with different letters in the same column are statistically different (Tukey HSD, p = 0.05). Level of significance of ANOVA: *= $P \leq 0.05$. **= $P \leq 0.01$. ***= $P \leq 0.001$ (media ± SD).

Table 2. Effect of NaCl-stress on morpho-physiological characteristics of basil plants at 50-days treatment (T_{20}) and inoculated with an arbuscular mycorrhizal fungus (*Rhizophagus fasciculatum*) strain as NaCl-stress mitigator

NaCl (mM)	AMF (g)	PH (cm)	RL (cm)	FBAP (g)	DBAP (g)	FRB (g)
0	AMF	45.25±0.96a	40.50±0.58a	146.27±4.99a	36.00±1.10a	125.08±6.60a
50		35.75±0.50b	31.50±1.29bc	94.01±2.19c	18.62±0.82b	95.65±4.59b
100		29.75±0.96c	29.75±0.50c	86.19±1.35c	15.75±0.62c	81.07±2.60c
0	Non-AMF	36.75±0.50b	32.00±0.82b	114.69±6.71b	17.65±0.95b	97.95±1.43b
50		26.75±2.22d	30.25±0.50c	74.77±5.14d	14.15±0.37c	92.42±5.31bc
100		25.00±1.41d	27.50±1.29d	65.86±4.26d	11.72±0.43d	69.87±7.32d
Significance level		**	***	*	***	***
NaCl (mM)	AMF (g)	DRB (g)	FA (cm^2)	RWC (%)	Ψw (MPa)	Col (%)
0	AMF	27.53±4.79a	2775.25±162.10a	96.00±2.13a	-0.24±0.05a	64.50±3.00a
50		10.75±0.74b	1401.30±73.75c	73.96±2.80a	-0.70±0.14b	56.25±4.35b
100		10.02±0.53b	1230.35±54.96cd	63.54±2.90a	-1.09±0.03cd	46.50±2.08c
0	Non-AMF	11.35±1.27b	2254.18±213.51b	86.60±3.73a	-0.92±0.10c	---
50		9.52±0.74b	1308.16±48.14cd	64.04±1.44a	-1.03±0.01c	---
100		8.00±1.30c	1080.36±24.45d	56.72±2.91a	-1.25±0.05d	---
Significance level		***	***	*ns*	***	***

NaCl= Sodium chloride (mM), AMF= Arbuscular mycorrhizal fungi (AMF= with AMF, Non-AMF= without AMF), PH= plant height (cm), RL= root length (cm), FBAP= fresh biomass of aerial part (g), DBAP= dry biomass of aerial part (g), FRB= fresh root biomass (g) DRB= dry root biomass (g), LA= leaf area (cm^2), RWC= relative water content (%), Ψw= water potential (MPa), Col (%)= colonization percentage (%). Average and standard deviation values with different letter in the same column are statistically different (Tukey HSD, $p = 0.05$). Level of significance of ANOVA: *= $P \leq 0.05$. **= $P \leq 0.01$. ***= $P \leq 0.001$ (media ± DE).

without AMF. Water potential (Ψw) had lower negative values at 0 mM followed by 50 mM NaCl, both with AMF while the most negative values were showed at 100 mM NaCl without AMF (Table 2).

Relative water content did not show significant differences among NaCl concentrations; although, the highest values were at 0 mM with and without AMF, followed by 50 with and without AMF (Table 2).

Mycorrhizal colonization percentage

The mycorrhizal colonization percentage decreased as NaCl increased, which was highest in control (0 mM) and decreased at 50 and 100 mM NaCl (Table 2).

Discussion

Previous studies have been reported that in saline soils, AMF increased nutrient intake in plants, especially P with precipitates of Ca^{2+}, Mg^{2+} and Zn^{2+} ions (Porras-Soriano *et al.*, 2009). In addition to nutritional improvement, AMF benefits physiological processes, such as water absorption when roots hydraulic conductivity increases and improves the osmotic balance of adaptation and carbohydrates composition (Sharifia *et al.*, 2007). Salinity affects plant development from reducing growth due to photosynthesis alteration, enzymatic activity and ionic homeostasis to increasing plant death (Fatma *et al.*, 2014). However, in the present study, a favorable response was observed in basil 'Nufar' cv. which confirmed that AMF inoculation was efficient in mitigating NaCl-stress as was reported by Hashem *et al.* (2015) and Mendes *et al.* (2016). In other species such as *Cucurbita pepo* var. *pepo* cultivated under drought and salinity conditions, the application of a mixed inoculum and a consortium of six AMF native species of the Sonoran Desert, improved dry weight of the shoot and root, foliar humidity percentage, water and osmotic potential, and radicle colonization percentage, while also decreasing physiological stress caused by drought and salinity, thus AMF inoculation was an efficient alternative as a mitigate agent in salinity stress (Harris-Valle *et al.*, 2011). In lettuce (*Lactuca sativa* L.), AMF inoculation also showed a mitigating effect with salinity (Aroca *et al.*, 2013). This effect has been associated to the mitigating effect of AMF because their associations with plants improve functions that improve efficiency of nutrients in the radicle area, starting from an increase in the volume of the soil explored, increase in toxin resistance, translocation and solubilization of essential elements and increase in tolerance to adverse abiotic conditions, such as drought and salinity (Evelin *et al.*, 2013). The results showed in the present study could be attributed to AMF which increased the root contact area with soil, assuring continuity among the absorbent radicle surface and soil solution, optimizing soil interaction with roots (Jurkiewics *et al.*, 2010). According to Hajiboland *et al.* (2010), the AMF are characterized by forming structures in the shape of a miniature tree in the radicle parenchyma cells, a structure called "arbuscule", which is the exchange site between the plant and fugus. Moreover, the mycorrhizal system is formed by a set of hyphas (mycelium) that are connected to the root tissue and branch out in soil. The mycelium found in soil forms a hypha net capable of interconnecting roots and allowing water and nutrient flux between them. These stimulating AMF functions improved the plant water state, which was observed in the relative water content, which increased in plants basil inoculated with AMF in the present study.

Basil plants response in the present study was related to morphometric, nutritional and physiological changes

induced in colonized plants contributing to increase their resistance to abiotic tension. These results are similar to those reported previously by Alqarawi *et al*. (2014) where they pointed out that AMF modified the root architecture, allowing a greater range for water and essential elements in soil than in plants stressed without AMF. Moreover, AMF also mitigated salinity stress by improving absorption and nutrient capture rhizosphere conditions (Parra-Rivero *et al*., 2014); photosynthetic activity and water use efficiency (Hajiboland *et al*., 2010); compatible solute accumulation and antioxidant enzyme production (Evelin et al., 2013). Most recently, Elhindi *et al*. (2017) reported an increase in growth, chlorophyll content, gas exchange, photosynthetic efficiency, proline content, water use efficiency and nutrient absorption in sweet basil plants subjected to salinity stress (5 and 10 dS m^{-1}) and inoculated with AMF *Glomus deserticola*, which favored greater growth, vigor and general cultivation productivity. In other species such as *Solanum lycopersicum* subject to salinity stress and inoculated with *Glomus cubense*, Mujica-Pérez and Fuentes-Martínez (2012) observed an increase in plant height, number of flowers and fruit and yield. In *Dianthus caryophyllus*, an ornamental species, Navarro *et al*. (2012) reported a beneficial effect in plant growth, number and size of flowers, leaves and concluded that the use of mycorrhizal (*Glomus intraradices*, *GII* and *GIII*) was efficient in mitigating NaCl-stress (1, 3, and 6 dS m^{-1}). In citric plants (*Poncirus trifoliata*) subjected to NaCl-stress (0 and 100 mM) and inoculated with *Glomus mosseae* and *Glomus vesiforme*, an increase in growth was observed in plants inoculated with AMF compared with those in the control group without inoculation (Qiang-Sheng *et al*., 2010). Different studies maintain and conclude that AMF strains mitigate NaCl stress in different plant species. Some AMF strains that have shown efficiency as NaCl-stress mitigators are *Funneliformis caledonius*, *Funneliformis mosseae* and *Rhizophagus irregularis*, among others with positive effects in several species, such as *Fragaria ananassa Duch* that has shown increases in leaves and shoots biomass, root length and biomass (Sinclair *et al*., 2014). The increase in nutrient absorption such as P and water even in abiotic tension, reduced Na and Cl absorption, affecting movement into the aerial parts (leaves and shoots) of the plant augmented water catchment maintained ionic equilibrium to improve nutrient absorption and stimulated selective catchment (Evelin *et al*., 2012); increased synthesis and efficiency of some enzymes (Wu *et al*., 2010) as proline (Ibrahim *et al*., 2011) and caused osmotic adjustment that maintained turgid pressure in leaves, improving equilibrium among photosynthesis, transpiration, water use efficiency and stomatal conductance in inoculated plants (Evelin *et al*., 2009). Likewise, AMF have improved rhizosphere soil characteristics and root architecture (Hodge and Storer, 2015). The results of the present study are in agreement with those reported by Al-Khaliel (2010), bsince AMF improved plant development benefiting nutrient absorbance and soil structure; had a direct effect in the quality of the soil while causing aggregates that improve humidity retention, such as increasing soil water potential and favoring water and nutrient absorption. These changes stimulate plant growth, hydraulic conductivity and decrease toxic ion effect induced by salinity (Seema and Garampalli, 2015). The response in basil can be attributed to the combination of physical, nutritional and cellular effects as reported previously by Ruiz-Lozano and Aroca (2010). Other studies have also demonstrated that fungus-host plant symbiosis altered water movement rate within and outward from the plants in other species, affecting water relationships and physiology (Ruiz-Lozano *et al*., 2006). Moreover, AMF have stimulated physiological mechanisms that increase plant stress tolerance and have the potential to increase growth in normal and induced environmental stress conditions (Abd-Allah *et al.,* 2015a, b). Previous studies showed significantly higher values in plant height, stem diameter, leaf area, total plant leaf number and total dry weight where AMF (*Rhizophagus intraradices*) was used in *Vitis vinifera* L. plants subjected to three salinity levels (0.65, 1.56 and 4.68 dS m^{-1}) compared with those non-inoculated (Khalil, 2013).

As expected, no colonization was observed in those non-inoculated basil plants since a sterile substrate were used, which did not allow the development of native species capable of colonizing basil plants. Then, in this study an authentic colonization was observed in the inoculated basil plants; nonetheless, colonization percentage decreased as NaCl increased. This result did not affect the benefit reported by this endophyte species, such as basil, which was evident in the increase in the assessed variables in inoculated AMF basil plants. These results are similar to those reported by Harris-Valle *et al*. (2011) where they found that mycorrhizal colonization of a mixed native AMF inoculum, decreased physiological stress (drought and salinity) in *Cucurbita pepo* var. *pepo*; nevertheless, mycorrhizal colonization decreased as salinity increased, which did not affect plant response in such a condition. According to Rivera *et al*. (2003) colonization percentage is representative of arbuscular mycorrhizal symbiosis functionality. The fact that inoculated AMF has survived, adapted and established under different habitats, indicates that it has a favorable influence in the rhizosphere and substrate conditions where basil plants were established. In arbuscular mycorrhizal symbiosis no threshold colonization value occurs in roots to produce plant growth improvement. This effect depends on plant and fungal species in symbiosis and specific cultivation conditions. The principle mechanism is generally assumed to be that a greater root colonization rate by the fungus improves AMF effects on growth and plant development (Seema and Garampalli, 2015).

Conclusions

The basil plants inoculated with AMF showed increases in their morpho-physiological variables even under NaCl-stress conditions, showing a degree of infectivity and effectiveness of the AMF strain in the rhizosphere zone and in the growing conditions. The percentage of colonization decreased as the NaCl concentrations increased, being highest in the control (0 mM) and decreasing in 50 and 100 mM NaCl. As expected, no colonization occurred in those non-inoculated basil plants, because of a sterile substrate was used, which did not allow for the development of native

species capable of colonizing the basil plants. The substrate used was suitable both for the development of the AMF strain and for the basil plants, as well as the number of inoculum spores.

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Authors' Contribution

YMAF, BMA and LGHM, designed the experiment. YMAF, DSB and CMOS contributed in the assembly of experiment in agricultural area, collection of samples and transfer to the laboratory. YMAF, DSB, and CMOS participated with laboratory analysis, data and preparation of manuscripts. LGHM and JMMS contributed with reagents another laboratory and field materials. BMA analyzed the data. YMAF and BMA wrote and edited the first draft of the manuscript. BMA wrote the final version of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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