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RESEARCH PAPER

Efficiency of microsymbiont in relation to salt stress in teak seedling

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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* (AS) + vesicular-arbuscular mycorrhizal (VAM) fungi and combination of all three *i.e. Azotobacter* +VAM+AS. Experiment was repeated for two years and data recorded on growth parameters *i.e.* shoot length, collar diameter, leaf area were increased at 4 months interval (4^{th}, 8^{th} and 12^{th} month) under each trail and biomass estimated at the end of experiment by recording shoot, root fresh and dry weight. Triple inoculation (*Azotobacter*+*Azospirillum*+VAM) significantly and positively influenced the growth and biomass of teak seedlings as compared to duel inoculation and uninoculated seedlings under salt stress conditions.

Key Words : Teak seedlings, Microsymbiont, Salt stress

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INTRODUCTION

Teak is the common name for the tropical hardwood tree species *Tectona grandis*. It 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 microsymbionts on the plant nutrition is well known, but their role in relation to salinity 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. Now a day, the use of chemical fertilizer is the most common approach to improve soil fertility. However, it results to be in increase the salt concentration in such condition biofertilizer appear more effective than the chemical fertilizer in maintaining health. Saline site conditions are usually associated with stunted growth and poor overall quality of teak. Hence, in the present study, an attempt to investigate the growth and development of economically important tree species *Tectona grandis* in the saline condition in association with mycorrhiza and other

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microbes was made with the objective of improving growth and biomass of teak seedlings.

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.* (ECe <4) collected from the Instructional Farm ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari and high salinity level soils *i.e.* ECe 4-8 and ECe 8-12 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 (ECe <4 soil), saline soil as S_2 (ECe 4-8) and highly saline soil as S_3 (ECe 8-12) as treatments and seedlings were inoculated with *Azotobacter* + vesicular-arbuscular mycorrhizal (VAM) fungi as (M_1), *Azospirillum* (AS) + vesicular-arbuscular mycorrhizal (VAM) fungi (M_2) and the combination of all three (M_3) *i.e. Azotobacter*+AS+VAM.

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 to field capacity. The uniform quantity of water was added throughout the period of investigation. No water was allowed to drain out of the bag. So that required salinity was maintained in the bag throughout the experimental period. Seedlings were finally removed after 12 month of investigation.

Oobservations recorded :

Various biometric observations were recorded at the 4 months interval during the experimental period. The details are given below.

Growth parameters :

Shoot length (cm) :

Shoot length of five seedlings per treatment was recorded at four months interval up to 12 month of experiment by using wooden scale to the nearest centimeter and average was worked out.

Leaf area per plant :

For leaf area measurement, fresh leaves were detached from the seedling belonging to the individual treatment and area of these leaves was measured on "automatic leaf area meter".

Collar diameter (cm) :

With the help of vernier caliper, the diameter at shoot collar was also recorded at 4 monthly intervals from each treatment and all repetition and average was worked out.

Plant biomass production :

Shoot fresh weight (g) :

Fresh weight of whole plant and fresh weight of stem (with leaves) and roots were recorded per plant separately at the end of the experiment (after 12 months) by using electronic balance and expressed in gram and average was worked out.

Dry weight of seedling :

After taking the treatment wise fresh weight of seedling and its different parts, all the seedlings were spread in a shade for 12 hours and then they were placed in paper bags and oven-dried at 40^0C, until no further weight loss occurred. Then the dry weight of seedling was taken with the help of electronic balance and expressed in gram.

RESULTS AND DISCUSSION

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

Growth parameters :

The mean data on shoot length, leaf area and collar diameter of teak recorded at 4th, 8th and 12th months after planting as influenced by microsymbiont in saline soils are presented in Table 2 and 3.

During the different seedling stages (4th, 8th and 12th months after planting) a significant increase in the shoot length, leaf area and collar diameter and biomass was noted under all microsymbiont treatment under study namely M_1 (VAM + *Azotobacter*), M_2(VAM + *Azospirillium*), M_3(VAM + *Azospirillum* + *Azotobacter*) as compared to M_4 uninoculated (Control). The shoot length of teak during both the years of experiment at 4th MAP increased at faster rate in M_3 as compared to control *i.e.* M_4 which was followed by M_2 and M_1 (Table 2). Most prominent effect was observed at 8th months after planting. The three microsymbiont under investigation followed the trend of $M_3 > M_2 > M_1$ *i.e.* triple inoculation with VAM + *Azospirillum* + *Azotobacter* was most effective. An examination of growth parameter and biomass under different salinity levels showed maximum values under S_1 *i.e.* soil of <4 ECe which was followed by S_2 soil of 4-8 ECe and S_3 soil of 8-12 ECe (Table 2) . Similar trend of results was found at all the interval months.

Interaction study :

The extent to which microsymbiont can alleviate stress due to salinity determined by evaluating interaction of both the factors on the basis of growth and biomass of teak seedlings under microsymbiont inoculation and uninoculated treatments. The shoot length of teak increased under normal soil (<4 Ece) when it inoculated with triple microsymbiont (M_3) (24.23 and 24.39 cm in 1st and 2nd trial, respectively at 12th month) as compared to the length of seedlings grown under uninoculated treatment (M_4) which was followed by interaction of M_2 (*Azospirillum* + VAM) under normal soil. In case of leaf area and collar diameter similar treatment combination *i.e.* M_3S_1 recorded maximum values in both the trials. Leaf area under M_3S_1 was (261.79 and 261.81 cm^2, respectively at the end of 1st and 2nd year) which was followed by M_2S_1. Whereas collar diameter of seedlings under M_3S_1 was 7.38 cm at the 12th month of each trial.

On the basis of biomass data presented in Table 3 it is revealed that maximum shoot fresh and dry weight (9.87 and 6.22 g in 1st year trial and 9.83 and 6.10 g in 2nd year trial was recorded under the treatment M_3S_1 which was followed by inoculation of *Azospirillum*+VAM (M_2S_1) under normal soils. Per cent increase in shoot and root fresh weight in the M_3 condition as compared to the uninoculated condition under different salinity levels are indicated in the Table 3 (in parenthesis). On the basis of data it can be stated that per cent increased in biomass in triple inoculated treatment ranged from 22.30 to 22.42 per cent for shoot fresh weight, 40.41 to 41.46 per cent for shoot dry weight, 10.32 to 10.01 per cent for root fresh weight and 13.60 to 13.06 per cent for root dry weight under normal soil conditions (S_1), whereas under highly saline soils (S_3) it ranged from 19.11 to 19.28 per cent for shoot fresh weight, 34.56 to 30.33 per cent for shoot dry weight, 6.20 to 5.96 per cent for root fresh weight and 8.89 to 8.42 per cent for root dry weight. From the Tables 1 and 2 it can be concluded that uninoculated soils showed their low performance as compared to inoculated. Even the seedlings grown under highly saline soils showed their better growth in terms of shoot length, leaf area and collar diameter and biomass accumulation as compared to the similar soils under uninoculated condition.

Significantly better growth parameters of seedlings grown under M_3 may be due to the fact that all three microsymbiont strongly correlated to improved accumulation of both N due to *Azospirillum* and *Azotobacter* and P due to VAM. And phosphorus is a constituent of the cell nucleus (DNA and RNA) and is essential for the cell division and for development of meristematic tissue (Russell, 1973). Similar findings were given in teak by (Verma *et al*., 2008) and other results are in agreement with the findings of Aseri and Rao

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 growth of teak seedling under different salinity levels

Microsymbiont	4th month						8th month						12th month					
Shoot length (cm)																		
	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	17.89	13.89	12.92	18.47	14.47	13.49	19.99	15.69	14.72	20.58	16.29	15.30	23.09	17.09	16.10	23.69	17.70	16.66
M_2	18.00	13.99	13.00	18.50	14.50	13.50	20.11	15.80	14.80	20.69	16.40	15.40	23.22	17.21	16.20	23.70	17.80	16.79
M_3	19.02	15.02	14.02	19.23	15.23	14.25	21.13	16.84	15.82	21.30	17.00	16.09	24.23	20.22	19.22	24.39	20.38	19.49
M_4	15.02	11.02	10.02	15.09	11.09	10.11	17.12	12.82	11.80	13.99	9.70	8.69	20.22	14.21	13.20	17.07	11.09	10.07
S.Em.±		0.21			0.24			0.20			0.22			0.20			0.22	
C.D. (P=0.05)		0.61			0.68			0.59			0.64			0.59			0.66	
Collar diameter (cm)																		
	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	4.80	4.04	2.72	4.75	4.04	2.77	5.85	4.54	3.11	5.85	4.54	3.27	7.08	5.24	4.01	7.08	5.24	4.01
M_2	4.78	4.07	2.80	4.78	4.07	2.80	5.90	4.57	3.24	5.88	4.57	3.30	7.09	5.27	4.01	7.02	5.28	4.03
M_3	5.05	4.34	3.01	5.05	4.34	3.07	6.15	4.84	3.53	6.15	4.84	3.57	7.38	5.54	4.31	7.38	5.56	4.30
M_4	4.58	3.87	2.46	4.55	3.84	2.45	5.68	4.34	3.05	5.65	4.34	2.95	6.88	5.04	3.79	6.88	5.04	3.68
S.Em.±		0.07			0.06			0.07			0.06			0.09			0.05	
C.D. (P=0.05)		0.22			0.17			0.21			0.17			0.26			0.16	
Leaf area (cm^2)																		
	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	145.78	86.31	44.10	146.72	87.22	45.10	186.77	106.29	64.11	187.74	107.28	65.09	259.76	137.33	95.10	260.79	138.30	96.11
M_2	146.77	87.30	45.11	146.75	87.33	45.11	187.78	107.32	65.10	187.76	107.32	65.11	260.77	138.30	96.10	260.80	139.22	97.09
M_3	146.80	87.32	45.12	147.78	88.33	46.14	187.80	107.33	65.13	188.78	108.33	66.13	261.79	139.22	97.11	261.81	139.35	97.11
M_4	143.78	84.34	42.10	144.78	85.22	43.10	184.77	104.30	62.09	185.78	105.22	63.14	257.78	135.31	93.00	258.77	136.33	94.10
S.Em.±		1.21			1.18			1.21			1.23			1.20			1.19	
C.D. (P=0.05)		3.42			3.38			3.42			3.45			3.40			3.39	

Table 3: Response of different microsymbiont inoculation to the shoot, root fresh and dry weight of teak seedlings after 12 months of planting under different salinity levels

	Shoot fresh weight (g)						Shoot dry weight (g)					
	2011-2012			2012-2013			2011-2012			2012-2013		
Microsymbiont and saline soils	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	8.54	6.52	4.71	8.50	6.48	4.67	4.89	2.87	1.34	4.77	2.75	1.28
	(5.82)	(1.09)	(4.67)	(5.85)	(1.09)	(4.71)	(10.38)	(2.14)	(5.51)	(10.93)	(2.61)	(4.92)
M_2	8.81	6.56	5.07	8.77	6.52	5.03	5.16	2.92	1.42	5.04	2.79	1.30
	(9.17)	(1.71)	(12.67)	(9.22)	(1.72)	(12.78)	(16.48)	(3.91)	(11.81)	(17.21)	(4.10)	(6.56)
M_3	9.87	6.85	5.36	9.83	6.81	5.32	6.22	3.21	1.71	6.10	3.08	1.59
	(22.30)	(6.20)	(19.11)	(22.42)	(6.24)	(19.28)	(40.41)	(14.23)	(34.56)	(41.86)	(14.93)	(30.33)
M_4	8.07	6.45	4.50	8.03	6.41	4.46	4.43	2.81	1.27	4.30	2.68	1.22
S.E.±		0.14			0.14			0.05			0.03	
C.D. (P=0.05)		0.39			0.39			0.16			0.10	
	Root fresh weight (g)						Root dry weight (g)					
	2011-2012			2012-2013			2011-2012			2012-2013		
Microsymbiont and saline soils	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	17.92	15.82	14.09	18.46	16.44	14.63	13.71	11.71	9.88	14.25	12.23	10.42
	(2.96)	(0.19)	(1.51)	(2.61)	(1.17)	(1.46)	(3.55)	(0.17)	(2.17)	(3.41)	(0.66)	(2.06)
M_2	18.19	15.94	14.45	18.73	16.48	14.99	13.98	11.73	10.24	14.52	12.27	10.78
	(4.24)	(0.95)	(4.11)	(4.11)	(1.42)	(3.95)	(5.59)	(0.34)	(5.89)	(5.37)	(0.99)	(5.58)
M_3	19.25	16.23	14.74	19.79	16.77	15.28	15.04	12.02	10.53	15.58	12.56	11.07
	(10.32)	(2.79)	(6.20)	(10.01)	(3.20)	(5.96)	(13.60)	(2.82)	(8.89)	(13.06)	(3.37)	(8.42)
M_4	17.45	15.79	13.88	17.99	16.25	14.42	13.24	11.69	9.67	13.78	12.15	10.21
S.E.±		0.18			0.18			0.17			0.18	
C.D. (P=0.05)		0.50			0.51			0.50			0.50	

Note : Figures in parenthesis is per cent increased in values to their control condition

(2005) for the ber and aonla. They stated that improvement in the microbial activity upon inoculation, as reflected by the enhancement in the activity of various enzymes status help to increase the leaf area. Between the soils under study, soil S_1 (< 4 ECe) performed significantly better than S_2 (4-8 ECe) and S_3 (8-12 ECe). In short, the teak grown on S_1 recorded higher values of growth attributing characters than S_3. Similar pattern of growth parameters were observed in the 2nd year trial and pooled analysis. This is in conformity with some earlier findings made by Srinivasrao *et al.* (2004) who reported that total shoot and root growth and leaf area of faba beans were significantly reduced by salinity. Increased salinity showed a significant reduction in leaf area may be due to reduction of photosynthetic rate with salinity (Shannon *et al*. 1994). Rosendahl and Rosendahl (1991) reported that the VAM fungi have the ability to protect plants from salt stress. Hirrel and Gerdemenn (1980) demonstrated improved growth of onion and bell pepper in saline soils by inoculation of VAM. Zang, *et al.* (2010) reported that AM fungi improve the drought tolerance of *Casurina equisetifolia* seedling by means of improving growth and biomass production. Supporting finding was made by Selvaraj (1989).

Conclusion :

Thus, it can be concluded that seedlings of teak can grow better and accumulate more biomass under microsymbiont inoculation especially combined application of VAM, *Azospirillum* and *Azotobacter* under the normal soils. Beside this the research trials has broadened our understanding of the response of microsymbiont in ameliorating saline condition in plants. The result suggests that VAM, *Azospirillum* and *Azotobacter* symbiosis protects plants against oxidative damages due to salinity which in turn enhances salt tolerance of teak.

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