

Management of *Meloidogyne incognita* and salinity on sweet pepper (*Capsicum annuum* L.) with different arbuscular mycorrhizal fungus species

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ABSTRACT

The deleterious effect of salinity and root-knot nematodes on sweet pepper is enormous. A screenhouse experiment was conducted to evaluate the efficacy of three arbuscular mycorrhizal fungi (AMF) in alleviating adverse effects of salinity and *M. incognita* on sweet pepper. A $2 \times 3 \times 4$ factorial experiment was laid out in a completely randomized design with three replications. The 24-treatment combinations were three mycorrhizal fungi (*Glomus mosseae*, *Glomus deserticola*, and *Gigaspora gigantea*), an uninoculated control, three salinity levels (0.16, 3.24, and 6.06 dS/m), and inoculation either with or without 5,000 eggs of *M. incognita*. The results showed that sweet pepper variety Tatase was highly susceptible to *M. incognita* infection with heavy galls on nonmycorrhizal plants. Nematode inoculation and salinity significantly ($P \leq 0.05$) impaired growth, AMF root colonization, and dry matter production compared with the control plants. Increase in salinity level significantly ($P \leq 0.05$) reduced root gallings and egg mass production. AMF inoculation significantly ($P \leq 0.05$) reduced root gallings and significantly enhanced growth and dry matter yield in the presence or absence of nematode infection at all salinity levels compared with the nonmycorrhizal plants. Among the AMF species, *G. deserticola* was the most efficient in ameliorating the injurious effects of salt and *M. incognita*.

1. INTRODUCTION

The cultivated and wild pepper belongs to the Solanaceae family of the genus *Capsicum*. The bell or sweet peppers (*Capsicum annuum* L.) and the pungent or “bird’s eye pepper” (*Capsicum frutescens* L.) are the two widely grown species [1]. The world production of pepper (sweet and hot varieties) is estimated at 26,537 million tons, with China leading with an output of 7072 million tons representing 27% of the world production [2]. In Africa, Algeria is the lead producer (317,500 tons), followed by Tunisia with 280,000 tons [2]. Pepper is an important spice crop, highly cherished for its pungent flavor. Pungency comes from the capsaicinoids, alkaloid compounds ($C_{18}H_{27}NO_3$) that abound only in the genus *Capsicum* [3]. Pepper fruit is enriched with vitamins A, C, and B₂, potassium, phosphorus, and calcium, and in industries, pepper serves as an ingredient for many pharmaceuticals, for example., antioxidant and anticancer products; in food and cosmetics, the red pigment extract of ripe fruits serves as a natural coloring agent [1].

Pepper production in Sub-Saharan Africa is hindered by a number of biotic and abiotic factors. In Nigeria, a large amount of the crop comes from the semiarid area where drought and salinity are potential production constraints; thus, pepper is mostly grown under irrigation. Pepper is a salt-sensitive crop [4]. Salt may originate from several sources, such as seawater and salt strata. In the arid and semiarid regions, it originates from surface run-off and capillary movement. Poor source/quality of irrigation water may also constitute a source. Excess salt in the root zone inhibits water and nutrient absorption by the plant and may induce toxic effects [5,6]. Some saline soils contain sufficient salts that can suppress plant growth by upsetting osmotic potential and enzyme activities in plants [7-9].

The best management option for the production of crops under saline conditions has been the use of salt-tolerant varieties [5,10] and the use of the extracts of some plants [6,9]. Recently, however, researches have shown that arbuscular mycorrhizal fungi (AMF) are effective in alleviating the stress induced by salts on plants in saline soils [4,11-14]. The physiological and biochemical mechanisms of salt stress amelioration by AMF remain topical issues. Enhanced nutrient acquisition, especially immobile elements such as P, Cu, and Zn [4,13], has been attributed in part to the induced salt-tolerance by AMF-colonized plants. In recent studies, other potential mechanisms have been advocated, which include greater osmotic adjustment, reduced oxidative damage, selective toxic ion absorption like Na⁺,

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vacuolar compartmentalization of toxic ions, and increased production of antioxidative enzymes [7,14,15]. In spite of the substantial scientific evidence that AMF colonization alleviates salt stress, some researchers have explicitly stated that salt has negative effects on AMF [16,17]. Apart from salinity, root-knot nematodes (*Meloidogyne* spp.) are a serious threat to pepper production in the tropics [10,11,18].

Chemical control of plant-parasitic nematodes is quite efficient, but its associated drawbacks including environmental and health issues present a serious challenge. Recently, many authors have reported the effectiveness of AMF in the control of root-knot nematodes in several crops [19–22]. The various mechanisms attributed to nematode inhibition by AMF are still under debate [23,24]. However, induced systemic resistance/tolerance and histological, physiological, morphological, and biochemical changes associated with AMF inoculation have been implicated in nematode control and improved crop growth [25,26]. Galling of plant roots by root-knot nematodes under the saline condition could lead to growth and yield impairment if not checked [27,28]. There is a paucity of information on the salt stress ameliorating ability and root-knot disease control by AMF with both stress factors occurring simultaneously. The objective of this work was to evaluate the efficacy of AMF in the control of *Meloidogyne incognita* on sweet pepper exposed to salt stress.

2. MATERIALS AND METHODS

2.1. Experimental Site

Calabar is located in the tropical rainforest zone of Nigeria within latitude 5°00' and 5°40'N and longitude 8°04' and 8°62'E with an elevation of 70 m above sea level. The research was done in a screenhouse of the Faculty of Agriculture, University of Calabar, from January 7 to July 30, 2013. The environmental parameters of the screenhouse during the experimental period were as follows: average temperature range 28°C–31°C, relative humidity 69% to 81%, and sun hours 325 h/month to 371 h/month.

2.2. Source of Materials

The pepper seeds (*Capsicum annum* cv. Tatase) used in the experiment were obtained from the crop germplasm collection of the Crop Science Department, University of Nigeria, Nsukka. The pepper cultivar is susceptible to *M. incognita* [10]. The starter culture of AMF species *Glomus mosseae* FR113 (Nicol. and Gerd.) Gerd. and Trappe, *Glomus deserticola* FL912 (Trappe, Boss, and Menge), and *Gigaspora gigantea* VA 105 (Nicol. and Gerd.) were procured from the Department of Agronomy, University of Ibadan, Oyo State, Nigeria. The accessions were collected from France, Florida, and Virginia and cultured by the International Institute of Tropical Agriculture, Ibadan, Nigeria. The salt used for the trial was sodium chloride (NaCl) and was bought from a chemical shop in Calabar.

2.3. Soil Sample Collection and Analysis

A composite surface soil (0–15 cm) was collected from the University of Calabar Teaching and Research Farm and was analyzed for its physicochemical properties according to the methods of Tel and Rao [29]. The soil from this farm was used to fill plastic pots which served as the growth medium for the pepper plants.

2.4. Multiplication of Starter Culture of AMF and *M. incognita* Inoculum

The AMF starter culture which comprises pieces of roots of maize (*Zea mays* L.) plants, glomerospores, spores, and soil was increased

in heat-sterilized soil. The soil was sterilized by heating in an earthen pot to a temperature of 100°C and maintained for an hour, allowed to cool for 5 d, and then planted with maize, and Hoagland's solution low in phosphorus was used with the irrigation water for 3 months. The spore densities for *G. mosseae*, *G. deserticola*, and *Gi. gigantea* were 51, 49, and 48 spores/10 g of soil, respectively, determined based on the method of Gerdemann and Nicolson [30]. The stock culture of *M. incognita* kept on *Talinum fruticosum* (L.) Juss. (waterleaf) in the vicinity of the screenhouse was multiplied in *Celosia argentea* L. (Cock's comb) in a sterilized soil. It served as an inoculum source.

2.5. Preparation of Nematode Inoculum

Roots of *C. argentea* heavily galled by *M. incognita* were carefully removed from the planting pots, washed with water, and later cut into pieces (1–2 cm segments) for egg extraction using the method of Hussey and Barker [31]. The galled root segments were shaken thoroughly for 4 min in a solution of 0.5% NaOCl in a 500 ml conical flask with the mouth of the flask tightly covered. The eggs in the solution were sifted by placing a 200-mesh sieve over a 500-mesh sieve. The eggs were carefully collected in a beaker by washing with a wash bottle, and the inoculum density was determined by counting with a stereomicroscope. The average of three counts gave approximately 500 eggs in 1 ml of suspension.

2.6. Nursery of Pepper Seedlings and Inoculation with AMF

Inoculation of pepper plants with the respective AMF species was done at the nursery stage. Sandy soil and poultry manure were mixed at the ratio of 3:1 and then heat-sterilized. Two and a half kilograms of the heat-sterilized soil admixture was placed in plastic baskets to which the top 5–6 cm layer was placed with the AMF inoculum. The pepper seeds were surface-sterilized with a 0.5% NaOCl solution (household bleach) and rinsed 3 times with distilled water. The seeds were planted in each basket by drilling which was later thinned to 25 plants per basket after emergence. Baskets that had seedlings with no AMF inoculation represented the control. The plants were watered appropriately. This inoculation method follows the procedure of Oyeekanmi *et al.* [32].

2.7. Inoculation of Pepper Seedlings with *M. incognita* and Irrigation with Saline Water

Five-week-old pepper seedlings (1.5–2 cm average leaf width and 3–5 cm average leaf length) were transplanted to the screenhouse in the evening and inoculated with the root-knot nematode (*M. incognita*). Seventy-two (72) plastic pots were used for the experiment. Holes were made at the bottom of each pot for water drainage. Pots were labeled appropriately for easy identification and treatment application. Each of the labeled pots was filled with 2.5 kg of sterilized soil. The pepper seedlings were transplanted and inoculated by making three holes around each seedling and pouring 10 ml of the inoculum suspension containing 5,000 eggs near the roots. Uninoculated seedlings served as the control. Salinity levels of 3.24 and 6.06 dS/m were obtained by dissolving 31.68 and 63.50 g of NaCl, respectively, in 18 L of tap water. The electrical conductivity of the saline irrigation water (EC_w) was measured with an electrical conductivity meter. Irrigation with saline water (150 ml/plant) commenced 2 weeks after transplanting on a daily basis. The control plants were irrigated with tap water with electrical conductivity (EC_w) of 0.16 dS/m.

2.8. Experimental Design

It was a 2 × 3 × 4 factorial experiment fitted into a completely randomized design (CRD) with three replications. The first factor was nematode inoculation, with uninoculated plants serving as control. This factor was combined with the three salt levels and the three AMF species' inoculation plus nonmycorrhizal plants as control. Thus, there were 24 treatment combinations with three replications amounting to 72 pots.

2.9. Data Collection, Statistical Analysis, and Evaluation of Mycorrhizal Colonization

Data were collected on plant height (cm) and the number of leaves per plant at 7 w after transplanting. At 7 w after transplanting, each plant was uprooted, and roots were washed thoroughly with flowing water. The numbers of galls and egg masses per root system were counted. The galling index was scored on a 0–5 scale rating as used by Taylor and Sasser [33], where 0 = no galls, 1 = 1 or 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, and 5 = more than 100 galls per root system. According to Sassar *et al.* [34], 0 = immune, 1 = highly resistant, 2 = resistant, 3 = moderately susceptible, 4 = susceptible, and 5 = highly susceptible. Egg masses were stained with phloxine B (0.15 g/l) for 15 min according to the procedure by Daykin and Hussey [35] for easy counting. The fresh root was separated from the shoot, and fresh root weight was obtained by weighing with an electronic balance, while the dry shoot weight was obtained after oven drying in an envelope at 70°C for 48 hr. Data obtained from the study were statistically analyzed using the analysis of variance (ANOVA) for a three-factor factorial experiment in CRD with GenStat 10th Release Version statistical software. Means were compared using Duncan's new multiple range test at the 5% probability level. There was a need to transform data on egg mass counts using square root transformation ($\sqrt{x+0.50}$) before statistical analysis as there were zero counts. The section of the roots colonized by AMF was determined by a grid-line intersecting method [36] after clearing the roots with KOH [37], roots stained with 0.05% trypan blue-lactophenol [38,39].

3. RESULTS

3.1. Soil Properties

The results of the soil analysis showed that the soil used for the study was loamy sand, with a pH of 5.80 (moderately acidic), electrical conductivity of the extract (ECe) = 0.13 dS/m, low total N (0.1%), exchangeable cations, ECEC, medium available P (24.63 mg/kg), and very high base saturation.

3.2. Gall Index/Root System

The result showed that the effects of AMF, salinity, and nematodes and their interactions on gall index were significant [Table 1]. No gall was found on the roots of pepper plants not inoculated with *M. incognita* [Table 2]. The plants irrigated with tap water without salt and uninoculated with AMF significantly ($P \leq 0.05$) had the highest gall index (GI) of 5.00 in the presence of *M. incognita* and were rated highly susceptible. In both AMF-inoculated and uninoculated plants, irrigation with saline water significantly reduced root galling. However, among the mycorrhizal plants, those inoculated with *G. deserticola* and irrigated with 6.06 dS/m salt water significantly had the least root gall index of 3.00 and were rated moderately susceptible.

Table 1: ANOVA for arbuscular mycorrhizal fungus (M), salinity (S), and *Meloidogyne incognita* (N) and their interactions for all the variables studied in sweet pepper.

| Variable | M | S | N | M×S | M×N | S×N | M×S × N |
|-----------------------|-----|-----|-----|-----|-----|-----|------------|
| Gall index | *** | *** | *** | *** | *** | *** | *** |
| Number of egg masses | *** | *** | *** | *** | *** | *** | *** |
| Fresh root weight | *** | *** | *** | *** | *** | *** | ** |
| Root colonization (%) | *** | *** | *** | *** | *** | *** | ** |
| Plant height | *** | *** | *** | *** | *** | *** | ** |
| Number of leaves | *** | *** | *** | ns | ** | ns | ns |
| Dry shoot weight | *** | *** | *** | *** | *** | *** | *** |

ns=Not significant, * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 2: Effects of AMF and salinity on gall index per root system of pepper inoculated with or without *Meloidogyne incognita*.

| Salinity level (dS/m) | Nematode | Arbuscular mycorrhizal fungus | | | |
|--------------------------|----------------|-------------------------------|----------------|----------------|----------------|
| | | M ₀ | M ₁ | M ₂ | M ₃ |
| S ₀ | N ₀ | 0.00e | 0.00e | 0.00e | 0.00e |
| | N ₁ | 5.00a | 4.00c | 4.00c | 4.33b |
| S ₁ | N ₀ | 0.00e | 0.00e | 0.00e | 0.00e |
| | N ₁ | 4.00c | 4.00c | 4.00c | 4.00c |
| S ₂ | N ₀ | 0.00e | 0.00e | 0.00e | 0.00e |
| | N ₁ | 4.00c | 4.00c | 3.00d | 4.00c |

Means followed by the same letter are not significantly different at the 5% probability level according to Duncan's new multiple range test. M₀=uninoculated control, M₁=*Glomus mosseae*, M₂=*Glomus deserticola*, M₃=*Gigaspora gigantea*, N₀=uninoculated control, N₁=inoculated with *Meloidogyne incognita*, S₀=0.16 dS/m, S₁=3.24 dS/m, and S₂=6.06 dS/m

3.3. Number of Egg Masses/Root System

The results showed that AMF, salinity, and nematodes and their interactions significantly ($P \leq 0.05$) affected the number of egg masses produced per plant [Table 1]. The control plants without nematode inoculation had no egg mass on their roots [Table 3]. In both mycorrhizal and nonmycorrhizal plants, there was a significant ($P \leq 0.05$) decrease in the number of egg masses produced by *M. incognita* with successive increases in the concentration of saline water. In general, inoculation of pepper plants with AMF significantly reduced egg mass production by *M. incognita* compared with the uninoculated plants. The lowest number of egg masses was observed in plants irrigated with 6.06 dS/m saline water and inoculated with *G. deserticola* in the presence of *M. incognita* infection. This was closely followed by *G. mosseae*-inoculated plants.

3.4. Fresh Root Weight

The result indicated that AMF, salinity, and nematodes and their interactions significantly ($P \leq 0.05$) affected fresh root weight [Table 1]. With the exception of the highest level of salinity (6.06 dS/m), *M. incognita* infection significantly reduced the fresh root weight of pepper in both AMF-inoculated and uninoculated pepper plants compared with uninfected plants [Table 4]. However, at that salt level, nematode infection significantly reduced fresh root weight with *G. deserticola* inoculation compared with noninfected plants. Also, in the presence or absence of nematodes, successive increases in salinity level led to a significant decrease in fresh root weight of both AMF-inoculated and uninoculated plants. Relative to the nonmycorrhizal plants, plants inoculated with AMF at all salinity

Table 3: Effects of AMF and salinity on the number of egg masses per root system of pepper inoculated with or without *Meloidogyne incognita*.

| Salinity level (dS/m) | Nematode | Arbuscular mycorrhizal fungus | | | |
|-----------------------|----------------|-------------------------------|----------------|----------------|----------------|
| | | M ₀ | M ₁ | M ₂ | M ₃ |
| S ₀ | N ₀ | 0.00 (0.71 l) | 0.00 (0.71 l) | 0.00 (0.01 l) | 0.00 (0.71 l) |
| | N ₁ | 86.55 (9.33a) | 52.79 (7.30c) | 30.30 (5.55g) | 60.34 (7.80b) |
| S ₁ | N ₀ | 0.00 (0.71 l) | 0.00 (0.71 l) | 0.00 (0.01 l) | 0.00 (0.71 l) |
| | N ₁ | 50.62 (7.15c) | 28.34 (5.37h) | 21.97 (4.74 i) | 43.06 (6.60 d) |
| S ₂ | N ₀ | 0.00 (0.71 l) | 0.00 (0.71 l) | 0.00 (0.71 l) | 0.00 (0.71 l) |
| | N ₁ | 39.70 (6.34e) | 10.92 (3.38j) | 8.99 (3.08k) | 33.37 (5.82 f) |

Means followed by the same letter are not significantly different at the 5% probability level according to Duncan's new multiple range test. Transformed data ($\sqrt{x+0.50}$) in parentheses. Values outside the parentheses are means of the original data. M₀=uninoculated control, M₁=*Glomus mosseae*, M₂=*Glomus deserticola*, M₃=*Gigaspora gigantea*, N₀=uninoculated control, N₁=inoculated with *Meloidogyne incognita*, S₀=0.16 dS/m, S₁=3.24 dS/m, and S₂=6.06 dS/m.

Table 4: Effects of AMF and salinity on the fresh root weight (g plant⁻¹) of pepper inoculated with or without *Meloidogyne incognita*.

| Salinity level (dS/m) | Nematode | Arbuscular mycorrhizal fungus | | | |
|-----------------------|----------------|-------------------------------|----------------|----------------|----------------|
| | | M ₀ | M ₁ | M ₂ | M ₃ |
| S ₀ | N ₀ | 2.73c | 3.02b | 4.00a | 2.57c |
| | N ₁ | 1.31ghi | 2.08d | 3.11b | 1.84e |
| S ₁ | N ₀ | 1.54fg | 1.94de | 3.07b | 1.36gh |
| | N ₁ | 0.99jk | 1.42g | 2.10d | 1.14hij |
| S ₂ | N ₀ | 0.88kl | 1.09ijk | 1.71ef | 1.06ijk |
| | N ₁ | 0.74l | 0.98jk | 1.16hij | 1.02jk |

Means followed by the same letter are not significantly different at the 5% probability level according to Duncan's new multiple range test. M₀=uninoculated control, M₁=*Glomus mosseae*, M₂=*Glomus deserticola*, M₃=*Gigaspora gigantea*, N₀=uninoculated control, N₁=inoculated with *Meloidogyne incognita*, S₀=0.16 dS/m, S₁=3.24 dS/m, and S₂=6.06 dS/m

levels and with *M. incognita* inoculation significantly had higher fresh root weight except for those inoculated with *Gi. gigantea* which had similar results to the control without AMF at 3.24 dS/m. Among the nematode-inoculated or uninoculated plants under salt stress, plants inoculated with *G. deserticola* produced significantly higher fresh root weight.

3.5. Percentage Root Colonization

The effects of AMF, salinity, and nematodes and their interactions were significant ($P \leq 0.05$) on percentage root colonization by AMF [Table 1]. No root colonization was observed in plants not inoculated with AMF [Table 5]. At salinity level of 0.16 dS/m, root-knot nematode infection significantly reduced root colonization by *G. mosseae* and *G. gigantea* but not that of *G. deserticola*. However, when pepper plants were irrigated with saline water nematode infection significantly reduced root colonization by *G. deserticola*. At all salinity levels in the presence or absence of nematodes, *G. deserticola* significantly had the highest percentage root colonization with the exception of *G. mosseae*-inoculated plants at 6.06 dS/m in the presence of *M. incognita* that did not differ from *G. deserticola*-inoculated plants.

3.6. Plant Height

The effects of AMF, salinity, and nematodes and their interactions on plant height were significant [Table 1]. Irrespective of the salinity level and AMF species, *M. incognita* inoculation significantly reduced the height of pepper plants compared with the nematode-free plants [Table 6]. In both mycorrhizal and nonmycorrhizal plants, increase in

Table 5: Effects of salinity and *Meloidogyne incognita* on percentage (%) root colonization of pepper plants by different AMF species.

| Salinity level (dS/m) | Nematode | Arbuscular mycorrhizal fungus | | | |
|-----------------------|----------------|-------------------------------|----------------|----------------|----------------|
| | | M ₀ | M ₁ | M ₂ | M ₃ |
| S ₀ | N ₀ | 0.00o | 54.00b | 59.00a | 46.33d |
| | N ₁ | 0.00o | 51.00c | 58.67a | 44.00e |
| S ₁ | N ₀ | 0.00o | 30.33i | 40.35fg | 19.33lm |
| | N ₁ | 0.00o | 29.67i | 38.67h | 18.33lm |
| S ₂ | N ₀ | 0.00o | 17.67m | 24.67i | 14.00n |
| | N ₁ | 0.00o | 18.67lm | 20.00l | 14.00n |

Means followed by the same letter are not significantly different at the 5% probability level according to Duncan's new multiple range test. M₀=uninoculated control, M₁=*Glomus mosseae*, M₂=*Glomus deserticola*, M₃=*Gigaspora gigantea*, N₀=uninoculated control, N₁=inoculated with *Meloidogyne incognita*, S₀=0.16 dS/m, S₁=3.24 dS/m, and S₂=6.06 dS/m

Table 6: Effects of AMF and salinity on plant height (cm) of pepper inoculated with or without *Meloidogyne incognita*.

| Salinity level (dS/m) | Nematode | Arbuscular mycorrhizal fungus | | | |
|-----------------------|----------------|-------------------------------|----------------|----------------|----------------|
| | | M ₀ | M ₁ | M ₂ | M ₃ |
| S ₀ | N ₀ | 26.93de | 34.83b | 45.20a | 28.37d |
| | N ₁ | 18.37lm | 27.07de | 34.83b | 24.50fgh |
| S ₁ | N ₀ | 23.37ghi | 26.17ef | 36.00b | 23.67ghi |
| | N ₁ | 15.10n | 22.67hi | 31.83c | 20.50jk |
| S ₂ | N ₀ | 19.43kl | 22.27ij | 28.83d | 19.67kl |
| | N ₁ | 12.67o | 18.50klm | 25.27efg | 17.03m |

Means followed by the same letter are not significantly different at the 5% probability level according to Duncan's new multiple range test. M₀=uninoculated control, M₁=*Glomus mosseae*, M₂=*Glomus deserticola*, M₃=*Gigaspora gigantea*, N₀=uninoculated control, N₁=inoculated with *Meloidogyne incognita*, S₀=0.16 dS/m, S₁=3.24 dS/m, and S₂=6.06 dS/m

salinity level in the presence or absence of *M. incognita* resulted in a significant decrease in plant height. In nematode-inoculated plants, at all salt levels, plants inoculated with AMF were significantly taller than their uninoculated counterparts. However, in all cases, *G. deserticola*-inoculated plants were significantly taller than others followed by *G. mosseae*-inoculated plants.

3.7. Number of Leaves

The results showed that only the individual effects of AMF, salinity, and nematodes and the interaction between AMF and nematodes were

Table 7: Effects of AMF and salinity on the number of leaves per plant (NL) of pepper inoculated with and without *Meloidogyne incognita*.

| AMF (M) | NL | Salinity level (S) (dS/m) | NL | Nematode (N) | NL | M×N interaction | NL |
|----------------|--------|---------------------------|--------|----------------|--------|-------------------------------|--------|
| M ₀ | 18.22d | S ₀ | 27.17a | N ₀ | 25.64a | M ₀ N ₀ | 18.89e |
| M ₁ | 24.78b | S ₁ | 23.75b | N ₁ | 22.42b | M ₀ N ₁ | 17.56f |
| M ₂ | 29.83a | S ₂ | 21.17c | | | M ₁ N ₀ | 26.78b |
| M ₃ | 23.28c | | | | | M ₁ N ₁ | 22.78d |
| | | | | | | M ₂ N ₀ | 32.33a |
| | | | | | | M ₂ N ₁ | 27.33b |
| | | | | | | M ₃ N ₀ | 24.56c |
| | | | | | | M ₃ N ₁ | 22.00d |

Means followed by the same letter are not significantly different at the 5% probability level according to Duncan's new multiple range test. M₀=uninoculated control, M₁=*Glomus mosseae*, M₂=*Glomus deserticola*, M₃=*Gigaspora gigantea*, N₀=uninoculated control, N₁=inoculated with *Meloidogyne incognita*, S₀=0.16 dS/m, S₁=3.24 dS/m, and S₂=6.06 dS/m

significant on the number of leaves produced by pepper plants [Table 1]. AMF inoculation significantly ($P \leq 0.05$) increased the number of leaves relative to the nonmycorrhizal plants [Table 7]. However, *G. deserticola*-inoculated plants produced a significantly higher number of leaves ahead of *G. mosseae*-inoculated plants. Successive increases in salt level significantly reduced the number of leaves. Furthermore, in both mycorrhizal and nonmycorrhizal plants, nematode inoculation significantly reduced the number of leaves. However, among AMF-inoculated plants in the presence of *M. incognita*, *G. deserticola*-inoculated plants produced a significantly higher number of leaves.

3.8. Dry Shoot Weight

The effects of AMF, salinity, and nematodes and their interactions were significant on the dry shoot weight of pepper [Table 1]. In both mycorrhizal and nonmycorrhizal plants, nematode inoculation significantly reduced dry matter accumulation in the shoot at all salinity levels with the exception of nonmycorrhizal plants at 3.24 dS/m and *Gi. gigantea*-inoculated plants at 6.06 dS/m [Table 8]. In some cases, there was a significant reduction in shoot dry matter as salinity level was increased from 0.16 dS/m (control) to 6.06 dS/m in both mycorrhizal and nonmycorrhizal plants in the presence or absence of *M. incognita*. In all cases, *G. deserticola*-inoculated plants in the presence or absence of nematodes at all salinity levels accumulated the highest dry matter in shoot compared with the other AMF species.

4. DISCUSSION

Evidence from this study showed that salinity significantly reduced plant height, fresh root weight, shoot dry weight, and the number of leaves in pepper. Salinity reduces the growth and development of plants [4,6,12]. These findings agree with Abdel Latef and Chaoxing [14], who reported that salinity stress significantly reduced assimilate partitioning to root, stem, leaf, and leaf area of tomato compared with the control plants. They attributed these to direct effects of ion toxicity or indirect effects of saline ions resulting in soil/plant osmotic imbalance. Salinity also affected nematode activities. It was observed that where there was nematode inoculation, a higher level of salt-reduced nematode activities. Root galling was reduced at the higher salt level. A similar observation was made by Hamdy *et al.* [28]. This could be due to the reduction of growth and development of the host plant which leads to the failure of the host tissue to keep pace with the nutritional demands of the nematodes [36]. This finding corroborates the report of Edongali and Ferris [27] and Hamdy *et al.* [28] that increase in salinity decreased *M. incognita* reproduction and root galling in susceptible varieties of tomato and okra, respectively.

Table 8: Effects of AMF and salinity on dry shoot weight (g/plant) of pepper inoculated with or without *Meloidogyne incognita*.

| Salinity level (dS/m) | Nematode | Arbuscular mycorrhizal fungus | | | |
|-----------------------|----------------|-------------------------------|----------------|----------------|----------------|
| | | M ₀ | M ₁ | M ₂ | M ₃ |
| S ₀ | N ₀ | 1.16i | 2.27e | 4.47a | 2.04f |
| | N ₁ | 0.96j | 1.97fg | 3.15b | 1.41h |
| S ₁ | N ₀ | 1.05ij | 2.03f | 3.08b | 1.89fg |
| | N ₁ | 0.90j | 1.84g | 2.89c | 1.15i |
| S ₂ | N ₀ | 0.96j | 1.81g | 2.62d | 1.02ij |
| | N ₁ | 0.68k | 1.46h | 2.02f | 1.03ij |

Means followed by the same letter are not significantly different at the 5% probability level according to Duncan's new multiple range test. M₀=uninoculated control, M₁=*Glomus mosseae*, M₂=*Glomus deserticola*, M₃=*Gigaspora gigantea*, N₀=uninoculated control, N₁=inoculated with *Meloidogyne incognita*, S₀=0.16 dS/m, S₁=3.24 dS/m, and S₂=6.06 dS/m

High levels of salinity suppressed root colonization by arbuscular mycorrhiza fungi. This is in agreement with the reports by several researchers [16,17,37]. They reported the negative effects of salinity, stating that the rate of spore germination, hyphal colonization of root, and hyphal growth of the fungus were impeded. Pepper seedlings with AMF significantly had reduced effects of salts. This was obvious with the fact that the pepper plants inoculated with AMF had higher fresh root weight and shoot dry weight and were taller. This conforms to the reports of several researchers who reported enhanced growth attributes and yield by AMF-inoculated plants under saline stress compared to the nonmycorrhizal plants. They stated that plant growth and biomass suffered a setback under salt stress. This may likely be due to nutrient deficiency and waste of energy to alleviate the toxic effects of salt [12,22,40].

Inoculation of pepper seedlings with AMF reduced galling and egg mass production. This agrees with Borowicz's [41] findings where he listed various results of many workers confirming the reduction in the reproduction of sedentary nematodes due to AMF inoculation. The decrease in the fecundity was attributed to physiological changes and/or physical or chemical barriers leading to the unattractiveness of the roots for easy penetration by plant-parasitic nematodes. The inhibition in root galls observed on pepper seedlings could have occurred as a result of competition between the nematode and the symbiont for infection sites, though other factors such as higher concentration of lignin and phenols could be involved [19,24]. Zhang *et al.* [19] recorded fewer galls and egg masses in cucumber plants inoculated with AMF than the uninoculated plants. This was attributed to the failure of the nematode to penetrate the root and initiate the

formation of giant cells as well as impairment of the nematode's development [23,42,43].

However, among the AMF species, *G. deserticola* was the most effective in reducing root-knot nematode infection and alleviation of salt stress in pepper plants. There are various reports on the variation in the efficacy of different AMF in nematode control and salinity amelioration [21,44]. Mohammed and Mittra [44] observed that an isolate of a stress-adapted AMF species, *G. deserticola*, was the most effective in alleviating salinity and heavy metal stresses as well as enhancement of *Solanum melongena* and *Sorghum sudanense* growth and dry matter yield compared to other AMF species. This is in line with the result of this trial. In general, this trial has illustrated the possibility of managing the abiotic stress factor induced by salinity and the biotic factor caused by *M. incognita* on pepper production with proper selection and inoculation of the symbiont, AMF. Under saline soils, appropriate and sufficient AMF propagules may be lacking, thus the need for inoculation of nursery seedlings with efficient AMF species.

5. CONCLUSION

AMF inoculation significantly reduced root gallings and egg masses by *M. incognita* and also enhanced growth and dry matter production of sweet pepper plants in a simulated salinity condition. This study revealed that the most efficient AMF species in salinity amelioration and reduction in nematode infectivity was *G. deserticola* followed by *G. mosseae*. In conclusion, *G. deserticola* and *G. mosseae* could be effectively utilized as eco-friendly management bioagents in pepper production where salinity and *M. incognita* are constraints.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

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All data generated and analyzed are included within this research article.

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