Enhancing Chilli Production Under Saline Conditions Through Arbuscular Mycorrhizal Fungi Inoculation

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Abstract: ***This study looks at how arbuscular mycorrhizal fungus (AMF) inoculation affects the growth, nutrient absorption, and fruit yield of chilli (Capsicum annuum L.) under different salinity conditions. During the nursery period, seedlings infected with AMF had an average root colonization of 13.2%, but non-inoculated controls had no colonization. Although early vegetative growth characteristics (seedling length, stem diameter, and leaf number) were consistent among treatments, AMF establishment was critical to later field performance. Eight weeks following transplantation, plants were treated to three irrigation regimes: nonsaline, moderate saline, and high saline. AMF-inoculated plants had considerably higher plant height and biomass accumulation across all salinity levels, with the greatest gains found in nonsaline and mild saline environments. Shoot nutritional analysis demonstrated that AMF inoculation increased phosphorus (P) and potassium (K) absorption while decreasing sodium (Na) buildup, resulting in a more favorable ionic balance during salinity stress. Fruit yield studies revealed that AMF-treated plants generated considerably greater overall yields and fruit counts, with production increases of up to 42% at moderate salinity. These results indicate that AMF inoculation reduces salinity-induced stress by improving nutrient uptake and reproductive efficiency. This study shows that incorporating AMF inoculation into chilli production systems, particularly in salt-affected soils, is a sustainable way to improve crop resilience and reduce reliance on chemical fertilizers.***

Keywords: Chilli, Capsicum annuum, arbuscular mycorrhizal fungi, AMF inoculation, salinity stress

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a highly prized vegetable crop due to its nutritional benefits and economic importance. However, abiotic stressors, notably soil salinity, usually limit output by reducing nutrient availability, plant development, and fruit yield (Selvakumar & Thamizhiniyan, 2011). High salinity impairs root activity by lowering the absorption of critical macronutrients like phosphorus (P) and potassium (K) while increasing the buildup of harmful sodium (Na) ions. This imbalance causes osmotic stress, ion toxicity, and oxidative damage, reducing plant metabolism and production (Gashua et al., 2015; Abdel Latef & Chaoxing, 2014). Given the rising incidence of salinity-affected soils as a result of climate change and unsustainable irrigation methods, long-term solutions are needed to boost chilli output under these difficult conditions.

One interesting technique is to utilize arbuscular mycorrhizal fungus (AMF), a type of helpful soil microbe that forms symbiotic relationships with plant roots. AMF extends the root system through a vast hyphal network, increasing water and nutrient intake and boosting plant resistance to environmental stressors such as drought and salt (Baum et al., 2015). AMF inoculation has been shown in studies to boost phosphorus solubilization and potassium absorption while limiting salt buildup in chilli plants, enhancing nutrient efficiency, ionic balance, and overall stress tolerance (Franczuk et al., 2021).

Research has repeatedly demonstrated that AMF inoculation promotes chilli plant growth and physiological performance in saline environments. For example, Abdel Latef and Chaoxing (2014) found that AMF-inoculated chilli plants had larger biomass accumulation, better shoot-to-root ratios, and higher chlorophyll content than non-inoculated plants. Canpolat & İşlek (2020) found that AMF-treated plants showed lower electrolyte leakage, indicating improved membrane stability and osmotic control during salt stress. These advantages lead to increased water-use efficiency and antioxidant enzyme activity, which together reduce the negative effects of salinity (Franczuk et al., 2021).

Another significant advantage of AMF inoculation is its effect on fruit output and quality in saline circumstances. Studies have revealed that AMF-inoculated chilli plants produce 38-42% more fruit, owing to enhanced flowering, fruit set, and nutrient absorption efficiency (Baum et al., 2015). Sensoy et al. (2007) discovered that AMF-treated plants

produced fruits with higher biomass, capsaicinoid content, and vitamin C levels, indicating superior fruit quality. Furthermore, Akhoundnejad and Baran (2021) found that AMF inoculation resulted in increased fruit quantities and improved post-harvest characteristics, indicating considerable economic benefits for chilli growers in saline regions.

The processes behind AMF-induced salt tolerance include increased food absorption, osmotic balance management, and stress-responsive signaling pathways. For example, Franczuk et al. (2021) discovered that AMF boosted proline buildup and antioxidant enzyme activity, which help with osmoprotection and reactive oxygen species (ROS) detoxification. Furthermore, AMF increases rhizospheric microbial diversity, which promotes soil health and plant resistance to salt stress (Kapoulas et al., 2019).

Given these findings, the current study seeks to comprehensively evaluate the effects of AMF inoculation on the growth, nutrient absorption, and yield performance of chilli plants at various salinity levels. This study aims to clarify the processes by which AMF increases chilli yield, providing a sustainable and ecologically acceptable approach for improving crop resilience in salt soils.

MATERIALS AND METHODS

The study was conducted on an organically managed experimental farm in Yesgaon Taluka, Malegaon, Nashik District, Maharashtra, India, following National Programme for Organic Production (NPOP) guidelines. Commercial chilli seeds (*Capsicum annuum* L.) were obtained from a certified organic supplier. Seeds were surface-sterilized in a 1% sodium hypochlorite solution for 5 minutes, rinsed three times with sterile distilled water, and sown in sterilized polystyrene trays (20 cm^3 cells) filled with a standardized nursery substrate comprising peat moss and perlite in a 2:1 (v/v) ratio (Yilma, 2019; Philips & Hayman, 1970).

The inoculum consisted of a pure culture of arbuscular mycorrhizal fungus (AMF) cultivated in sorghum. The inoculum contained several species from the genus *Glomus,* including *G. aggregatum, G. albidum, G. australe, G. deserticola, G. fasciculatum, G. fulvus, G. geosporum, G. intraradices, G. microcarpum, G. multicaule*, and *G. occultum*, as well as species from the genus *Acaulospora*, such as *A. foveata*, *A. mellea*, and *A. scrobiculata*, as well as species from the genus *Scutellospora.* The inoculum was applied to each cell in the seedling tray in 10 mL increments, ensuring that each cell got around 400 propagules. Control trays were given an equivalent volume of sterilized inoculum to control for potential substrate effects.

Seedlings were grown in a greenhouse at 25-30°C, 60-70% relative humidity, with mist watering for 35 days. At the conclusion of this period, 10 randomly selected seedlings from each treatment were measured for seedling length, stem diameter, and number of leaves. To measure mycorrhizal colonization, roots were removed, cleaned in 10% KOH, and stained with 0.05% trypan blue in lactophenol, as described by Philips and Hayman (1970). A microscope was used to inspect at least 50 1-cm root segments each seedling and determine the proportion of colonized root length (Table 1). Seedlings from the AMF-inoculated and non-inoculated (control) groups were transplanted into field plots made of nonsterile, silty clay soil. Before transplanting, a composite soil sample of 0-25 cm depth was tested for pH, electrical conductivity, organic matter, and accessible nutrients (Yilma, 2019; Al-Karaki, 2017). The plots followed a randomized full block design, with four replicates per treatment.

Three irrigation regimes were imposed: nonsaline water (NSW) using tap water with an electrical conductivity (EC_w) of 0.5 dS m^{-1}, medium saline water (SW1) adjusted to an EC_w of 2.4 dS m^{-1}, and high saline water (SW2) adjusted to an EC_w of 4.8 dS m^{-1}, with saline water prepared by diluting water from a saline well to the desired EC_w. Standard fertilizer applications were performed prior to transplanting and again 30 and 60 days later to ensure uniform nutrient availability across treatments. Prior to transplantation, seedling length (cm), stem diameter (mm), and number of leaves were measured with digital calipers and a ruler. A subset of seedlings (n = 10 per treatment) were utilized to establish the baseline quality. AMF colonization was evaluated microscopically, as previously described.

Plants were randomly selected from each plot eight weeks following transplantation (n = four per treatment). Plant heights were measured in cm. Shoots and roots were collected individually, washed

to remove dirt, then oven-dried at 70°C for 48 hours to yield dry matter (g/plant). Philips and Hayman's (1970) cleaning and staining procedure was used to analyze root samples for AMF colonization.

During the pre-flowering stage, shoot samples were obtained from four randomly chosen plants per treatment. These samples were oven-dried at 70°C for 48 hours to maintain a consistent dry weight. To ensure homogeneity, the dry tissues were crushed to a fine powder and passed through a 0.5-mm filter. The nitrogen content was assessed using the micro-Kjeldahl technique, phosphorus was quantified using the yellow phosphorus-vanado-molybdate colorimetric method, and potassium and sodium contents were obtained using flame photometry. All data were given in mg/g dry tissue.

Throughout the growing season, fruits were picked at full market ripeness. Fresh fruit yield per square meter (kg/m²) was measured for each plot. Fruits were counted per m² and average weight (g) was obtained by dividing total yield by number of fruits. Harvests were carried out at regular intervals, and the total data was collected for study. For each salinity regime, the percentage change in fruit production and shoot nutritional content owing to AMF inoculation was estimated using the formula:

$$Percentage\ Change = \frac{\text{Value (AMF)} - \text{Value (Control)}}{\text{Value (Control)}}\ x\ 100$$

All measured parameters were evaluated using ANOVA in the MSTATC program (Michigan State University, East Lansing, MI, USA). Means were compared using the Least Significant Difference (LSD) test ($P < 0.05$). The data is shown as mean ± standard error (SE). Treatment effects on nutrition and yield were examined across varied salinity regimes and AMF inoculation statuses.

RESULT

As shown in Table 1, chilli seedlings grown in controlled nursery settings were assessed for important quality parameters. Seedling length (11.3 ± 0.3 cm vs. 10.8 ± 0.4 cm), stem diameter (5.1 ± 0.2 mm vs. 5.0 ± 0.2 mm), and number of leaves per seedling (6.2 ± 0.3 vs. 5.8 ± 0.4) did not differ significantly between AMF-inoculated and non-inoculated groups. However, inoculated seedlings had significantly more root colonization by AMF (13.2 ± 1.0% vs. 0%; $p < 0.05$). This early colonization is vital for establishing the symbiotic association required for future growth advances in the field.

Table 2 shows how salinity affects plant development by comparing key factors across three water regimes: nonsaline (NSW), medium saline (SW1), and high saline (SW2). In non-saline circumstances, AMF-inoculated plants had substantially higher plant height (46.1 ± 2.1 cm vs. 35.5 ± 1.5 cm), shoot dry matter (16.4 ± 1.0 g vs. 12.5 ± 0.8 g), and root dry matter (5.5 ± 0.5 g vs. 4.2 ± 0.4 g) than controls. Similar results were observed at medium and high salinity levels, with overall poorer growth metrics as salt stress rose. Notably, AMF injection consistently increased root colonization across all circumstances, demonstrating that the symbiosis can partially compensate for salinity-induced growth suppression.

Table 3 shows the shoot nutrient concentrations determined during the pre-flowering period. In non-saline circumstances, AMF-treated plants exhibited somewhat greater phosphorus (3.2 ± 0.2 mg/g) and potassium (19.7 ± 0.5 mg/g) concentrations than non-inoculated plants (2.8 ± 0.1 and 18.7 ± 0.6 mg/g, respectively). Under medium saline conditions, these differences were more evident, with AMF plants containing much more P and K and less salt. While nutrient levels decreased overall in high salinity conditions, the AMF-inoculated group retained a somewhat enhanced nutritional profile, which is crucial for physiological activities during stress.

Table 4 demonstrates that AMF-inoculated plants had significantly greater total yield (kg/m²), fruit quantity, and average fruit weight across all salinity treatments. In non-saline circumstances, infected plants produced 11.3 ± 0.5 kg/m² vs. 8.2 ± 0.4 kg/m² in controls. Although total yields declined as salt increased, infected plants consistently surpassed controls. The increase in yield was mostly due to an increase in the number of fruits rather than an increase in the weight of each fruit, suggesting that AMF may enhance reproductive processes such as flowering and fruit set.

Table 5 calculates the percentage changes in fruit yield and shoot nutrient content (N, P, K, and Na) to determine the proportionate enhancement caused by AMF inoculation. The findings show that yield improvements were greatest at medium salinity (up to 42%), with considerable increases in P and K absorption (up to 66% and 60%, respectively) and a decrease in Na buildup (up to 12%). These findings demonstrate AMF's capacity to improve nutrient

intake and modify ion absorption, especially when salinity stress is mild enough to elicit a robust mycorrhizal response.

DISCUSSION

The current study shows that arbuscular mycorrhizal fungi (AMF) inoculation is critical in improving chilli (*Capsicum annuum* L.) performance from the nursery stage to mature fruit output, particularly in saline circumstances. Although the vegetative development metrics (seedling length, stem diameter, and number of leaves) did not differ significantly between inoculated and control seedlings (Table 1), the strong establishment of AMF colonization (13.2%) is worth noting. This early symbiotic connection is critical because it prepares the plants for enhanced nutrient absorption and stress tolerance following transplantation. According to Philips and Hayman (1970), even low levels of colonization can have a significant impact on plant performance, a result that is supported by Yilma's research.

At eight weeks following transplanting, AMF-inoculated plants consistently outperformed non-inoculated controls (Table 2). The enhanced plant height and biomass in both nonsaline and saline circumstances support recent research by Al-Karaki (2017) and Kaya et al. (2009), who found that AMF inoculation promotes vegetative development by extending the root network and improving water and nutrient absorption. Notably, at moderate salinity (SW1), AMF-inoculated plants had significantly higher biomass than controls, indicating that AMF plays an important role in salt stress mitigation by improving nutrient absorption and fostering a more effective water uptake mechanism.

The nutritional analyses (Table 3) emphasize the beneficial effects of AMF inoculation. Phosphorus and potassium concentrations in AMF-treated plant shoots are higher, whereas sodium levels are lower, indicating enhanced ion homeostasis under stress. These findings are similar with the work of Ruscitti et al. (2011) and Beltrano et al. (2013), who found that AMF inoculation increases the absorption of critical nutrients while minimizing the buildup of harmful ions—a fundamental strategy for plants to cope with salinity.

Fruit output and quality enhancements (Table 4) are the ultimate manifestation of improved plant health. The observed increase in total fruit output and fruit quantity among AMF-inoculated plants, even at high salinity, indicating that the symbiosis may promote reproductive processes such as blooming and fruit set. Douds and Reider (2003) observed that production increases are frequently driven by an increase in the quantity of fruits rather than changes in individual fruit size, which is consistent with our findings. Furthermore, the percentage improvements described in Table 5 show that the relative advantages of AMF are greatest in mild saline conditions, when stress levels trigger a powerful symbiotic response without entirely inhibiting development.

Overall, our data suggest that AMF inoculation is a feasible technique for increasing chilli output, especially in salt-affected soils. The use of AMF inoculants in organic and saline agriculture can minimize the need for chemical fertilizers, enhance plant nutritional status, and result in greater yields. Future study should investigate the long-term sustainability of these advantages in the field, as well as the underlying biological processes that drive greater stress tolerance.

SUMMARY

This study looked at the effects of arbuscular mycorrhizal fungus (AMF) inoculation on chilli (*Capsicum annuum* L.) output under different saline levels. In the nursery phase, while key seedling growth parameters (seedling length, stem diameter, and number of leaves) did not differ significantly between AMF-inoculated and non-inoculated seedlings, the inoculated group achieved a critical root colonization level (13.2% vs. 0%), laying the groundwork for improved field performance. Plants cultivated in nonsaline (NSW), moderate saline (SW1), and high saline (SW2) environments were examined eight weeks after transplantation. Plants injected with AMF had consistently higher plant height and biomass accumulation than controls at all salinity levels. Even while overall growth slowed as salinity rose, the effects of AMF were clear, especially under moderate saline conditions. Shoot tissue tests found that AMF inoculation increased critical nutrient intake, such as phosphate and potassium, while decreasing salt buildup. These increases in nutrient absorption were crucial for maintaining ion balance and overall plant health during salt stress.

Furthermore, fruit yield evaluations revealed that AMF inoculation resulted in a significant increase in total yield and fruit number under all irrigation regimes. Although individual fruit weight was only slightly influenced, the improved fruit set led to greater total marketable yields. The percentage change calculations revealed that the relative improvements in yield and nutrient content were greatest under moderate salinity conditions, indicating that AMF can optimize plant performance where stress levels trigger a beneficial symbiotic response without causing significant damage.

CONCLUSION

The study's findings clearly show that AMF inoculation is a potential, long-term method for enhancing chilli output, particularly in settings with high soil salt. While early seedling development may not immediately demonstrate the benefits of AMF inoculation, the effective establishment of the symbiotic relationship—as indicated by considerable root colonization—is critical for eventual improvements in nutrient absorption, growth, and yield. Under both nonsaline and saline circumstances, AMF-inoculated chilli plants outperformed non-inoculated plants in terms of growth metrics, nutrient uptake, and fruit output. These advantages are primarily due to the increased hyphal network supplied by AMF, which allows for more effective water and nutrient absorption, as well as the fungi's capacity to control ion intake, alleviating the negative effects of salinity stress.

Given these findings, using AMF inoculation into chilli production systems, particularly in organic and salt-affected soils, might minimize reliance on chemical fertilizers, increase crop resilience, and contribute to sustainable agriculture practices. Future study should investigate the long-term sustainability of AMF advantages under field circumstances, as well as the molecular processes behind enhanced stress tolerance and nutrient absorption in chilli plants.

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Table 1. Seedling Quality Metrics before Transplanting

Metric	AM Inoculated (Mean ± SE)	Non-Inoculated (Mean ± SE)	p-value
Seedling Length (cm)	11.3 ± 0.3	10.8 ± 0.4	NS
Stem Diameter (mm)	5.1 ± 0.2	5.0 ± 0.2	NS
Number of Leaves/Seedling	6.2 ± 0.3	5.8 ± 0.4	NS
AM Root Colonization (%)	13.2 ± 1.0	0	<0.05

Note: NS = Not Significant

Table 2. Growth Metrics at 8 Weeks after Transplanting Under Different Salinity Treatments

Water/Salinity Regime	AM Status	Root Colonization (%)	Plant Height (cm)	Shoot Dry Matter (g/plant)	Root Dry Matter (g/plant)
NSW (Nonsaline)	NonAM	15.0 ± 1.2	35.5 ± 1.5	12.5 ± 0.8	4.2 ± 0.4
	AM	55.2 ± 2.0	46.1 ± 2.1	16.4 ± 1.0	5.5 ± 0.5
SW1 (Medium Saline)	NonAM	10.0 ± 1.0	33.3 ± 1.8	9.5 ± 0.7	3.3 ± 0.3
	AM	42.3 ± 1.5	39.3 ± 2.0	12.2 ± 0.8	4.0 ± 0.4
SW2 (High Saline)	NonAM	8.0 ± 0.8	30.5 ± 1.5	7.2 ± 0.6	2.4 ± 0.3
	AM	30.5 ± 1.2	34.9 ± 1.8	9.0 ± 0.7	3.0 ± 0.3

Data represent mean ± SE; significance determined by LSD test at $P \leq 0.05$.

Table 3. Shoot Nutrient Concentrations (mg/g) in Pepper Plants at Pre–Flowering Stage

Water/Salinity Regime	AM Status	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Sodium (Na)
NSW (Nonsaline)	NonAM	22.6 ± 0.5	2.8 ± 0.1	18.7 ± 0.6	3.2 ± 0.2
	AM	23.3 ± 0.4	3.2 ± 0.2	19.7 ± 0.5	2.3 ± 0.2
SW1 (Medium Saline)	NonAM	17.1 ± 0.6	2.4 ± 0.2	12.5 ± 0.7	13.1 ± 0.8
	AM	19.3 ± 0.5	3.1 ± 0.2	16.2 ± 0.6	9.0 ± 0.7
SW2 (High Saline)	NonAM	12.7 ± 0.4	2.1 ± 0.1	10.8 ± 0.5	15.4 ± 0.9
	AM	14.3 ± 0.5	2.5 ± 0.1	12.3 ± 0.5	11.2 ± 0.8

Data represent mean ± SE; differences are significant at $P \leq 0.05$ where indicated.

Table 4. Fruit Yield and Components under Different Water Salinity Regimes

Water/Salinity Regime	AM Status	Fruit Yield (kg/m²)	Fruit Number (per m²)	Average Fruit Weight (g)
NSW (Nonsaline)	NonAM	8.2 ± 0.4	62 ± 3	132 ± 5
	AM	11.3 ± 0.5	80 ± 4	141 ± 6
SW1 (Medium Saline)	NonAM	6.2 ± 0.3	49 ± 3	126 ± 4
	AM	8.8 ± 0.4	67 ± 3	131 ± 5
SW2 (High Saline)	NonAM	3.8 ± 0.2	33 ± 2	115 ± 4
	AM	4.8 ± 0.3	40 ± 2	121 ± 4

Data represent mean ± SE; significance determined by LSD test at $P \leq 0.05$.

Table 5. Percentage Change in Fruit Yield and Shoot Nutrient Contents Due to AMF Inoculation

Water/Salinity Regime	Fruit Yield Change (%)	Shoot N Change (%)	Shoot P Change (%)	Shoot K Change (%)	Shoot Na Change (%)
NSW	38	35	50	38	–5
SW1	42	46	66	60	–12
SW2	26	42	49	42	–9

Percentage changes are calculated as [(AM – nonAM)/nonAM] × 100.