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ORIGINAL ARTICLE

Beneficial impact of Fungal Inoculation on Growth and development of *Terminalia arjuna* and *Terminalia bellirica*

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ABSTRACT
Terminalia bellirica and Terminalia arjuna are deciduous and ever green trees found throughout the Indian forests and plains. Both are belong to Combretaceae family. In Ayurved as well as through research it has been proved that both the plants having tremendous medicinal properties. To study the growth and establishment of these two plants and the effect of fungal inoculants, a pot experiment was carried out on 30 days old seedlings of Terminalia bellirica and Terminalia arjuna under nursery condition. The plants were inoculated with seven day old liquid culture of Aspergillus kanagawaensis, Penicillium chrysogenum, Aspergillus japonicas, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus separately. Growth of plants were measured in terms of plant height, leaf no, leaf size, biomass, Net assimilation rate (NAR), Relative growth rate (RGR), Leafarea ration (LAR) after 120days. Data recorded on growth performance of Terminalia bellirica and Terminalia arjuna plant exhibited the positive impact of fungal inoculation especially in case of Aspergillus niger and Aspergillus kanagawaensis over un-inoculated control.
Key words:*-Terminalia, Aspergillus, Fusarium, Rhizopus, NAR, RGR, LAR*

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INTRODUCTION

Growth and development of plants of any types reduce sufficient and balanced nutritional content certainly; NPK plays a vital role in their development and subsequent growth in natural environment. Hence the edaphic factors are generally important to look forward for developing strategies besides environmental factors. To combat with the nutritional depletion in the soil, supplementations of chemical fertilizers are the suitable proportion in this regard. However, the organic the organic forms of fertilizers are not useful for long term usage, as it reduce timely and periodical supplementation which makes its uses more costly. Simultaneously it goes to bonded form is most of the tropical soil, which makes it more costlier as we have to supplement again and again. In such case, the roles of mineral solubilizing microbes are quite visible and imperative. Fungi are known as good source of various enzymes and secondary metabolites [1,2,3,4]. Many of them are reported as mineral solubilizer especially phosphate form in vitro laboratory condition [5]. Few of them are proved as useful agent for the growth and development of plants in field condition [6] . Bio-inoculation of such mineral solubilizers especially phosphate solubilizing have exhibited growth promoting properties in case of same forest trees particularly. These plantation are important for medicinal and fuel etc.[5,7,8]. The application of such bioinoculants for the production of quality planting material has also been reported well [9,10]. However, the development of microbial consortium along with the adquet soil composition for the application and raise of forest tree nursery is reduced [11].

Terminalia arjuna, belong to Combretaceae family, is an important tree species in tropical plains and important due to its various medicinal properties. The detailed survey of literature indicated highly important curable properties of its bark for anemia, cardiovascular ailments, hypolipidemic activity and many other heart related infection and diseases [12,13]. Besides its ability to prevent myocardial abnormalities, it has antimicrobial properties also [14]. Hence, due to its high demand a proportionate diversity of plantation is reduced.

Terminalia bellirica has also potential source raw material reduced in drug formulation useful as anthelmintic, antiseptic, astringent, expectorant, laxative tonic. It also helps in treating many respiratory and ophthalmic diseases. It is well known constituents of Ayurvedic preparation of 'Triphala' [15] and its medicinal value; these tree species are also in high demand.

The populations of both trees are depleted, as users reduced to utilize their full potential but do not work for its conservation and mass propagation. It is therefore, may researchers came forward to work with its mass propagation through various biological techniques pertaining to vegetative propagation [16]. To this context, present study has been planned to evaluate the potential of phosphate solubilizing fungus for growth and development of *Terminalia bellirica* and *Terminalia arjuna* in nursery condition in order to develop healthy and quality planting material for mass propagation.

MATERIALS AND METHODS

The study was carried out during January-June, 2016 in experimental fields of the Regional Plant Resource Centre, Bhubaneswar, India. The experiment done on poly bags (size: 12 x16 cm containing 5.5 kg red laterite soil) received average 35±2ºC temperature & 60- 80±5% relative humidity. Textural class of the soil was loamy sand the soil pH was 5.61. Average nitrogen (N), average phosphate (P_2O_5) and average potassium (K_2O) of the soil was 232.0Kg/Ha, 25.2kg/Ha and 99.12kg/Ha, respectively. Soil was fumigated with 1% formalin (25ml /pot) for 48 hrs. prior to the experiment.

The seeds *Terminalia bellirica* are spherical to ovoid, 1.2 to 3.5 cm in diameter. The dry fruits are grayish brown in colour and thick coated. *Terminalia arjuna* fruits are (drupe) ovoid-oblong, 5-7 hard angles or wings. Seeds were depulped with treatment of watery organic manure. Air dried seeds were sown in experimental poly bags 1 inch below the upper surface Plants were watered twice a day through sprinkler. Inoculation experiment was designed for 30days old seedlings of *Terminalia bellirica* and *Terminalia arjuna* separately. Preselected 6 phosphate solubilizing phosphate solublizing and morphologically identified fungal cultures such as *Aspergillus kanagawaensis, Penicillium chrysogenum, Aspergillus japonicas, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus* were used for the inoculation studies. 25ml of 7 days old liquid cultures of fungi was developed in Czapek dox medium (4.5 pH) and supplemented to each plant of both *Terminalia bellirica* and *Terminalia arjuna.* Same treatments were continued to the seedlings in 30 days interval upto 120 days. Observations after 4 months from DAS (date of sowing) were recorded for shoot height, number of leaves, leaflets, branches, fresh and dry biomass of leaves and stem , collar diameter. The average and standard deviation of the growth parameters were calculated and analyzed for variation and correlation according to the standard procedures.The data were evaluated for RGR (Relative growth rate), NAR (Net Assimilation Rate) and LAR (Leaf area ratio) and QI (Quality index) [17,18,19].

RESULT AND DISCUSSION

Both the tree species *Terminalia arjuna* and *Terminalia bellirica* performed well in nursery condition during experimentation with bioinoculation of different fungi. Among six fungi used in the present study, all of them exhibited positive impact in growth of experimental plants of *T. arjuna* of 120 days age (Table-1). Significant enhancement in shoot height, fresh and dry biomass of rootr and shoot was revealed in inoculated plants as compared to the control ($P<0.05$). *Aspergillus niger* and *Aspergillus kanagawaensis* improved in the plant shoot height and gave highest value comparatively i.e. 60.67±7.9c.m and showed 55.17±6.77c.m. This was followed by *Penicillium chrysogenum* which showed 48.33±7.37c.m in height. However, this fungi did not differ significantly ($P<0.05$). In similar way, these three fungi performed well and helped significant enhancement of *T. arjuna*. Though, higher than control and other treatments, no significant difference have been found in growth enhancement capability of these fungi ($P<0.05$). The other fungal inoculants *Aspergillus flavus*, *Aspergillus japonicas* and *Fusarium oxysporum* though better as compared uninoculated control, did not exhibit very promising growth promoting behavior towards *T. arjuna* seedlings at nursery conditions.

The effect of fungal inoculation on growth and biomass enhancement was also quite visible in case of *Terminalia bellirica* also (Table-2). Inoculation of *Aspergillus kanagawaensis* and *Aspergillus niger* significantly enhance the shoot height and plant biomass ($P<0.05$). Interestingly, plants treated with *Aspergillus niger* showed higher leaf area 15707.7±2086.9 mm^2 as compared to other treatments along with uninoculated control (Table-2). Other morphological growth parameters did not show much significant difference among themselves as well as control experimental sets. A significant higher shoot biomass was observed in plants of treated with *Aspergillus kanagawaensis* and *Aspergillus niger.* However, *Aspergillus flavus* also exhibited good dry biomass of roots along with these fungi. It is very important to note that *Aspergillus kanagawaensis* and *Aspergillus niger* both were more or less similar in improving plant growth as differences in numerical data is not very significant ($P<0.05$).

It has been observed that shoot height and shoot fresh biomass of *T. arjuna* was correlated positively ($R^2=0.7606$, $P<0.05$). Similarly, fresh and dry biomass of these plants were also strongly and positively correlated ($R^2=0.9428$, $P<0.05$). Correlation among shoot height and fresh biomass of *Terminalia bellirica* was strongly positive were R^2 measured 0.8694 at $P<0.05$. In similar way correlation calculated for fresh vs. dry biomass also measured positively and strongly correlated ($R^2=0.9395$, $P<0.05$). This could also be confirmed through data recorded on relative growth rate of both the tree species. The maximum RGR recorded was in case of *Aspergillus kanagawaensis* and *Aspergillus niger*. Net assimilation rate was also improved due to enhancement in biomass and plant height.

However, *Aspergillus niger* and *Aspergillus kanagawaensis* shared NAR which was 2.06 and 1.04 ($g.m^{-2}.d^{-1}$) in *Terminalia bellirica* and in treatment of *Aspergillus kanagawaensis* and *Aspergillus niger* it was 0.58 and 0.38 ($g.m^{-2}.d^{-1}$) in *Terminalia arjuna* plant. *Aspergillus japonicas* shared higher LAR followed by *Aspergillus flavus* in these forest tree species. Data recorded on R/S is also depicted in Table 1 and 2 exhibited the superior response of *Aspergillus japonicas* and *Aspergillus flavus* followed by *Fusarium oxysporum* over control in *Terminalia arjuna* and *Aspergillus japonicas* over control in *Terminallia bellirica*.

The organic and chemical fertilizer are needed for the better growth and development of host plants as the most of the tropical soil is phosphate fixing and does not available for plant usage. In this context the role play by mineral solubilizers are very vital and important. They do not only make phosphate available to the plant simultaneously also retain the physiochemical properties of the soil. The present experiments done on inoculation of phosphate solubilize with *Terminalia arjuna* and *Terminalia bellirica* also exhibited the very promising and growth promoting properties. The idea of using liquid culture for the experiment is to increase the fungal population in the rhizosphere of the host plant, so that phosphate content level of the soil may enhance indirectly [20].

Table-1 Growth performance of *T.arjuna* growth under inoculated condition

Parameters	Treatments					
	Control	*A.flavus*	*A.japonicas*	*A. kanagawaensis*	*A. niger*	*F. oxysporum*	*P. chrysogenum*
Shoot Height (in cm)	36.67±8.48	44.5±7.23	41.83±2.93	55.17±6.77	60.67±7.69	43.0±2.83	48.33±7.37
Root Length (in cm)	35.67±15.10	41.50±10.21	44.50±14.35	44.33±16.85	49.50±2.59	30.50±4.59	42.67±3.98
No. Of Branch	7±4.26	6±3.87	5±5.81	11±4.10	9±3.78	6±3.85	8±7.19
No. Of leaves	42.2±15.73	40.2±18.12	47.8±19.23	56.8±21.48	62.7±19.05	50.7±13.11	44.8±17.29
Leaf Area (in mm^2)	1855.2±96.01	2729.2±65.52	2740.0±154.88	3795±135.56	3165.2±123.29	2085.5±146.98	2844.7±258.27
Wet Biomass Shoot (in g)	15.50±4.55	20.53±7.29	16.97±3.83	29.14±6.42	23.66±4.55	17.69±2.85	22.68±5.70
Wet Biomass (in g) Root	3.47±1.77	6.70±1.64	6.75±1.91	9.97±1.77	9.14±2.30	4.76±0.65	7.26±1.33
Dry Biomass Shoot (in g)	6.73±2.23	9.10±2.26	8.51±2.29	16.44±6.47	13.66±4.13	8.36±1.76	12.60±3.95
Dry Biomass Root (in g)	1.88±1.07	3.34±0.73	3.75±1.18	5.82±0.69	5.81±1.75	2.49±0.46	4.46±1.01
Wet RGR (d^{-1})	0.162	0.218	0.179	0.314	0.253	0.187	0.242
Dry RGR (d^{-1})	0.073	0.100	0.093	0.181	0.150	0.091	0.138
NAR ($g.m^{-2}.d^{-1}$)	0.09	0.21	0.20	0.58	0.38	0.13	0.31
LAR ($m^2.g^{-1}$)	1067.821	888.289	949.727	559.081	626.036	886.197	653.018
QI	0.214	0.263	0.278	0.384	0.309	0.234	0.333
RSR	0.973	0.933	1.064	0.804	0.816	0.709	0.883

Means in column with different levels are significantly different ($p<0.05$)

Table-2 Growth performance of *T.bellirica* growth undeinoculated condition

Parameters	Treatments						
	Control	*A.flavus*	*A.japonicas*	*A. kanagawaensis*	*A. niger*	*F. oxysporum*	*P. chrysogenum*
Shoot Height(in cm)	35.0±1.0	10.3±7.6	31.3±3.8	55.3±1.2	57.3±7.6	40.0±5.3	39.7±4.5
Root Length (in cm)	42.3±12.7	62.3±14.6	43.3±8.1	58.0±9.5	60.7±22.3	54.3±25.5	38.0±5.6
No. Of Small Branches	3.7±2.1	2.0±2.0	1.3±1.5	4.0±2.0	1.0±1.7	1.0±1.0	1.0±1.0
No. Of leaves	23.7±6.8	20.3±12.7	19.0±7.2	36.7±12.5	24.7±9.1	21.0±8.9	19.3±8.5
Leaf Area (in mm^2)	6843.3±405.4	9395.7±133.3	9124.3±221.0	8630.0±708.1	15707.7±2086.9	8104.0±130.8	8034.0±228.9
Wet Biomass Shoot (in g)	16.98±4.3	19.157±2.7	14.21±7.1	37.51±2.5	31.85±7.6	15.84±1.8	18.62±6.9
Wet Biomass (in g) Root	8.96±2.9	10.74±1.5	11.72±3.0	13.99±2.8	13.36±4.2	7.79±1.9	4.85±0.6
Dry Biomass Shoot (in g)	8.12±2.2	18.7±5.6	7.84±2.9	6.07±2.0	15.66±2.6	5.97±2.0	8.60±3.0
Dry Biomass Root (in g)	5.33±1.7	7.10±2.2	4.00±0.8	7.05±1.6	7.17±1.4	4.27±1.1	3.03±0.8
Wet RGR (d^{-1})	0.148	0.137	0.122	0.334	0.283	0.137	0.162
Dry RGR (d^{-1})	0.073	0.070	0.054	0.138	0.141	0.053	0.077
NAR ($g.m^{-2}.d^{-1}$)	0.42	0.58	0.43	1.04	2.06	0.37	0.53
LAR ($m^2.g^{-1}$)	448.977	634.347	684.796	254.769	516.129	555.760	472.429
QI	1.352	1.385	1.605	1.110	1.124	1.328	1.114
RSR	1.210	1.545	1.936	0.687	0.948	1.558	1.092
Means in column with different levels are significantly different ($p<0.05$)							

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