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# **Development of Eco-friendly Technology for the Management of Dry Land Saline Soil**

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## *Authors' contributions*

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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# **ABSTRACT**

To develop an eco-friendly and economically viable biological technology to alleviate dry land salinity, an incubation experiment was carried out at Tamil Nadu Agricultural University, Coimbatore during 2021-22 in saline soils with varied EC values viz., 4, 5 and 6 dS m<sup>-1</sup> collected from Adivalli village of Coimbatore district, Tamil Nadu. The soils were treated with microbial cultures developed from both TNAU (*Bacillus subtilus*) and CSSRI (CSR-GROW-SURE bio-stimulant which consists of CSR-M-16 (*Bacillus licheniformis*), CSR-A-11 (*Lysnibacillus fusiformis*), CSR-A-16 (*Lysinibacillus* sphaericus)) at different rates namely 1, 2 and 3 L ha<sup>-1</sup> under two varied moisture regimes this is 75% and 100% field capacity. The results of the study showed that application of irrespective of microbial cultures (CSSRI / TNAU) and moisture regimes (75% /100% field capacity) were effective in reducing the soil EC and SAR. The CSR-GROW-SURE at 3 L ha<sup>-1</sup> had reduced the salinity to the tune of 7 to 9 % and SAR from 26 to 27 % and it was on par with TNAU culture at 3 L ha<sup>-1</sup> which also reduced the EC from 6 to 9 % and SAR between 25 and 26 % after 90 days of incubation. The results of both the moisture regimes namely 75% and 100% FC were comparable but a little higher effect was pronounced at 100% FC.

*Keywords: TNAU culture (Bacillus subtilus); CSR-GROW-SURE bio-stimulant; saline soils; 75% and 100% field capacity; salinity parameters.*

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# **1. INTRODUCTION**

Soil salinity is one of the damaging environmental stresses, resulting in significant decrease in cultivated land area [1]. In the world, one of the adverse effects of soil sustainability is soil salinity, particularly in the arid and semi-arid regions [2]. According to estimates, high salinity affects 20% of total farms and 33% of irrigated agricultural regions around the world [3]. By 2050, it is anticipated that around half of all arable land will be affected by salinity stress [4]. Natural salt accumulation over time is the fundamental cause of the salinity of the soil. The breakdown of sodium, calcium, and magnesium containing rocks, salt deposition by the wind and surface runoff water are the natural processes of salinity, and the secondary salinity forming process is by anthropogenic activities. When the electrical conductivity of a soil exceeds 4.00 d Sm<sup>-1</sup> (approximately 40 mM NaCl), it is classified as saline soils [3]

Using microbes for the remediation of saltaffected soils is a cheap and eco-friendly approach [5]. Microbial populations in soil are more plentiful on the planet, and they are responsible for a variety of ecological and economic functions [6,7,8,9]. These microorganisms are versatile and adaptive to various challenging environmental conditions like salt stress, and they are considered to be the first life forms to have evolved [10].

Bacteria such as Achromobacter, Arthrobacter, Bacillus, Chryseobacterium, Enterobacter, Ochrobactrum, and Pseudomonas isolated from saline soil have been shown in numerous studies to enhance soil properties without any adverse effects on soil [11,12,13]. Among these, Bacillus spp. can be sustained in adverse conditions [14]. Bacillus spp., a halophilic bacterial genus, has the ability to alleviate drought and salinity stress in soil [15,16,17] by producing 1 aminocyclopropane-1-carboxylate (ACC) deaminase [18], increase nutrient availability in soil by converting insoluble compounds to soluble compounds [19], and make them available by producing organic acids, siderophore, and polysaccharide capsule As well, these microbes conserve the nutrient reserves [21]. Further, usage of microbes for management of saline soils can reduce fertilizer consumption [22,23,24] and increase the soil fertility by mobilisation of nutrients in soil [25,26]. It also improves the properties of soil [27]. There are many ecofriendly uses for using microorganisms to reclaim saline soils. The present study is aimed at reclaiming the saline soils by using microbial cultures which are halophilic in nature that is Bacillus spp.

# **2. MATERIALS AND METHODS**

# **2.1 Collection of Soil Samples**

Soil samples of 4.03, 5.01 and 6.03 dS  $m^{-1}$  were collected from Adivalli village, Udumalpet talk of Coimbatore district with latitude of 10°41'44" N, 10°41 '33" N, 10°41 ' 29" N respectively and longitude of 77 °09' 21" E, 77°09' 18" E, 77°09 04" E respectively**.**

# **2.2 Collection of Microbial Cultures**

For the present investigation, microbial consortia CSR-GROW-SURE Bio stimulant containing salt tolerant bacterial strains *viz.,* CSR-M-16 (*Bacillus licheniformis*), CSR-A-11 (*Lysnibacillus fusiformis*), CSR-A-16 (*Lysinibacillus sphaericus*) was obtained from Central Soil Salinity Research Institute, Karnal, Haryana and another salinity resistant culture *Bacillus subtilis* isolated by the Tamil Nadu Agricultural University, Coimbatore was also utilized.

## **2.3 Details of the Incubation Experiment**

A known weight of air-dried soil (2 mm sieved 4.03, 5.01 and 6.03 dS m<sup>-1</sup>) was taken (250g) in a incubation cups and imposed with different rates of microbial culture. The treatment structure comprised of graded levels of microbial culture at the rate of 1,2 and 3 L ha<sup>-1</sup> of soil (w/w basis) and replicated thrice in a completely randomized<br>design. Three sets were maintained for Three sets were maintained for destructive sampling. The microbial population of  $(1.0 \times 10^7 \text{ CFU per ml})$  in TNAU Culture and  $(1.0 \text{ FU})$  $x$  10<sup>7</sup> CFU per ml) in CSR-GROW-SURE biostimulant were thoroughly mixed with the soil and required quantity of distilled water was added to achieve a final moisture content equivalent to field capacity (75 and 100 %). The soils were incubated at two moisture regimes (75 and 100% field capacity) for three months (90 days) period and based on the weight loss distilled water was added once in two days to the container to maintain an uniform moisture content throughout the incubation period. Destructive sampling was done at intervals *viz.,* 30, 60 and 90 days after incubation and analyzed for important soil characteristics. Moisture factor was computed and applied to express the results on an oven dry basis.

<b>Treatments</b>	<b>Other Details</b>
$T_1$ – Control	Soil EC: 4.03, 5.01, 6.02 dS m <sup>-1</sup>
$T_2$ - TNAU Culture @ 1 L ha <sup>-1</sup>	
$T_3$ - TNAU Culture @ 2 L ha <sup>-1</sup>	Moisture Regimes: 75 and 100% field capacity
$T_4$ - TNAU Culture @ 3 L ha <sup>-1</sup>	
$T_5$ - CSR-GROW-SURE @ 1 L ha <sup>-1</sup>	Incubation Periods: 30, 60 and 90 Days
$T_6$ - CSR-GROW-SURE @ 2 L ha <sup>-1</sup>	
$T_7$ - CSR-GROW-SURE @ 3 L ha <sup>-1</sup>	Replication: 3

**Table 1. Treatment details**

**Table 2. Initial experimental soil properties**

<b>Soils</b>	Cations (meg kg <sup>"</sup> )				Anions (meq kg"			
	$Ca2+$	$Mq^{2+}$	$Na+$	v+	HCO <sub>2</sub>	Сľ	<b>SO</b>	<b>SAR</b>
$4.03$ dS m <sup>-1</sup>	10.34	5.48	15.27	7.99	3.42	22.10	13.56	5.43
5.01 dS $m^{-1}$	12.64	6.98	19.103	9.89	3.76	27.003	17.85	6.10
6.02 dS $m^{-1}$	14.58	8.58	23.76	12.04	3.98	35.15	19.83	6.98

## **2.4 Soil Analysis**

The soils were analyzed for pH and EC by 1:2.5 Soil water extract [28]. Exchangeable cations like calcium, magnesium, sodium, potassium, and anions namely bicarbonates, chlorides by standard methods [29], sulfates by turbidimetric<br>method [30] and computed for salinity method [30] and computed for salinity parameters *viz.,* sodium absorption ratio [29].

#### **2.5 Statistical Analysis**

The data obtained from the experiment were subjected to statistical analysis using AGRESS software version 7.01. Critical Difference (CD) values were calculated for the P < 0.05 whenever "F" test was found significant [31].

## **3. RESULTS AND DISCUSSION**

#### **3.1 Effect of Microbial Culture on Soil EC**

The soil EC decreased from the initial value due to addition of microbial cultures with varied rates and the per cent of decrease was increased with increasing rates. Significantly lower mean EC values of 3.77 & 3.76; 4.77 & 4.76 and 5.69 & 5.68 dS m<sup>-1</sup> were recorded in CSR-GROW-SURE at 3 L ha<sup>-1</sup> and it was on par with TNAU culture  $@$ 3 L ha-1 with mean EC of 3.78 & 3.77; 4.78 & 4.77 and 5.70 & 5.69 dS  $m^{-1}$  in 4.03, 5.01 and 6.02 dS  $m^{-1}$  salinity soils under 75 and 100% FC moisture respectively. However, comparing the different moisture regimes, slightly higher values were registered under 100%. Due to cationic adsorption by exopolysacrides makes the EC less in soil treated with *Bacillus spp* [32]. Similar works also done earlier with the application of microbial culture resulted in reduction of EC [33,34].

Comparing different periods of incubation, there was a significant decrease in the EC value (7 to 9%) with increasing periods of incubation up to three months. For example, the initial EC of soils of 4.03, 5.01 and  $6.02$  dS  $m^{-1}$  due to addition of bio-stimulants gradually decreased with advancement of incubation periods and it recorded the mean values of 3.90, 3.81 and 3.75; 4.89, 4.81 and 4.75; and 5.87, 5.73 and 5.64 dS m<sup>-1</sup> at 30, 60 and 90 DAI respectively at 75% FC moisture. However higher per cent 3 - 5 % of reduction was observed at 30 DAI and there after increased with decreasing rate (1 to 2 %) up to 60 and 90 DAI. The data provide evidence for the priming effect of microbes. Similar trend with little higher reduction was observed in 100% FC moisture regime. The reasons are apparent. With the advancement of incubation period, the population of the microbes would have been increased and their by the amount of production of Indole Acetic acid and Absasic acid also increased might be the reason. Similar findings showed increasing period of incubation increased the microbial population and their activity in soil, which caused significant reduction in soil EC [35].

The interaction effect between cultures with varied rates and incubation periods on soil EC was significant. Among the treatments, significantly higher reduction in EC was recorded in CSR-GROW-SURE @ 3 L ha<sup>-1</sup> at 90 DAI with the EC of 3.69 & 3.68; 4.69 & 4.68 and 5.56 & 5.55 in 4.03, 5.01 and 6.02 soils at 75 and 100% FC moisture level respectively and it was at par



## Table 3. Effect of microbial cultures on EC and SAR at different moistures of 4 .03 dS m<sup>-1</sup> soil (Mean of three values)



## Table 4. Effect of microbial cultures on EC and SAR at different moistures regimes of 5.01 dS  $\text{m}^{\text{-}1}$  soil



 ${\sf T}_6$   $\hspace{1cm}$  5.83 (3.21)  $\hspace{1cm}$  5.67 (5.99)  $\hspace{1cm}$  5.56 (7.95)  $\hspace{1cm}$  5.69  $\hspace{1cm}$  6.36 (9.29)  $\hspace{1cm}$  5.77 (18.99)  $\hspace{1cm}$  5.38 (25.97)  $\hspace{1cm}$  5.84  ${\sf T}_7 \hspace{1.5cm} 5.82 \, (3.38) \hspace{1.5cm} 5.66 \, (6.16) \hspace{1.5cm} 5.55 \, (8.12) \hspace{1.5cm} 5.68 \hspace{1.5cm} 6.32 \, (10.04) \hspace{1.5cm} 5.74 \, (19.51) \hspace{1.5cm} 5.34 \, (26.75) \hspace{1.5cm} 5.80$ 

Cultures(C) Duration(D)  $C \times D$  Cultures(C) Duration(D)  $C \times D$ <br>0.01 0.03 0.04 0.03 0.03 0.06

Mean 5.86 5.72 5.63 6.46 5.96 5.62

SEd 0.01 0.03 0.04 0.03 0.03 0.06 CD (0.05) 0.03 0.06 0.09 0.06 0.07 0.13

Table 5. Effect of microbial cultures on EC and SAR at different moistures of 6.02 dS  $\text{m}^{\text{-1}}$  soil



**Fig. 1. Effect of microbial cultures on EC, SAR with different incubation periods at 75% field capacity of 4.03 dS m -1 soil**



**Fig. 2. Effect of microbial cultures on EC, SAR with different incubation periods at 100% field capacity of 4.03 dS m -1 soil**



**Fig. 3. Effect of microbial cultures on EC, SAR with different incubation periods at 75% field capacity of 5.01 dS m -1 soil**



**Fig. 4. Effect of microbial cultures on EC, SAR with different incubation periods at 100% field capacity of 5.01 dS m -1 soil**



**Fig. 5. Effect of microbial cultures on EC, SAR with different incubation periods at 75% field capacity of 6.02 dS m -1**



**Fig. 6. Effect of microbial cultures on EC, SAR with different incubation periods at 100% field capacity of 6.02 dS m -1 soil**

with TNAU culture  $@3$  L ha<sup>-1</sup> at 90 DAI with the EC of 3.70 &3.69: 4.70 & 4.69 and 5.57 & 5.56 in 4.03; 5.01 and 6.02 dS  $\text{m}^{-1}$  soils at 75 and 100% FC moisture levels respectively. Comparing different soil moisture regimes, both 75 and 100% field capacity moistures produced same trend of reduction in EC however, slightly higher reduction was found at 100% field capacity moisture regime. Hence, it has become clear that application of either CSR-GROW-SURE @ 3 L ha $^{-1}$  or TNAU culture @ 3 L ha $^{-1}$  that can act as a soil amendment and has the capacity to reduce soil salinity from 7 to 9 and 8 to 9% respectively.

Further the effect of CSR-GROW-SURE and TNAU cultures showed almost similar trend of results in all the EC soils *viz.,* 4.03, 5.01 and 6.02  $dS m^{-1}$ .

# **3.2 Effect of Microbial Culture on Soil SAR**

The results of SAR of bio stimulant amended soils showed a significant reduction under all the doses of application. The treatment CSR-GROW-SURE @ 3 L ha<sup>-1</sup> application had recorded the higher mean reduction (4.52 & 4.47; 5.02 & 5.13 and 5.84 & 5.80 in 4.03, 5.01 and 6.02 dS  $m^{-1}$  salinity soils at 75 and 100% FC moisture respectively) and it was at par with application of TNAU culture @ 3 L ha-1 (4.55 & 4.52; 5.07 & 5.02 and 5.87 & 5.84 in 4.03, 5.01 and 6.02 dS  $m^{-1}$  salinity soils at 75 and 100% FC moisture respectively) while, the highest value was recorded by control *viz.* soil without microbial culture (5.46, 6.13 and 7.00 in 4.03, 5.01 and 6.02  $dS$  m<sup>-1</sup> salinity soils respectively). The reduction in SAR could be due to greater absorption of Na<sup>+</sup> by the microbial culture amended soils since the CSR- Grow Sure and TNAU cultures used in the experiment had more affinity to Na<sup>+</sup>. The sodium content in soil was reduced due to uptake of sodium @ 1.272 meq / L by *Bacillus subtilis* and 1.122 meq / L by *Bacillus pumilis* at 1 M NaCl concentration [36].The sodium which is taken by halotolerant bacteria is replaced by the potassium accumulation in cell through K<sup>+</sup>/Na<sup>+</sup> ion transporters, Na<sup>+</sup>/H<sup>+</sup> antiporters [37]. And also due to displacement of sodium by sulfuric acid produced by *Bacillus spp* [38].

Significant reduction in SAR values was found under different period of incubation and there was a progressive decrease under all periods of incubation up to 90 days except in the control treatment. However significantly higher reduction

was found at 90 DAI. The effect of incubation period on SAR was statistically significant at both the moisture regimes but slightly higher reduction was found at 100% field capacity moisture. In general, the added microbes might have increased its population with days of incubation and accounted for the decrease in Na<sup>+</sup> content in the saline soils. Many studies proved that application of microbes reduced the sodium content in the soil [10,39].

Comparing different treatments, application of CSR- GROW-SURE  $@ 3 L$  ha<sup>-1</sup> at 90 DAI reduced SAR significantly to the tune of 26 to 27 % with the mean SAR values of 4.19 & 4.15; 4.65 & 4.61 and 5.38 & 5.34 in 4.03, 5.01 and 6.02 dS m -1 EC soils at 75 and 100% FC moisture respectively. And it was on par with application of TNAU culture  $@3$  L ha<sup>-1</sup> With mean values of 4.22 &4.19; 4.69 & 4.65 and 5.41 & 5.38 in 4.03, 5.01 and 6.02 dS  $m^{-1}$  EC soils at 75 and 100% FC moisture respectively. However a little higher reduction was noted under 100% FC condition. At all times, application of either CSR-GROW-SURE or TNAU culture at the rate of  $3 L$  ha<sup>-1</sup> recorded the lowest values followed by application of these culture  $@$  2 and 1 L ha<sup>-1</sup>. The highest value was recorded in control treatment. The reduction in Sodium absorption ratio was due to binding of cations by *Bacillus spp* [40].

# **4. CONCLUSION**

As chemical amendments are causing damage to soils, it is better to use microorganisms in the reclamation of saline soils. Hence, an ecofriendly technology was developed by using TNAU microbial culture (*Bacillus subtilis*) and CSSRI culture namely CSR-GROW-SURE, which consists of highly efficient salt-tolerant bacterial strains CSR-M-16 (*Bacillus licheniformis*), CSR-A-11 (*Lysnibaciullus fusiformis*), CSR-A-16 (*Lysinibaciullus sphaericus*). Both the cultures CSR-GROW-SURE bio-stimulant and TNAU culture, are effective in reducing the saline soil EC and SAR. The cultures showed almost equal efficiency in all the three 4, 5 and 6 ds  $m^{-1}$  saline soils. The use of microbial consortia for saline soil management is good for improvement of saline soil properties and a reduction in the use of chemical amendments by the farmers, which will save the cost of production.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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