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RESEARCH PAPER

Alleviating effect of micro-symbionts on nutrient status and survival of teak seedlings under salt stress

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Abstract : Seedlings of *Tectona grandis* L. were planted under different salinity levels *viz.* normal soil (<4 ECe soil), saline soil (4-8 Ece) and highly saline soil (8-12 Ece) and seedlings were inoculated with *Azotobacter* + Vesicular-arbuscular mycorrhizal (VAM) fungi, *Azospirillum* + vesicular-arbuscular mycorrhizal (VAM) fungi and combination of all three. Experiment was repeated for two years and data were recorded at the end of each experiment on nutrient content in different plant parts (leaves, stem and root), chlorophyll content, root colonization and survival per cent. Triple inoculation (*Azotobacter* + *Azospirillum* + VAM) significantly and positively influenced the nutrient status and survival per cent of teak seedlings as compared to uninoculated seedlings under salt stress condition. It was followed by dual inoculation of *Azospirillum* and VAM.

Key Words : Micro-symbiont, Salinity levels, Nutrient content, Chlorophyll content, Root colonization, Survival per cent

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INTRODUCTION

Teak is the common name for the tropical hardwood tree species *Tectona grandis*. *Tectona grandis* is native to South and southeast Asia, mainly India, Indonesia, Malaysia, and Myanmar, but is naturalized and cultivated in many countries, including those in Africa and the Caribea. Myanmar accounts for nearly one third of the world's total teak production. Salinity is a general term used to describe the presence of elevated levels of different salts such as sodium chloride, magnesium and calcium sulphates and bicarbonates in soil and water (Ouda, 2008). The beneficial effect of micro-symbiont on the plant nutrition is well known, but the role in salinity related factor has been studied very less frequently. Screening of plants for salt stress in the net house has mostly been conducted in soil medium. This mimics some field condition more closely than any other method especially when factors such as toxicity of reduced ions and redox potential of soil are considered. Saline soils are distributed throughout the world especially in the arid and semiarid regions. Nutrient deficiency frequently compounds the problems of saline soil of the tropics. High salinity affects plant growth through the osmotic effect; toxicity of salt ions; the changes in physical and chemical properties of soil. It also suppresses the nutrient uptake by plant roots and reduces nutrient status of plant. The use of chemical fertilizer is the most common approach to improve soil fertility and it sometimes results in increased the salt concentration. In such condition biofertilizer along with an organic manure appears to be more effective choice than the chemical fertilizer in maintaining soil health. Saline site conditions are usually associated with stunted growth and poor overall quality of teak. Hence, the present study was an

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attempt to investigate the growth and development of economically important tree species *Tectona grandis* in the saline condition in association with mycorrhiza and other microbes with the objectives to improve survival per cent and nutrient status at nursery stages.

MATERIAL AND METHODS

The present investigation was under taken during the year 2011-2012 and 2012-2013 at College of Forestry, Navsari Agricultural University, Navsari (Gujarat). Teak stumps used in the experiment were collected from Experimental Farm of College of Forestry. The bulk surface soil samples having neutral value *i.e.* (<4 ECe) was collected from the Instructional Farm ASPEE College of Horticulture and Forestry, Navsari Agricultural University and high salinity level soils *i.e.* 4-8 and 8-12 ECe were collected from the Danti farm of Navsari Agricultural University, Navsari and was processed to pass through 2 mm sieve. From the collected bulk soil sample, a representative sample was preserved for initial analysis of soil properties. The details regarding physical, chemical and fertility parameters of these soils are furnished in Table 1. The experiment was carried out in Completely Randomized Design with factorial concept in three salinity levels normal soil as S_1 (<4 ECe soil), saline soil as S_2 (4-8 Ece) and highly saline soil as S_3 (8-12 Ece) as treatments and seedlings were inoculated with *Azotobacter* with vesicular-arbuscular mycorrhizal (VAM) fungi as (M_1), *Azospirillum* with vesicular-arbuscular mycorrhizal (VAM) fungi (M_2) and the combination of all three (M_3).

Pot culture technique used :

Polythene bags of size 20 cm × 15 cm and 200 gauge (thickness) were used. In order to maintain the salinity at the required level no hole were made in the polythene bags for drainage of excessive soil solution. Each bag was filled with 1.6 kg of the treated soil as per the treatments. In each bag one seedling was planted and bags were maintained at field capacity. The uniform quantity of water was added throughout the period of investigation. No water was allowed to drain out of the bag (watering was done with rain water so less chances of extra salt accumulation) so that required salinity was maintained in the bag throughout the experimentation period. Seedlings were finally removed after 12 month of investigation for further observation.

Plant chemical analysis :

The plant shoot and root samples were drawn from experiment after recording dry weight of seedlings, they well ground and then analyzed for various nutrient content in leaves, stem and root, for nitrogen (Chromic acid method, Trivedi *et al.*, 1999), phosphorus (Spectrophotometric method, Jackson, 1967) and potassium (Flame photometric method, Jackson, 1967), chlorophyll content in leaves was determined by DMSD (dimethylsulphoxide) method described by Arnon (1949).

The amount of chlorophyll present in the sample was calculated using standard formula :

Total chlorophyll (mg/g) = 22.2(O.D at 663)+8.02 (O.D at 645) x v x1000x w

where,

V= Volume of extract in DMSO

W=Fresh weight of tissue extract (mg).

Root colonization :

The level of root colonization was estimated by the frequency of root colonization and by evaluating the intensity of each colonization. Colonization by VAM is restricted to the root cortex and is usually most prevalent in young feeder roots. The intra-metrical morphology of VAM fungi consist of network of intercellular coenocytic hyphae, running through the layers of cortical cell, usually bearing intra or extra cellular vesicles, sometimes spores and differentiating intracellular arbuscules.

The most common method used to detect VAM root colonization is microscope examination after staining with acid fuchsin and trypan blue. Nonsystematic root scanning under dissecting microscope, which rapidly gives broad categories of root colonization in percentage.

One centimeter root section was mounted on slide. The length of colonized root tissue was measured and compared to total length of roots observed.

Survival per cent :

Survival per cent under different micro-symbiont inoculated treatments and salinity levels were recorded at the intervals of 4 months.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Nutrient content :

Data clearly indicated significant difference in N, P and K content in the leaves, stem and roots. Leaves showed most prominent and significantly higher N, P, and K content which was followed by stem and roots (Table 2). The nitrogen content was recorded significantly maximum in triple inoculation of M_3 (VAM + *Azospirillum* + *Azotobcter*) in normal ECe soil (0.709, 0.540 and 0.308 per cent in leaves, stem and roots, respectively in 1st year trial) and (0.710, 0.540 and 0.309 in 2nd year trial) which increased approximately by 10 per cent as compared to uninoculated treatments. And which was followed by combination of (VAM + *Azospirillum*) and (VAM + *Azotobacter*). Whereas, minimum amount of nitrogen content

in all plants parts was noted under highly saline soils without any inoculation of micro-symbiont.

Similar pattern of results was recorded in plant parts of seedlings grown under different inoculation treatments for phosphorus content under different salinity levels. On the basis of data of both years, triple inoculation exceeds other treatment to maintain P content in the teak seedlings *i.e.* M_3 (0.149, 0.110 and 0.060 % in leaves, stem and roots, respectively in the 1st year trial) and (0.159, 0.123 and 0.070 % in leaves, stem and roots, respectively in the 2nd year trial). In case of salinity levels it was noted that phosphorus content in the seedlings increased with increasing salinity, as plants grown under highly saline soils recorded more phosphorus content as compared to the normal soils.

Data presented in Table 2 for potassium content showed similar trend of result as observed in case of nitrogen, significantly maximum K per cent recorded in the leaves of teak seedlings which were grown under triple inoculation with highly saline soils. Looking to the interaction effect of micro-symbiont and salinity it was noted that triple micro-symbiont inoculation treatment in moderately and highly saline soils aid to improve nutrient content as compared to uninoculated treatments.

Other combinations also play better to remove nutrients as compared un-inoculated treatment. Increased phosphorus concentration observed in the inoculated plants seems to be linked to mycorrhizal effect and increased N and K due to N fixing micro-symbiont (Marcela *et al.*, 2008). Similar results were described for *Araucaria angustifolia* (Duarte *et al.*, 2002). Lower nutrient uptake in soil S_3 might be due to poor inherent fertility status of soil and consequently the nutrient availability. Anilkumar and Kurup (2003) reported that different level of salinity affected uptake of N and K in low and normal salinity stresses in *Phaseolus aureus*. They also reported maximum P content in the *Cicer ariatum* and *phaseolus aureus* under high salinity.

Chlorophyll content :

At the end of experiment Chlorophyll content in the leaves of the teak was analysed and it was found that chlorophyll content was significantly influenced due to different micro-symbiont inoculation. Significantly higher amount of chlorophyll content was noted in the leaves of teak seedling under M_3 (triple inoculated treatment) and increase in salinity level adversely affected the chlorophyll content in the teak leaves (Table 3). Srinivasrao *et al.* (2004) reported total chlorophyll content in the faba beans was significantly reduced by salinity. The decrease in chlorophyll content under

Table 1 : Initial physico-chemical properties of soils

Particulars	2011-2012			2012-2013		
Soil type	S_1 (Navsari)	S_2 (Danti)	S_3 (Danti)	S_1 (Navsari)	S_2 (Danti)	S_3 (Danti)
Initial chemical properties						
pH (1:2.5)	7.20	7.98	8.23	7.23	8.03	8.26
EC	0.32	2.03	3.32	0.30	2.17	3.28
ECe (dSm^{-1})	1.17	7.23	10.98	1.22	7.48	10.56
Organic carbon (%)	0.58	0.60	0.54	0.56	0.60	0.55
Available N ($kg\ ha^{-1}$)	226	204	186	228	198	189
Available P_2O_5 ($kg\ ha^{-1}$)	49.23	44.56	52.93	52	48	54
Available K_2O ($kg\ ha^{-1}$)	542.0	972.0	1485.0	564	986	1517
Available S (ppm)	13.16	14.36	17.43	12.47	15.13	16.78
Exchangeable Ca (me/100g)	37.23	28.52	23.15	38.12	27.92	22.78
Exchangeable Mg (me/100g)	14.26	15.32	16.24	14.48	16.32	17.37
Exchangeable Na me/100g	0.38	1.54	2.22	0.42	1.59	2.35
Exchangeable K (me/100g)	0.50	0.90	1.32	0.52	0.95	1.46
CEC (me/100g)	53.28	46.51	43.26	53.47	46.48	43.32
ESP	0.71	3.31	5.13	0.79	3.42	5.42
DTPA extractable micro nutriment cations (ppm)						
Fe	14.56	12.86	11.42	17.4	13.5	9.6
Mn	18.32	14.23	15.23	18	16	6.5
Zn	1.12	0.98	0.75	2.2	1.60	0.27
Cu	3.47	2.32	2.16	3.8	2.1	1.2
Water stable aggregates (%)						
<1.0 mm	28.20	22.50	24.36	27.4	18.6	10.1
>1.0 mm	52.34	38.40	27.72	57.8	39.2	18.5

Table 2 : Response of different microsymbiont inoculation on the N, P and K content in leaves, stem and roots of teak seedling under different salinity levels

Microsymbiont	Leaves						Stem						Root					
	N content (%)																	
	2011-2012			2012-2013			2011-2012			2012-2013			2011-2012			2012-2013		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
M_1	0.680	0.578	0.467	0.682	0.571	0.466	0.518	0.439	0.352	0.518	0.439	0.352	0.296	0.250	0.201	0.300	0.254	0.205
M_2	0.691	0.586	0.476	0.693	0.586	0.477	0.526	0.445	0.360	0.528	0.444	0.361	0.300	0.254	0.205	0.296	0.250	0.201
M_3	0.709	0.589	0.479	0.710	0.586	0.478	0.540	0.447	0.362	0.540	0.448	0.360	0.308	0.255	0.206	0.309	0.255	0.206
M_4	0.644	0.569	0.455	0.642	0.568	0.455	0.490	0.432	0343	0.490	0.432	0.342	0.280	0.246	0.196	0.280	0.246	0.196
S.Em.±		0.08			0.09			0.004			0.005			0.002			0.003	
C.D. (P=0.05)		0.24			0.24			0.013			0.014			0.007			0.004	
P content (%)																		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
M_1	0.141	0.238	0.347	0.146	0.258	0.359	0.100	0.187	0.266	0.113	0.200	0.279	0.054	0.104	0.149	0.064	0.114	0.159
M_2	0.148	0.252	0.358	0.154	0.263	0.370	0.108	0.193	0.274	0.121	0.206	0.287	0.059	0.107	0.154	0.068	0.117	0.164
M_3	0.149	0.259	0.380	0.159	0.268	0.387	0.110	0.195	0.288	0.123	0.208	0.301	0.060	0.109	0.162	0.070	0.118	0.172
M_4	0.123	0.237	0.313	0.133	0.248	0.322	0.091	0.180	0.238	0.104	0.193	0.251	0.049	0.100	0.133	0.059	0.110	0.143
S.Em.±		0.002			0.004			0.004			0.004			0.002			0.002	
C.D. (P=0.05)		0.007			0.013			0.013			0.013			0.007			0.007	
K content (%)																		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
M_1	0.397	0.295	0.184	0.383	0.281	0.170	0.309	0.230	0.143	0.310	0.231	0.144	0.176	0.131	0.081	0.169	0.124	0.075
M_2	0.408	0.303	0.193	0.393	0.288	0.179	0.317	0.235	0.150	0.318	0.236	0.151	0.180	0.134	0.085	0.174	0.127	0.079
M_3	0.426	0.306	0.196	0.411	0.291	0.182	0.331	0.238	0.153	0.332	0.239	0.154	0.188	0.135	0.086	0.182	0.129	0.080
M_4	0.361	0.286	0.172	0.347	0.272	0.158	0.281	0.223	0.134	0.282	0.224	0.135	0.160	0.126	0.076	0.153	0.120	0.069
S.Em.±		0.002			0.003			0.002			0.002			0.001			0.001	
C.D. (P=0.05)		0.008			0.010			0.006			0.006			0.003			0.004	

Table 3 : Response of different microsymbiont inoculation to the chlorophyll content in leaves, root colonization and survival per cent of teak seedlings under different salinity levels

Microsymbiont	Salinity levels								
	Chlorophyll content (mg/g)			Root colonization (%)			Survival per cent (%)		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
2011-2012									
M_1	6.39	5.35	4.39	59.44	38.55 (-35.14)	6.44 (-89.17)	100.00	93.33 (-6.67)	80.00 (-15.79)
M_2	6.41	5.36	4.40	59.44	39.11 (-30.28)	6.77 (-71.03)	100.00	95.56 (-4.44)	84.44 (-15.56)
M_3	6.63	5.58	4.62	62.00	41.44 (-33.16)	17.22 (-72.23)	100.00	98.89 (-1.11)	85.56 (-14.44)
M_4	6.20	5.15	4.19	00.00	00.00	00.00	95.00	93.33 (-1.76)	78.89 (-21.11)
S.E.±		0.04			1.56			2.00	
C.D. (P=0.05)		0.12			4.43			5.64	
2012-2013									
M_1	6.39	5.34	4.38	59.11	39.44 (-33.28)	6.11(-89.66)	100.00	96.67 (-3.33)	80.44 (19.56)
M_2	6.40	5.35	4.39	60.66	38.77 (-36.09)	7.22 -(88.10)	100.00	98.89 (-1.11)	81.11 (-18.89)
M_3	6.62	5.57	4.61	61.88	40.44 (-34.65)	17.23 (-72.16)	100.00	98.89 (-1.11)	86.67 (-13.33)
M_4	6.19	5.14	4.18	00.00	00.00	00.00	94.44	87.78 (-7.05)	71.11(-24.70)
S.E.±		0.05			1.17			2.05	
C.D. (P=0.05)		0.14			3.33			5.78	

Note-Figures in parenthesis is per cent reduction in values as compared to their control condition

saline condition was attributed to salt induced chlorophyllase activity (Al-Tahir and Abdul- Salam, 1997).

Root colonization :

Triple inoculated seedlings (M_3) gave significantly higher root colonization (62.00 and 61.88%, respectively for 2011-2013 and 2012-2013) as compared to the other micro-symbiont treatments. Second place was occupied by the combination of *Azospirillum* and VAM inoculation (M_2). In case of salinity level root colonization significantly decreased with increased salinity as compared to normal soil conditions (S_1) there was about 59-62 per cent root colonization noted which was reduced by 71-90 per cent in the highly saline soils (S_3) (Table 3). An increase in per cent root colonization by AM- fungi upon inoculation with N_2 fixers were observed by Aseri and Rao (2005). Giovanetti (1985) also reported that enhancement of colonization in dual inoculation with *Azotobacter* and *Azospirillum*. This might be due to physiological state of the host plant and the nutritional status. Anilkumar and Kurup (2003) reported percentage of mycorrhization gradually decreased as the level of salinity stress increased.

Survival per cent :

Upto the initial eight month there was no any mortality observed. After 12 months mortality was recorded in the uninoculated treatment. In case of normal soil (S_1) in conjunction with micro-symbionts 100 per cent survival of seedlings was recorded which reduced in uninoculated and highly saline soils (S_3) by 21.11 per cent in 1st year and 24.70 per cent in 2nd year (Table 3). Whereas survival per cent under M_3 was 100 per cent which was reduced in highly saline soils by 14.44 per cent in 1st trail and 13.33 per cent in 2nd trail. Besides individual results when time comes to note performance of interaction of micro-symbiont and salinity levels it is revealed that triple inoculated or in fact all the inoculated treatment improved survival per cent of Teak seedlings in normal as well as highly saline soils. This was interpreted on the basis of performance of uninoculated treatment. Higher survival per cent in the triple inoculation and normal soil might be due to inherent capacity of soil to provide nutrients and beneficial symbiosis among three micro-symbiont.

Conclusion :

Thus, it can be concluded that seedlings of teak can grow better under micro-symbiont inoculation especially VAM, *Azospirillum* and *Azotobacter* under the normal soils, beside that, this research trials has broadened our understanding to the response of micro-symbiont symbiosis in ameliorating effect in saline condition in plants. The results suggested that VAM, *Azospirillum* and *Azotobacter* symbiosis protects host plants against oxidative damages due to salinity which in turn enhances salt tolerance of teak seedlings.

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