



RESEARCH ARTICLE

Poultry manure and arbuscular mycorrhiza fungi remediation of sodium chloride induced substrate salinity for Pepper production

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ABSTRACT

Pepper (*Capsicum annum*) is an important spice in the world, but the production is constrained by soil salinity. Hence, this study aims to explore the use of organic manure and Arbuscular Mycorrhiza Fungi in reducing the soil saline state for pepper cultivation. Two genotypes of cherry pepper were planted in the following treatment combinations; Cocopeat + poultry manure, Cocopeat + 10 mg/ml NaCl, 10 mg/ml NaCl + 250 g/4kg cocopeat, 10 mg/ml NaCl + 250 g/4kg cocopeat + 5 g of AMF, Cocopeat + 20 mg/ml NaCl, 20 mg/ml NaCl + 250 g/4kg cocopeat and 20 mg/ml NaCl + 250 g/4kg cocopeat + 5 g of AMF. The experiment was arranged in a completely randomized design with three replicates. The agronomic and yield parameters were collected and analysed using ANOVA and means were separated using DMRT at 5% significance level. Results obtained showed that the tallest plants, highest number of nodes, number of fruits and fruit weight (kg) were observed in the control (14.85±0.49), (20.50±0.68), (8.67±0.52) and (1.38±0.08), respectively, although they were not significantly different from the addition of 250 g of poultry manure and 5 g of AMF to 4 kg cocopeat substrate.

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INTRODUCTION

Pepper (*Capsicum annum*) is an important spice predominantly cultivated in Southern America (Mexico) were its native to, and has been domesticated in other parts of the world (Adhikari et al., 2016). The fruit is highly rich in different micro and macro elements (magnesium, calcium, potassium, phosphorus and iron), sugars, phytochemicals like polyphenols, carotenoids (Julio et al., 2020). Due to the wide nutritional properties of pepper, it has been used by humans in different delicacies like salads and soup making in different parts of the world, which has made pepper an established cash crop (Jadzack et al., 2010).

Due to the large demands for pepper worldwide, the need to increase productivity is highly necessary, and this comes with the wide range of land exploration in the cultivation of pepper, however, its production is constrained by both biotic (insect pest and diseases) and abiotic (salinity, heat, cold and drought) factors which has caused severe damages beyond economic injury levels and crop losses yearly (Atkinson and Urwin, 2012). Soil salinity is a typical example of an abiotic factor limiting the productivity of pepper, as those farmers in the arid regions are frequently faced with this challenge in pepper production (Tayyab et al., 2016). An example of a saline soil is that caused by the excessive content of sodium chloride in the soil which inhibits plant growth by causing water deficit effect or the release of excess ions thereby disrupting normal metabolism by the plants (Abbaspour, 2012).

In Nigeria, efforts have been channeled into reducing the effect of excess salt in the farmlands by avoiding areas prone to saline effects because pepper responds better to a moderate salt stressed condition (Marco et al., 2011). However, the rain or river water depended upon by the farmers further exposes the plant to saline stress. An alternative way to solve the problem of excess ions released to the soil in a saline state is to fertilize the soil with an organic manure that will release a counteractive ion into the soil through timely mineralization as demonstrated by Dong et al. (2012). To help in the breakdown of these organic manures for the timely absorption by the plants, the use of an Arbuscular Mycorrhiza Fungi (AMF) has been reported (Kumar et al., 2019). It is then important to evaluate the combined role of AMF and organic manure in improving the excess saline

state in pepper growth environment using the hydroponics system that is devoid of any saline state.

MATERIALS AND METHODS

The experimental site and environmental condition

The *ex-vitro* experiment was conducted between May 04 - June 04, 2021, at the Department of Microbiology, University of Ibadan, Nigeria in a greenhouse. The site is located at 7.4433° N, 3.9003° E.

Source of experimental materials

Buffered cocopeat blocks were purchased from Afri-Agri Company, Lagos. Well cured poultry manure was purchased from Ajayi Farms. Analytical salt (Sodium chloride (NaCl)) was purchased from Sigma Aldrich. The AMF (*Claroideoglomus etunicatum*) was obtained from the Botany Department of University of Ibadan. The radish seeds (two genotypes of pepper (fireball and cherry stuffer)) and hydroponic troughs were purchased from the Soilless FarmLab Limited, Abeokuta, Ogun State, Nigeria.

Experimental procedure

The cocopeat blocks were dissolved with water (60 litres per cocopeat block) and poured into 4 kg hydroponic pots, and 250 g of organic manure (poultry manure) were measured into the 4 kg cocopeat substrate (except the control and two containers as arranged in the treatments below). 100 g of NaCl salt was dissolved in 1 L of water to give a concentration of 10 mg/ml, while 200 g of the NaCl was dissolved in 1 L of water to give a concentration of 20 mg/ml as watering treatments done thrice per week. The radish seeds were sown one per pot and were arranged in a treatment combination below:

1. S0: control (Cocopeat + poultry manure),
2. S10MAMF: 10 mg/ml NaCl + 250 g/4kg cocopeat + 5 g of AMF,
3. S1: Cocopeat + 10 mg/ml NaCl,
4. S10M: 10 mg/ml NaCl + 250 g/4kg cocopeat,
5. S2: Cocopeat + 20 mg/ml NaCl,
6. S20M: 20 mg/ml NaCl + 250 g/4kg cocopeat,
7. S20MAMF: 20 mg/ml NaCl + 250 g/4kg cocopeat + 5 g of AMF

Experimental design and data collection

The experiment was a 2 (genotypes) by 7 (growth environment) factorials in a Completely Randomised Design (CRD) with three replicates. At bi-weekly

interval, data were taken on the plant height and number of leaves, also the number of days to 50% flowering, days to fruiting, number of fruits and the average fruit weight was collected.

Data analysis

Data were subjected to analysis of variance (SAS 9.0 ver.) and differences in treatment means were separated using DMRT at 5% level of significance.

RESULTS

Results obtained showed that at 2 weeks after planting, the number of leaves produced by the cherry pepper was highest in S2OMAMF (6.50±0.27), however, it was not significantly different from the control (6.17±0.27) but was significantly higher than the rest treatments. At 4 weeks after planting, height of the plants in the control (10.17±0.50) was statistically same with the S1OMAMF (9.97±0.50), and they were both significantly higher than the rest treatments. Also, the number of leaves produced at 4 weeks after planting in the control (10.50±0.53) was not significantly different from S1OMAMF

(10.33±0.53) and S2OMAMF (10.67±0.53), but was significantly higher than the rest treatments. At 6 weeks after planting, the tallest plants were observed in the control (14.85±0.49), however, it was only significantly higher than S1 (12.88±0.49), S2OM (13.28±0.49) and S2 (12.95±0.49), respectively. Also, the number of leaves produced was highest in the control (20.50±0.68), which was not significantly different from S1OMAMF (19.33±0.68) and S2OMAMF (19.67±0.68), but was significantly higher than the rest treatments (Table 1).

The genotypic performances at 4 weeks after planting shows that the number of leaves produced by the fireball (10.80±0.34) was significantly higher than the cherry stuffer (9.73±0.34) genotype. However, at 6 weeks after planting, the height of the cherry stuffer genotype (14.37±0.31) was significantly taller than the fireball (13.40±0.31) genotype. The interaction between genotypes and treatments was significant for the number of leaves at 2, 4 and 6 weeks after planting, and for the plant height at 4 and 6 weeks after planting respectively (Table 2).

Table 1. Effect of salt stress, organic manure and arbuscular mycorrhiza fungi on the growth parameters of cherry pepper (*Capsicum annum*)

Stress level	PH2	NOL2	PH4	NOL4	PH6	NOL6
S0	3.27 ^a	6.17 ^{ab}	10.17 ^a	11.50 ^a	14.85 ^a	20.50 ^a
S1OMAMF	3.18 ^a	5.17 ^c	9.97 ^a	10.33 ^{ab}	14.50 ^{ab}	19.33 ^{ab}
S1OM	3.07 ^a	5.00 ^c	8.70 ^{ab}	9.33 ^b	14.15 ^{ab}	17.17 ^c
S1	3.07 ^a	5.11 ^c	8.00 ^b	9.01 ^b	12.88 ^c	15.56 ^c
S2OMAMF	3.52 ^a	6.50 ^a	9.18 ^{ab}	10.67 ^{ab}	14.65 ^a	19.67 ^{ab}
S2OM	3.48 ^a	5.50 ^{bc}	8.25 ^b	9.50 ^b	13.28 ^{bc}	18.17 ^{bc}
S2	3.18 ^a	5.13 ^c	7.99 ^b	8.77 ^b	12.95 ^{bc}	15.17 ^c
SE	0.17	0.27	0.5	0.53	0.49	0.68

Means with the same letter are not significantly different from each other at 5% level of significance, S0: control, S1OMAMF: 10 mg/ml NaCl + 250 g/4kg cocopeat + 5 g of AMF, S1: 10 mg/ml NaCl, S1OM: 10 mg/ml NaCl + 250 g/4kg cocopeat, S2: 20 mg/ml NaCl, S2OM: 20 mg/ml NaCl + 250 g/4kg cocopeat, S2OMAMF: 20 mg/ml NaCl + 250 g/4kg cocopeat + 5 g of AMF, SE: Standard error. PH2: Plant height at 2 weeks after planting, NOL: Number of leaves at 2 weeks after planting

Table 2. Agronomic performance cherry pepper (*Capsicum anum*) as influenced by Effect of salt stress, organic manure and arbuscular mycorrhiza fungi

Genotypes	PH2	NOL2	PH4	NOL4	PH6	NOL6
Fireball	3.80 ^a	5.80 ^a	8.87 ^a	10.80 ^a	13.40 ^b	18.40 ^a
Cherry stuffer	3.31 ^a	5.53 ^a	9.60 ^a	9.73 ^b	14.37 ^a	19.53 ^a
SE	0.11	0.17	0.31	0.34	0.31	0.43
Gen × Stress level interaction	0.13 ^{ns}	1.87 [*]	4.79 [*]	5.12 [*]	6.36 ^{**}	8.22 [*]

Means with the same letter are not significantly different from each other at 5% level of significance, SE: Standard error, Gen: Genotype, ns: not significant, *: significance at 95% and **: significance at 99%

The number of days taken by the pepper plants to produce fruits was highest in the control (60.17±1.22), which was only significantly higher than the plants grown in the S10M (55.17±1.22). The number of fruits produced in S0 (8.67±0.52), S10MAMF (8.17±0.52) and S20MAMF (8.00±0.52) were statistically same and were all significantly higher than S10M (5.33±0.52), S1 (5.00±0.52), S20M (5.17±0.52) and S2 (5.13±0.52), respectively. The

average fruit weight obtained in the control (1.38±0.08) g was not significantly different from the S10MAMF (1.15±0.08) g, but was significantly heavier than the rest treatments (Table 3). However, the weight of the fruits produced by Fireball (1.12±0.05) genotype was significantly higher than the Cherry stuffer (0.96±0.05) genotype. Also, the interaction between genotypes and treatments was only significant in the fruit weight (Table 4).

Table 3. Effect of salt stress, organic manure and arbuscular mycorrhiza fungi on the yield parameters of cherry pepper (*Capsicum annum*)

Stress level	DT50 %F	DTF	NOF	FW (kg)
S0	35.00 ^a	60.17 ^a	8.67 ^a	1.38 ^a
S10MAMF	34.50 ^a	59.17 ^a	8.17 ^a	1.15 ^{ab}
S10M	34.00 ^a	55.17 ^b	5.33 ^b	0.92 ^{bc}
S1	36.12 ^a	60.88 ^a	5.00 ^b	0.80 ^c
S20MAMF	34.67 ^a	56.67 ^{ab}	8.00 ^a	0.92 ^{bc}
S20M	36.17 ^a	58.67 ^{ab}	5.17 ^b	0.85 ^c
S2	36.77 ^a	58.02 ^{ab}	5.13 ^b	0.78 ^c
SE	1.18	1.22	0.52	0.08

Means with the same letter are not significantly different from each other at 5% level of significance, S0: control, S10MAMF: 10 mg/ml NaCl + 250 g/4kg soil + 5 g of AMF, S1: 10 mg/ml NaCl, S10M: 10 mg/ml NaCl + 250 g/4kg soil, S2: 20 mg/ml NaCl, S20M: 20 mg/ml NaCl + 250 g/4kg soil, S20MAMF: 20 mg/ml NaCl + 250 g/4kg soil + 5 g of AMF, SE: Standard error. PH2: Plant height at 2 weeks after planting, NOL: Number of leaves at 2 weeks after planting, DTF: Days to fruiting, DT50%F: Days to 50% flowering, NOF: number of fruits and FW: Fruit weight.

Table 4. Yield performance cherry pepper (*Capsicum annum*) as influenced by Effect of salt stress, organic manure and arbuscular mycorrhiza fungi

Genotypes	NOF	DTF	DT50% F	FW (kg)
Fireball	6.80 ^a	34.60 ^a	59.07 ^a	1.12 ^a
Cherry stuffer	6.20 ^a	35.13 ^a	56.87 ^a	0.96 ^b
SE	0.33	0.75	0.77	0.05
Gen × Stress level interaction	1.62 ^{ns}	5.22 ^{ns}	8.55 ^{ns}	0.12 [*]

Means with the same letter are not significantly different from each other at 5% level of significance, SE: Standard error, Gen: Genotype, ns: not significant, *: significance at 95%, DTF: Days to fruiting, DT50%F: Days to 50% flowering, NOF: number of fruits and FW: Fruit weight.

DISCUSSION

The geometric increase in human population has consequently led to the overexploitation of arable lands and human enhanced rock weathering activities to form soil (breaking down of small rock sediments) in a bid to ensure food security for the populace (Kumar et al., 2019). These activities had led to the

release of excess minerals to arable lands, thereby creating saline soil as a result of the excess salt, thus the soil possesses high pH level which is detrimental to plants.

The use of cocopeat in the hydroponics system of crop cultivation presents an alternative to soil, as the substrate contains an acceptable amount of pH,

electrical conductivity, easily manipulated and can be easily fertilized adequately (Balogun et al. 2018). This provides an adequate medium for the study and manipulation of the nutrient status of any growth medium for a given plant.

At the early growth stage of the pepper plants, the growth rate in the treatments were even, possibly because the saline states had not been fully translocated through the young roots to other parts of the plant, hence the salinity effect is gradually building up. However, as the plants grow older, the effect became more visible as the zero-salt stress condition enhanced better agronomic performances considering the leaf production and plant height, although the plants growth rate in the unstressed plants was same as the stressed ones amended with both organic manure and AMF.

According to Yadav et al. (2019), the level of sodium chloride in the soil directly affects plant growth, and the presence of this salt in the soil is compounded by the world's climate change menace, irrigation and high evapotranspiration that induces salt accumulation in the soil (Kumar et al., 2019). This makes the absorption of both water and nutrients by the plants difficult due to the water deficit condition created by the excess salt and the physiological development of the plants will be impeded (Yadav and Yadav, 2018).

Irrespective of the stress conditions, the two genotypes were similar in their performances, and the salt stress did not have significant effect on the days to flowering. However, despite the even flowering rate, the ameliorating effect of organic manure on the salt stress in addition enhanced the quick fruiting of the pepper plants, even as the unstressed condition and the organic manure and AMF control effect on salt stress performed best in terms of fruit production. This shows the ability of the combination appropriate amount of organic manure and AMF to neutralize the saline state of the soil caused by salts.

The use of AMF to ameliorate the effect of salt stress on plants had earlier been reported by Kumar et al. (2019). This becomes necessary as the organisms colonizes the root zone of most plants as they form a symbiotic relationship with the plants that supplies them carbohydrate and they in turn extends the surface area of the plant roots. The ability of the plants to absorb water from the soil is improved

through the symbiotic set up as most AMF are known to thrive under saline conditions (Zhang et al., 2018).

Although, in terms of number of fruit production, both genotypes performed equally, but the sizes of the fireball fruits were heavier than the cherry stuffers. However, fruit sizes may not correlate directly with pungency, but in terms of sale, the bigger size generates higher income to the farmer.

CONCLUSION

The nutritive contents of pepper had made it a worldwide sort after fruit, thereby increasing the pressure on the production. Its production cannot be holistically achieved without available arable farmland with conducive nutrient contents. Due to the stretch on land usage, weathering, irrigation and excess use of mineral fertilizers, salt availability in the soil has become excess in arid and semi-arid regions leading to the saline nature of the soil. The findings of this study had shown the ameliorating effect of the combination of organic manure and AMF strains that are known to thrive under saline state to restore normalcy for the cultivation of pepper plants in these regions of the world.

DISCLOSURE STATEMENT

There is no conflict of interest reported by the authors.

AUTHORS CONTRIBUTION

Akpeji Stephanie carried out the experiment and collected the data and interpreted the analysed data, Ossai Chukwunalu designed the experiment and analysed the data and carried out the final editing of the manuscript, Oroghe Elohor led the write up of the manuscript. The manuscript was read by all the authors and approved it.

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