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Studies on the Effect of Microbial Inoculants on the Growth of Silver Oak

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Abstract. A green house experiment was conducted at College of Forestry, Ponnampet, which is a sub-campus of University of Agricultural Sciences, Bangalore, Karnataka state, INDIA, to evaluate the performance of microbial inoculants such as *Trichoderma, Bacillus coagulans* (P solubilizer) and *Azatobacter* (N Fixer) for the growth promoting activity on Silver oak. The experiment was conducted in Completely Randomized Design (CRD) with ten treatments and three replications. Height, girth and total number of leaves of the plants were recorded at 30, 60, 90 and 120 days after transplanting. The growth of the plants was significantly different between the treatments in all the growth stages for different parameters studied except for number of leaves. The number of leaves per plant decreased in later stages *i.e.* 120 days after transplanting (DAT) in all the treatments. This could be due to abiotic factors like Temperature, RH *etc*. This study suggests that in the nursery the potting mixture has to be provided with bioinoculants *viz*, *Trichoderma*, P-solubilizers and if necessary the Nitrogen fixers based on the nutrient status of the potting mixture and the plant requirement.

Keywords: *Trichoderma, Bacillus coagulans*, *Azatobacter,* P solubilizer, N Fixer, Silver oak.

1. Introduction

Though silver oak is fast growing species, its growth in India is not as fast as in Australia. In spite of application of chemical fertilizers its growth is not as expected. Many different microorganisms used as biofertilizers have both direct and indirect effects in the plant growth promotion, nitrogen fixation, solubilization of phosphate, biocontrol *etc.* Boosting the nursery growth has an impact on early establishment, higher growth and production of quality planting stock of this commercially important tree species and also reduces the cost of production. Therefore, realizing the importance of this, Bioinoculants study was conducted in order to know its effect on growth of silver oak at nursery level.

2. Methodology

To evaluate growth promoting activity of *Trichoderma*, P solubilizer and N fixers on Silver oak, a green house experiment was conducted at The College of Forestry, Ponnampet, which is a high rainfall area, a sub-campus of The University of Agricultural Sciences (UAS), Bangalore which is located in the southern parts of Karnataka state, INDIA. Bioinoculants such as *Trichoderma, Bacillus coagulans* (P solubilizer) and *Azatobacter* (N Fixer) were obtained from the Department of Agricultural Microbiology, UAS, Bangalore. One month old Silver oak seedlings were procured from a nursery in Chikkamagalore, Karnataka state, INDIA. Potting mixture was prepared by mixing sand, soil and FYM at 1:1:1 ratio and one kilo grams of the potting mixture was filled in 1000 polythene covers. Single Silver oak plant was planted in each polythene bag. Totally one thousand bags were planted with one plant each and maintained for one month with proper watering and weeding.

The experiment was conducted in Completely Randomized Design (CRD) with ten treatments and three replications per treatment. Three hundred seedlings were transplanted at thirty per each treatment having three replications at 10 plants per each replication. The fungal and bacterial microbial inoculants were grown in Potato dextrose broth and Nutrient broth respectively in 250 ml conical flask for 15 days at 26 ± 2^{o}C.The mycelial mat of *Trichoderma* was separated by muslin cloth, macerated in sterile water in a warring blender. The *Azatobacter* and *Bacillus coagulans* suspension was prepared by thorough shaking of the cultures and these inoculants were poured at10 ml per plant according to the treatments. Chemical fertilizer like urea, rock phosphate and murate of potash was mixed at 1:1:1 ratio and applied at 5g/ plant. Neem cake was applied at 10 g/ plant. This recommendation of nutrients is commonly practiced in the nursery condition at The College of Forestry, Ponnampet.

Silver oak plants height of was measured from the base to the shoot tip of the seedling at 30, 60, 90 and 120 days after transplanting. Stem girth was measured at the color region of the main shoot using Vernier Calipers at 30, 60, 90 and 120 days after transplanting. The girth was calculated using the formula, Girth= (Main Scale reading + Vernier scale reading) X least count. Total number of leaves per plant was counted and registered at 30, 60, 90 and 120 days after transplanting.

Population studies of *Trichoderma, Bacillus coagulans* and *Azatobacter* were conducted using serial dilution plate technique an interval of 30 days from the rhizosphere soil of treated plants. The plating dilutions used were 10^{-3} for *Trichoderm,* 10^{-4} for *Bacillus coagulans* and *Azatobacter* using *Trichoderma* specific medium (Santhosh *et. al.,* 2006.), *Bacillus coagulans* specific medium (Marshall., *et. al.,* 1967) and Waksmann No. 77 medium for *Azatobacter*.

The data collected over the experimental study were subjected to statistical analysis suitable for completely randomized experiment (Sunderaraj *et.al*., 1972)

3. Results and Discussion

Microbial inoculants have been advocated to provide benefits to growing plants in terms of direct promotion of vegetative growth through atmospheric N fixation, P solubilization and release of growth promoting substances in the rhizosphere which alter root physiology (Kloepper, 1993). The present study was aimed to assess the effects of *Bacillus coagulans, Azatobacter* and *Trichoderma* isolates on the growth of silver oak plants in the nursery condition. The average height, girth and number of leaves per silver oak plant at different growth stages studied for different treatments are tabulated in Table 1.

The data pertaining to the height of silver oak showed an increase at all the stages studied and there was a significant difference between the treatments at different stages studied. Maximum height was observed in *Trichoderma* inoculated plants (17.91 cm) and minimum was observed in Neem cake inoculated plants (9.60 cm) initially. At 30 DAT the same trend was observed, maximum was in *Trichoderma* inoculated plants (22.42 cm) and minimum was in Neem cake inoculated plants. At 60 DAT there was some difference in the results. Maximum was in *Trichoderma* + *Bacillus coagulans* (30.37 cm) and minimum was in control plants. The same trend was observed at 90 DAT, maximum in *Trichoderma* + *Bacillus coagulans* (32.20 cm) and minimum in control plants (21.01 cm). At 120 DAT maximum and minimum heights were 33.47 cm and 22.03 cm in *Trichoderma* + *Bacillus coagulans* and controlled plants respectively.

Initially the color diameter was maximum in plants inoculated with *Trochoderma* (2.95 mm) and minimum in Neem cake inoculated plants (1.84 mm). At 30 DAT and 60 DAT the same trend was observed. At 90 DAT the result was slightly different. The maximum girth was seen in *Bacillus coagulans* treated plants (3.90 mm) and it was on par with *Trichoderma treated* plants (3.44mm), the minimum was in Neem cake treated plants (2.97 mm). There was no significant difference between the other treatments. The result at 120 DAT was similar to that of 90 DAT, *ie.,* the maximum girth was in *Trichoderma* treated plants (4.38 mm) and it is on par with the plants treated with *Trichoderma* + *Bacillus coagulans* (4.18) and minimum was in Neem cake treated plants (3.54 mm).

The results obtained with respect to the number of leaves in this study showed an interesting pattern. In all the stages studied there was no significant difference between the treatments with respect to the number of leaves per plant. The maximum number of leaves per plant was observed in plants which received the

treatment of *Trichederma* and *Bacillus coagulans* at 60 DAT (21.17). Initially it was 21.33 in plants inoculated with *Trichoderma* and *Bacillus coagulans* which is maximum compared to all other treatments and there was no significant difference between the other treatments in the study. At 60 DAT in almost all the treatments there was a decrease in the number of leaves. This may be due to the abiotic factors like temperature, relative humidity *etc.* which may alter the plants' physiology and these need to be studied in future.

The overall increase in length and girth of the Silver oak could be attributed to the release of growth promoting substances and increased nutrient availability by *Bacillus, Pseudomonas* and *Trichoderma* (Baker *et.al.,* 1986). The growth promoting substances are known to cause enhanced cell division and root development (Arshad and Francenberger. Jr., 1993). The results obtained during the current investigation uphold the results observed by Daiho and Upadhyay (1995) and Bochew (1992), where in pot experiment, sand maize culture of *Trichoderma* resulted in an increase in the height and better root development of Soybean plants.

The population dynamics of *Trichoderma, Bacillus coagulans* (P solubilizer) and *Azatobacter* (N Fixer) was monitored during the study (Table 2. & Fig. 1, 2 &3). In almost all the treatments initially the population size was less and slowly the population size increased at 30 DAT and 60 DAT. This could be due to the rhizosphere effect. The plants which were treated with *Trichoderma, Bacillus coagulans* (P solubilizer) and *Azatobacter* (N Fixer) and co- inoculated with combinations showed higher population compared to the control and uninoculated plants, this might be due to the enrichment of the soil with the microbial inoculants and could also be due to rhizosphere effect . At 120 DAT the population was decreased, this might be because of the exhaustion of the nutrients from the rhizosphere.

Many strains of *Bacillus, Pseudomonas* and *Trichoderma* have been implicated in improvement of overall growth of many crop plants (Eneback *et.al*., 1998). In the present study, the growth of the plants inoculated with the bioinoculants showed a significant increase over that of the control plants in all the stages studied. Here, the treatment inoculated with *Trichoderma* and *Bacillus coagulans* showed a maximum growth. This indicates that the plant needs additional phosphorous and other plant hormones for its maximum growth which is not provided in sufficient quantity by the potting mixture. The growth of the plants treated with the chemical fertilizers and Neem cake were almost on par with each other and the performance of the plants treated with the bioinoculants was better than those treated with chemical fertilizers and Neem cake separately.

To conclude, the potting mixture at Forestry college nursery has to be provided biofertilizer *viz, Trichoderma, Bacillus coagulans* and if necessary the *Azatobacter* based on the nutrient status and the plant requirement. The optimum temperature and relative humidity should be standardized and should be maintained in the green house where silver oak plants are grown. This might reduce the dropping of leaves from the plants. Similar experiment can also be conducted outside the green house to find out the reasons for the leaves drop. By the use of bioinoculants we can reduce the cost incurred for growing silver oak in nursery condition.

4. Acknowledgements

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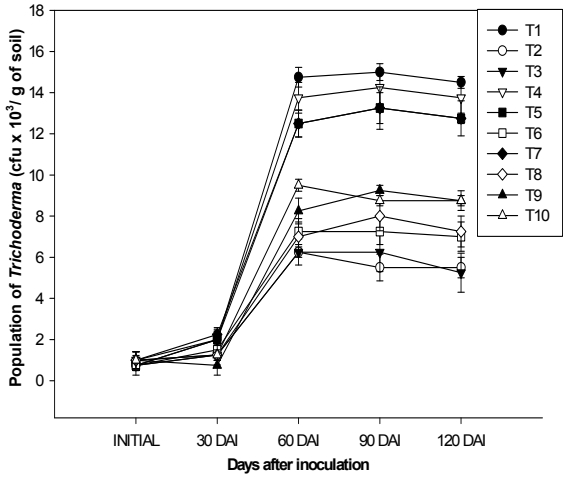

Fig. 1:

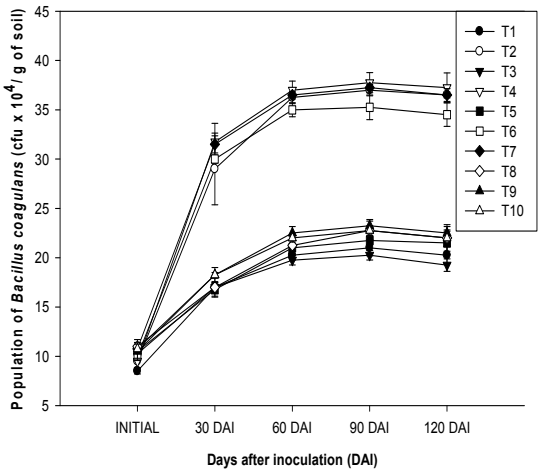

Fig. 2:

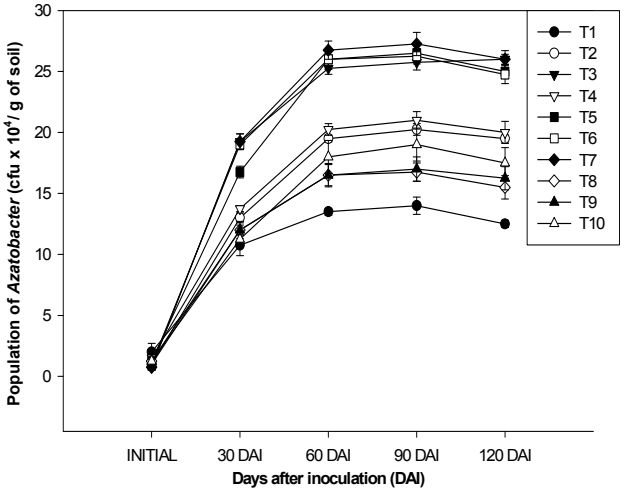

Fig. 3:

Fig.1, 2 & 3: Populations of *Trichoderma, Bacillus coagulans and Azatobacter* at different growth stages of Silver oak.

Table 1: Effect of microbial inoculants on the height, girth and number of leaves of Silver oak seedlings.

Table 1: (Part 1)

Treatments	Height(cm)					Girth (mm)				
	Initial	30 DAT	60 DAT	90 DAT	120 DAT	Initial	30 DAT	60 DAT	90 DAT	120 DAT

T_1 – *Trichoderma*	17.91	22.42	27.80	29.01	29.27	2.95	3.05	3.44	3.80	4.38
T_2 – *Bacillus coagulans*	14.55	20.89	26.67	26.77	28.53	2.73	2.82	2.92	3.38	3.89
T_3 – *Azatobacter*	14.23	19.35	25.47	26.08	28.12	2.66	2.93	2.97	3.64	3.78
T_4 - *Trichoderma* + *Bacillus*	14.90	21.52	30.37	32.20	33.47	2.66	2.71	2.76	3.90	4.18
T_5 - *Trichoderma* + *Azatobacter*	12.88	19.20	27.27	27.72	29.63	2.32	2.40	2.50	3.31	3.93
T_6 - *Bacillus* + *Azatobacter*	13.29	18.57	26.33	27.70	28.67	2.35	2.51	2.63	3.42	3.67
T_7 - *Trichoderma* + *Bacillus* + *Azatobacter*	11.64	18.03	25.12	26.03	27.93	2.22	2.36	2.49	3.10	3.78
T_8 – Chemical Fertilizer	10.93	15.03	21.35	24.13	24.57	2.01	2.21	2.41	3.01	3.77
T_9 – Neem Cake	9.60	14.29	20.70	23.08	23.43	1.84	2.01	2.22	2.97	3.54
T_{10} – Control (Only potting mixture)	10.55	14.47	19.03	21.01	22.03	2.27	2.33	2.71	3.37	3.74
***F* - Value**	*	*	*	*	*	*	*	*	*	**NS**
S.Em	**0.473**	**0.849**	**1.585**	**1.460**	**2.830**	**0.098**	**0.114**	**0.108**	**0.108**	**0.420**

DAT - Days after Transplanting

Table 1: (Part 2)

Treatments	**Number of leaves**				
	Initial	**30 DAT**	**60 DAT**	**90 DAT**	**120 DAT**
T_1 – *Trichoderma*	2.92	25.25	26.67	20.67	15.38
T_2 – *Bacillus coagulans*	20.17	24.33	27.00	25.58	20.17
T_3 – *Azatobacter*	18.42	22.67	24.00	22.50	17.67
T_4 - *Trichoderma* + *Bacillus*	21.33	25.33	28.08	24.50	21.17
T_5 - *Trichoderma* + *Azatobacter*	19.33	21.92	24.50	25.42	19.58
T_6 - *Bacillus* + *Azatobacter*	18.00	23.33	24.08	24.75	21.17
T_7 - *Trichoderma* + *Bacillus* + *Azatobacter*	18.17	21.75	22.92	22.92	18.75
T_8 – Chemical Fertilizer	18.00	21.08	21.92	23.25	18.25
T_9 – Neem Cake	19.42	24.50	26.25	25.50	21.17
T_{10} – Control (Only potting mixture)	20.17	25.08	25.92	22.33	17.92
***F* - Value**	**NS**	**NS**	**NS**	**NS**	**NS**
S.Em	**1.580**	**2.021**	**1.828**	**1.828**	**3.040**

DAT - Days after Transplanting

Table 2: Effect of microbial inoculants on the populations of *Trichoderma, Bacillus coagulans* and *Azatobacter*.

Table 2: (Part 1)

	Trichoderma **cfu X 10^3/g of Soil**			***Bacillus coagulans*** **cfu x 10^4/g of soil**		
Treatments	**Initial**	**60 DAT**	**120 DAT**	**Initial**	**60 DAT**	**120 DAT**
T_1 – *Trichoderma*	0.75	14.75	14.50	8.50	20.25	20.25
T_2 – *Bacillus coagulans*	0.75	6.25	5.50	9.50	36.25	36.50
T_3 – *Azatobacter*	0.75	6.25	5.25	10.25	19.75	19.25

T_4 - *Trichoderma + Bacillus*	1.00	13.75	13.75	9.50	37.00	37.25
T_5 - *Trichoderma + Azatobacter*	0.75	12.50	12.75	10.50	21.00	21.50
T_6 - *Bacillus + Azatobacter*	0.75	7.25	7.00	10.00	35.00	34.50
T_7 - *Trichoderma + Bacillus + Azatobacter*	1.00	12.50	12.75	10.75	36.50	36.50
T_8 – Chemical Fertilizer	1.00	7.00	7.25	11.00	21.25	22.00
T_9 – Neem Cake	1.00	8.25	8.75	10.50	22.50	22.50
T_{10} – Control (Only potting mixture)	1.00	9.50	8.75	10.75	22.00	22.00

DAT - Days after Transplanting

Table 2: (Part 2)

	Azatobacter **cfu x 10^4/g of soil**		
Treatments	**Initial**	**60 DAT**	**120 DAT**
T_1 – *Trichoderma*	2.00	13.50	12.50
T_2 – *Bacillus coagulans*	1.00	19.50	19.50
T_3 – *Azatobacter*	0.75	25.25	26.00
T_4 - *Trichoderma + Bacillus*	1.50	20.25	20.00
T_5 - *Trichoderma + Azatobacter*	1.25	26.00	25.00
T_6 - *Bacillus + Azatobacter*	1.00	26.00	24.75
T_7 - *Trichoderma + Bacillus + Azatobacter*	0.75	26.75	26.00
T_8 – Chemical Fertilizer	1.25	16.50	15.50
T_9 – Neem Cake	1.00	16.50	16.25
T_{10} – Control (Only potting mixture)	1.25	18.00	17.50

DAT - Days after Transplanting