Rhizospheric Inoculation Influence on Seedling Growth, Development and Biomass Yield in Oroxylum indicum (L.) Benth. ex Kurz

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Abstract: ***Oroxylum indicum is a medicinally important forest tree species. Due toindiscriminate exploitation for medicinal purpose, habitat destruction, the natural population of O. indicum is reported endangered. Therefore an experiment was conducted to to study the effect of bioinoculation on the growth and quality of the seedlingsand conserve the plant species. The seedlings were inoculated with plant growth promoting microbes mainly, bacteria (B), fungus(F) and mycorrhiza (M), both alone and consortium. The total biomass was maximum (156.01±1.94) in T_F treatmentand minimum (118.78±0.06) in Control treatment. The biovolume index was maximum (64.15±3.088) in $T_{M+B+F}$and minimum (14.43±0.33) in Control treatment. The Quality index (Qi) of the seedlings was maximum (1.041±0.089) in T_B (seedlings treated with bacteria) while, Qi was minimum (0.761±0.090) in Control treatment.***

Key words: *Oroxylum indicum*, endangered, bioinoculation, *biovolume index*,quality index.

1. Introduction

Oroxylum indicum (L.) Benth. ex Kurz is a medicinally significant forest tree species, which is reported to be endangered and vulnerable in different parts of North east India as well as the entire Indian subcontinent.Problems associated with its natural propagation and indiscriminate exploitation for medicinal purpose has pushed *O. indicum* to the list of endangered plant species of India [1].This plant is used in many ayurvedic preparations and widely used by people for health care. The important medicinal principles obtained from it are *Dashmula*, *Shyonaka patpak* and *Bruhat pancha mulayadi kwath* [2],*Chyawanprasha* [3] and is one of the best known health care products of Ayurveda.This tree has been harvested so heavily for medicinal purposes that its survival is in jeopardy.

The production, consumption and international trade of medicinal plants and phytomedicine (herbal medicine) is increasing. In order to satisfy growing market demands, there is need to develop new strategies for better yield and quality of medicinal plants, which can otherwise be achieved through variousbiotechnological methods. It mayhelp in conserving many valuable tree species in the process and pave new vistas in forest biotechnology [4].

Plant growth is influenced by the presence of bacteria and fungi, and their interactions are particularly common in the rhizospheres of plants with high relative densities of microbes [5].Microbial communities in the soil or rhizosphere contribute to plant growth by recycling nutrients and making them available [6], increasing root health through competition with root pathogens [7] or enhancing nutrient uptake [8].

Arbuscular mycorrhizal fungi (AMF) form symbiotic association which is ubiquitous and known to improve the nutritional status of the host plants by facilitating absorption of relatively immobile micronutrients such as Zn and Cu besides P through external mycelium that assists in nutrient transport [9,10,11,12,13,14]. AMF inoculation not only promotes the growth of medicinal plants but also improves the productivity and quantity of chemicals [15].*Trichoderma* sp. also helps to mobilize and take up soil nutrients, which makes it more efficient and competitive than many other soil microbes [16]. Similarly, certain bacteria provide plants with growth promoting substances and play major role in phosphate solubilizing [17]. Phosphate solubilizing microorganisms are another sort of bio-fertilizers which have the ability to solubilize organic and inorganic phosphorus compounds by producing organic acid or phosphatase enzyme [18].Mixed inoculation with diazotrophic bacteria and arbuscular-mycorrhizal fungi creates synergistic interactions that may result in a significant increase in growth, in the phosphorus content in plants, enhanced mycorrhizal infection and an enhancement in the uptake of mineral nutrients such as phosphorus, nitrogen, zinc, copper, and iron [19,20,21,22,23,24,25,26,27, 28].

The literature review revealed that bioinoculation studies has been mainly carried on various fields including the influence of bioinoculants on growth and mycorrhizaloccurrence in the rhizosphere, plant growth stage, fertiliser management and bio-inoculation of arbuscular mycorrhizal fungi, impact of endomycorrhizal fungi and other bioinoculants on growth enhancement. There are many reports, projects and research work carried out on *Oroxylum indicum* such as propagation of *O.indicum*, through organogenesis [29] genetic diversity in *O.indicum*, by random amplified polymorphic DNA marker

[30], need and importance of conservation of endangered tree *O. indicum*,[31]. *In vitro* propagation of *O. indicum*: a medicinally important forest tree [32], antibacterial activity of stem bark extracts of *O. Indicum*[33], phytochemical and antimicrobial study of *O. indicum*[34], study on the secondary metabolites of *O. indicum* [35]. But there is no report on the effect of bioinoculation on growth and quality of the seedlings of *Oroxylum indicum* (L.) Benth. ex Kurz: Hence, the present study was undertaken.

2. Material and Methods

In order to analyse the the effect of bioinoculation on growth and quality of the seedlings of *O. indicum*, an experiment was setup in the nursery of Rain Forest Research Institute, Jorhat. The experiment was designed in Randomized Block Design (RBD), where three replications of each treatment were taken. Different treatments like single and combined/ synergistic/influential were applied for the present investigation. In control sets, no bioinoculant (inoculum) was added. Seedlings were raised in polyethene bags (30cm×50cm) containing 5 kg of substrates (sandand soil) in a ratio of 1:3. The inoculum was applied very close to the rhizosphere of the stumps at the depth of 5-10 cm.Three-stage inoculation was done e.g. at 0 day (1st stage, when the experiments will be initiated), 60 day (2nd stage) and 120 days (3rd stage). The inocula was added as per the previous studies conducted at concerned laboratory e.g. 10% w/w or w/v (1st stage), 20% w/w or w/v (2nd stage) 30% w/w or w/v (3rd stage) according to growing phases of seedlings. Data on plant growth like height, girth, the increment in diameter, was observed after definite intervals e.g. 90, 180, 270 days after inoculation (DAI). The effect of bio-inoculation on height, diameter and girth was recorded periodically and tabulated. Thebiomass, biovolume index and quality index of the seedlings was determined after 270 days of inoculation.

3. Biomass Estimation

Shoot biomass and Root biomass of a plant were calculated using the following formulae,

$$\text{Shoot biomass} = \frac{F_{w(s)} - D_{w(s)}}{F_{w(s)}} \quad 100$$

$$\text{Root biomass} = \frac{F_{w(r)} - D_{w(r)}}{F_{w(r)}} \quad 100$$

$$\text{Total biomass} = \text{Shoot biomass} + \text{Root biomass}$$

Where,$F_{w(s)}$ = Fresh weight of shoot,$F_{w(r)}$ = Fresh weight of root, $D_{w(s)}$ = Dry weight of shoot,$D_{w(r)}$ = Dry weight of root.

Biovolume index: - The biovolume index of the seedlings was calculated using the following formula [36, 37].

$$B_i = H \text{ X } D$$

Where B_i = Biovolume index, H= Height of seedlings in cm, D= Diameter of stem in mm/cm

Quality index: - Quality index to access the quality of seedlings was calculated using the following formula [36, 37].

$$Qi = \frac{M_1}{\frac{H}{D} + M_2}$$

Where Q_i = Quality index, M_1 = Seedlings whole biomass (shoot biomass + root biomass),M_2 = Seedlings top biomass (except root biomass, H = Height of seedlings in,D = Diameter of stem in mm/cm

The results were analyzed statistically using standard error of mean and the test of significance [38].

4. Results

The effect of bioinoculation on the seedlings of *O.indicum*was studied and the data was tabulated accordingly. Table 1.1 represents the initial data of the height, diameter and girth of the seedlings of *Oroxylum indicum* before inoculation. T denotes treatment, M denotes Mycorrhiza, B = Bacteria (*Pseudomonas* sp.), F= Fungi (*Trichoderma harzianum*), ± SEM (Standard Error of mean), * Average of Three replications

The initial height of the seedlings varied from 8.16±4.71 to 16.1±9.29 cms, the initial diameter varied between 0.233±0.017 to 0.439±0.041cms and the initial girth varied from 0.733±0.053 to 1.380±0.226 cms. The data on the increase in height (cm), diameter and girth was recorded after 90 Days, 180 Days, and 270 days after inoculation. The data on the effect of bio-agents inoculation on height, diameter and girth of *Oroxylum indicum* after first stage inoculation 90 (DAI) of *Oroxylum indicum* was tabulated (Table 1.2). Table 1.3 shows the increase in height, diameter and girth of the seedlings due to bioinoculation. The maximum increase in height (11.533±3.066) in $T_{M+B+F,}$ whereas, minimum (2.366±1.484) increase in height was recorded in treatment T_{M+B}. The increase in diameter after 90 days of inoculation was recorded maximum (0.12±0.0221) in T_{M+B+F} whereas minimum (0.040±0.016) was found in Control. The increase in girth was maximum (0.376±0.069) in$T_{M+B+F,}$ treatment, whereas, minimum (0.126±0.051) increase in girth was recorded in Control. Table 1.4shows the effect of bioinoculation on height, diameter and girth of *Oroxylum indicum* after second stage inoculation i.e. 180 (DAI).T_{M+B+F}showedmaximum height (46±1.00), whereas, T_{M+B} showed minimum height (24.06±0.52) after 180 days of inoculation. Diameter of the seedlings after 180 DAIshows maximum (0.59±0.064 and 0.59±0.048) diameter in T_B andT_F respectively. Control showed minimum (0.322±0.0319) diameter, while, girth was maximum (1.867±0.151) in T_F while it was minimum (1.011±0.100) in Control. The study of the effect of inoculation on increase in height, diameter and girth of *O. indicum* after second stage inoculation 180 (DAI) as tabulated inTable 1.5 reveals that the maximum increase in height was recorded in T_{M+B+F} while it was minimum in T_{M+B} . The maximum (0.242±0.058) increase in diameter of *O.indicum* was found in T_{F+B}while minimum increase of diameter (0.088±0.015) was found in Control. The maximum (0.760±0.183) increase in girth was found in T_{F+B}while it was minimum (0.277±0.048) in Control. After 270 days of inoculation,the effect on height, diameter and girth of *Oroxylum indicum* was recorded in Table 1.6. The height was maximum (77.83±4.15) in T_{M+B+F} treatment, while it was minimum (39.2±3.90) in Control. Maximum (0.825±0.026) diameter was found in T_{M+B+F} while minimum (0.377±0.029) diameter was recorded in Control. Table 1.7shows the effect of bioinoculation on increase in height, diameter and girth on the seedlings of *O.indicum*. $T_{M+B+F,}$ treatment showed maximum increase in height (65±3.09), whereas, minimum increase in height (28.93±3.61) was found in Control. $T_{M+B+F,}$ showed maximum increase in diameter (0.404±0.058), whereas, T_{M+B} treatment showed minimum increase in diameter (0.206±0.092). Maximum (1.598±0.068)increase in girth was recorded in T_{M+B+F} treatment, whereas, minimum increase in girth (0.438±0.048) was recorded in Control.

Table 1.8 and Table 1.9showthe fresh and dry weight of the root and shoot of the seedlings of *O.indicum*respectively. It was found that the fresh weight of shoot (gm) was maximum (64.1±4.59) in case of T_{M+B+F} while it was minimum in case of Control, the fresh weight of root (gm) was maximum (152.4±7.52) in T_{M+B+F} while it was minimum (57.3±4.16) in Control. The total fresh weight (gm) was maximum (210.3±11.41) in case of T_F while it was minimum (91.93±6.35) in case of Control. Similarly, the total dry weight (gm) of both root and shoot of the seedlings was taken. It was found that the dry weight of shoot was maximum (18.2±0.51) in $T_{BF,}$ while it was minimum (16.2±3.00) in case of T_{MF}, whereas, the dry weight of root was maximum (26.66±1.16) in $T_{M+B+F,}$ while it was minimum (18.1±2.45) in case of $T_{MB.}$ The total dry weight (gm) was maximum (43.3±2.3) in case of T_F while it was minimum (34.9±4.44) in case of T_{MB}.

The biomass of the seedlings after 270 days of inoculation was calculated and tabulated in Table 1.10. The shoot biomass was maximum (73.92±1.77) in T_{M+B+F} treatment and it was minimum (52.45±0.91) in Control. The root biomass was maximum (85.28±0.52) in T_F treatment, whereas it was minimum (66.33±0.89) in Control. . The total biomass was maximum (156.01±1.94) in $T_{F\ treatment}$and minimum (118.78±0.06) in Control.

Table 1.11 shows the effect of bioinoculation on biovolume index. The initial biovolume index was maximum (6.75±0.003) in T_M treatment and minimum (4.266±0.469) in Control (2.407±0.0003) before the initiation of experiment. The biovolume index was maximum (10.66±1.035) in T_{M+B+F} and minimum (4.266±0.469) in Conrol after 90 days of inoculation. After 180 days of inoculation the biovolume index was again maximum (24.83±1.294) in $T_{M+B+F}$and minimum (8.147±0.369) in Control. The biovolume index was maximum (64.15±3.088) in T_{M+B+F} and minimum (14.43±0.33) in Conrol after 270 days of inoculation. Table1.12shows the Quality index (Qi) of seedlings of *O.indicum*after 270 DAI. The Qi was maximum (1.041±0.089) in $T_B$while, Qi was minimum (0.761±0.090) in Control.

Table 1.1: Initial height, diameter and girth of the seedlings ofOroxylum indicum before inoculation 90(DAI)*.

Treatments	Initial Height (cm)	Initial Diameter (cm)	Initial girth (cm)
Control	10±5.77	0.233±0.017	0.733±0.053
T_M	16.1±9.29	0.418±0.02	1.313±0.151
T_B	12.6±7.27	0.439±0.041	1.380±0.226
T_F	11.5±6.63	0.386±0.017	1.214±0.093
T_{M+B}	10.83±6.25	0.31±0.011	0.973±0.061
T_{M+F}	9±5.19	0.351±0.048	1.102±0.262
T_{F+B}	8.16±4.71	0.323±0.005	1.016±0.027
T_{M+B+F}	15.5±7.89	0.316±0.004	0.993±0.025

Anova: F obtained value 89.22, p value 3.73, $F_{critical}$ value 3.008

Table 1.2: Effect of bioinoculation on seedling height, diameter and girth of Oroxylum indicum after first stage inoculation 90 (DAI)*

Treatments	*Height after 90 DAI*	*Diameter after 90 DAI*	*Girth after 90 DAI*
Control	15.53±0.176	0.274±0.027	0.860±0.085
T_M	18.46±2.37	0.495±0.031	1.554±0.098
T_B	17.33±2.88	0.488±0.02	1.532±0.064
T_F	20.03±2.161	0.429±0.01	1.349±0.054
T_{M+B}	14.7±1.49	0.394±0.026	1.239±0.083
T_{M+F}	13.73±2.96	0.423±0.056	1.328±0.178
T_{F+B}	15.33±2.198	0.427±0.047	1.342±0.150
T_{M+B+F}	24.36±1.70	0.436±0.218	1.370±0.068

Table 1.3: Effect of bioinoculation on increase in seedling height, diameter and girth of Oroxylum indicum after first stage inoculation 90 (DAI)*

Treatment	*Increase in height after 90 DAI*	*Increase in Diameter after 90 DAI*	*Increase in Girth after 90 DAI*
Control	5.2±0.503	0.040±0.016	0.126±0.051
T_M	2.433±0.578	0.076±0.019	0.24±0.060
T_B	9±1.951	0.0483±0.023	0.151±0.074
T_F	6.2±1.625	0.043±0.003	0.135±0.011
T_{M+B}	2.366±1.484	0.084±0.036	0.265±0.116
T_{M+F}	4.566±3.117	0.072±0.0105	0.226±0.033
T_{F+B}	5.333±1.716	0.103±0.023	0.325±0.153
T_{M+B+F}	11.533±3.066	0.12±0.0221	0.376±0.069

Table 1.4: Effect of bioinoculation on height, diameter and girth of Oroxylum indicum after second stage inoculation 180(DAI)*

Treatment	*Height after 180 DAI*	*Diameter after 180 DAI*	*Girth after 180DAI*
Control	25.56±1.33	0.322±0.0319	1.011±0.100
$\mathbf{T_M}$	39.6±1.59	0.586±0.055	1.840±0.173
$\mathbf{T_B}$	35.16±6.99	0.59±0.064	1.856±0.202
$\mathbf{T_F}$	39.53±4.39	0.59±0.048	1.867±0.151
$\mathbf{T_{M+B}}$	24.06±0.52	0.420±0.060	1.320±0.191
$\mathbf{T_{M+F}}$	26.4±8.31	0.426±0.050	1.339±0.159
$\mathbf{T_{F+B}}$	29.6±2.94	0.565±0.053	1.776±0.168
$\mathbf{T_{M+B+F}}$	46±1.00	0.539±0.018	1.694±0.059

Table 1.5: Effect of bioinoculation on increase in height, diameter and girth of Oroxylum indicum after secondstage inoculation 180 (DAI)*

Treatment	*Increase in Height after 180 DAI*	*Increase in Diameter after 180 DAI*	*Increase in Girth after 180 DAI*
Control	15.23±1.12	0.088±0.015	0.277±0.048
T_M	23.56±2.90	0.167±0.273	0.526±0.086
T_B	26.837.85	0.151±0.075	0.475±0.235
T_F	25.7±4.80	0.208±0.031	0.653±0.098
T_{M+B}	11.73±1.85	0.110±0.068	0.346±0.215
T_{M+F}	17.23±5.39	0.075±0.002	0.236±0.008
T_{F+B}	19.6±2.05	0.242±0.058	0.760±0.183
T_{M+B+F}	33.16±2.36	0.223±0.015	0.700±0.047

Table 1.6: Effect of bioinoculation on height, diameter and girth of Oroxylum indicum after third stage inoculation 270 (DAI)*

Treatment	*Height after 270 DAI*	*Diameter after 270 DAI*	*Girth after 270DAI*
Control	39.2±3.90	0.377±0.029	1.172±0.091
T_M	57.66±8.17	0.701±0.095	2.203±0.299
T_B	54.8±4.88	0.703±0.077	2.208±0.242
T_F	61.1±7.129	0.744±0.040	2.336±0.127
T_{M+B}	65.96±5.57	0.516±0.082	1.622±0.259
T_{M+F}	52.6±17.26	0.575±0.057	1.807±0.179
T_{F+B}	65.43±4.59	0.717±0.057	2.254±0.179
T_{M+B+F}	77.83±4.15	0.825±0.026	2.592±0.824

Table 1.7: Effect of bioinoculation on increase in height, diameter and girth of Oroxylum indicum after third stage inoculation 270 (DAI)*

Treatment	*Increase in height after 270 DAI*	*Increase in Diameter after 270 DAI*	*Increase in Girth after 270 DAI*
Control	28.93±3.61	0.281±0.049	0.438±0.048
T_M	41.63±7.17	0.283±0.069	0.889±0.217
T_B	46.46±5.58	0.263±0.102	0.827±0.322
T_F	47.26±7.20	0.357±0.024	1.122±0.078
T_{M+B}	53.62±5.07	0.206±0.092	0.649±0.289
T_{M+F}	56.26±2.96	0.224±0.009	0.704±0.028
T_{F+B}	58.53±2.54	0.394±0.062	1.237±0.195
T_{M+B+F}	65±3.09	0.404±0.058	1.598±0.068

Table 1.8. Fresh weight of root and shoot of seedlings of O.indicum after 270 DAI*

Treatment	*Fresh weight shoot (gm)*	*Fresh weight root (gm)*	*Total fresh weight (gm)*
Control	34.63±2.24	57.3±4.16	91.93±6.35
T_M	50.03±4.70	127.33±5.42	177.36±10.13
T_B	58±4.85	123.43±9.07	181.43±12.30
T_F	57.9±5.91	152.4±7.52	210.3±11.41
T_{MB}	59.03±8.66	103.8±4.29	162.86±4.83
T_{MF}	53.66±11.24	82.76±5.55	136.43±16.36
T_{BF}	45.33±1.89	113.2±2.99	158.53±4.87
T_{MBF}	64.1±4.59	143.36±8.19	207.46±8.37

Table 1.9: Dry weight of root and shoot of seedlings of O.indicum after 270 DAI*

Treatment	*Dry weight shoot (gm)*	*Dry weight Root (gm)*	*Total dry weight (gm)*
Control	16.46±1.14	19.26±1.28	35.73±2.37
T_M	18.46±0.55	22.83±1.07	41.3±1.33
T_B	17.66±1.49	20.43±3.25	38.1±3.13
T_F	16.83±1.44	22.43±1.49	39.2±1.20
T_{MB}	16.86±2.21	18.1±2.45	34.9±4.44
T_{MF}	16.2±3.00	19.4±1.42	35.6±4.33
T_{BF}	18.2±0.51	18.6±1.56	36.8±1.85
T_{MBF}	16.7±1.51	26.66±1.16	43.3±2.3

Table 1.10: Biomass of seedlings of O.indicum after 270 DAI*

Treatment	*Shoot biomass*	*Root biomass*	*Total biomass*
T_c	52.45±0.91	66.33±0.89	118.78±0.06
T_M	62.49±3.30	82.07±0.14	144.57±3.28
T_B	68.99±4.29	83.38±2.53	152.38±1.75
T_F	70.72±1.89	85.28±0.52	156.01±1.94
T_{MB}	71.3±0.57	82.33±2.93	153.63±2.86
T_{MF}	69.55±1.10	76.58±0.28	146.13±1.33
T_{BF}	59.80±0.62	83.59±1.10	143.40±0.69
T_{MBF}	73.92±1.77	81.35±0.52	155.28±2.04

Table 1.11: Biovolume index (Bi) of seedlings of O.indicum

Treatment	*Biovolume initial*	*Biovolume after 90 DAI**	*Biovolume after 180 DAI**	*Biovolume after270 DAI**
T_c	2.407±0.0003	4.266±0.469	8.147±0.369	14.43±0.33
T_M	6.75±0.003	9.118±1.277	23.38±3.061	39.12±3.339
T_B	3.700±0.001	8.518±1.62	19.9±1.555	38.69±6.336
T_F	5.369±0.001	8.675±1.288	23.35±2.409	45.68±6.397
T_{MB}	3.807±0.0008	5.874±0.952	10.12±1.474	33.24±2.733
T_{MF}	3.466±0.003	5.838±1.606	12±5.074	38.16±6.288
T_{BF}	3.226±0.490	6.731±1.522	16.97±3.102	49.27±4.818
T_{MBF}	4.047±0.0008	10.66±1.035	24.83±1.294	64.15±3.088

Table 1.12: Quality index (Qi) of seedlings of O.indicum after 270 DAI*

Treatment	*Quality index (Qi)*
T_c	0.761±0.090
T_M	1.001±0.151
T_B	1.041±0.089
T_F	1.026±0.061
T_{MB}	0.775±0.135
T_{MF}	0.795±0.013
T_{BF}	0.920±0.036
T_{MBF}	0.923±0.039

5. Discussion

The growth and sustainability of a plant species is dependent on the biotic and abiotic factors associated with the particular plant species. Microorganisms play vital role in the cycling of the nutrients in the rhizosphere of the plant.Many plant growth promoting rhizobacteria, mycoflora and mycorrhizal fungi also stimulate plant through direct or indirect interactions with the plant roots. In the present study also results are same with the findings of Rani et al. [39]. Gill and Singh [40]; Parkash and Aggarwal [41], Parkash et al.[42]. They reported that the mutualistic association was accounted for better colonization and plant growth due to interchange of carbon, phosphate and nitrogen between host fungi and bacteria. Thus, it can be conferred that a synergistic interaction of the above mentioned microorganisms have direct impact on the growth and development of the plant species.In the present investigation also combined synergistic treatment showed positive growth effect on *Oroxylum indicum* seedlings.

Due to indiscriminate collection, over exploitation, uprooting of whole plants bearing roots, this plant has become endangered and vulnerable in different parts of the Indian subcontinent. The existence of O. indicum in naturalpopulation is highly threatened and has been categorized as vulnerable by the government of India [43]. Local healers and traders are collecting this species from the wild which is causing a severe threat to the existence of this plant species due to poor seed viability[44]. Referring to the availability and various uses of the target plant species, it was essential to take up the concerned study toconserve the target plant species by modern biotechnological eco-friendly methods to produce healthy and quality stock of superior germplasm.

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