Chapter

Trichoderma harzianum Rifai: A Beneficial Fungus for Growth and Development of *Abroma augusta* L. Seedlings with Other Microbial Bio-Inoculants

Vipin Parkash, Akshita Gaur, Rahul Agnihotri and Ashok Aggarwal

Abstract

Rhizospheric microbes play an important role in plant health. Rhizosphere is an area around the roots of plants where all microbes repose and influence the health of plants. These microbes require organic matter for their activity and provide nutrients to the plants and maintain the plant health. In this research paper, these useful microbes like fungi (*Trichoderma harzianum*), endomycorrhizae (Arbuscular Mycorrhiza) and bacteria (*Pseudomonas putida*) were isolated and after mass multiplication applied as bio-inoculants in alone and in combination to see the effect on growth and development of *Abroma augusta* seedlings which is a threatened medicinal plant in north-eastern part of India. *T. harzianum* alone and in combined form showed significant growth and development effect on seedlings. The effect of alone and combined treatments of *T. harzianum* on growth and development of this important medicinal plant species has been discussed in detail in this research paper.

Keywords: endomycorrhizae, microbes, *Pseudomonas putida*, rhizosphere, threatened medicinal plant

1. Introduction

Abroma augusta L. or Devil's Cotton is commonly known as *Ulat Kambal* (*Pisachkarpas* in Ayurvedic system, *Gorokhia koroi* in Assam, *Dadhubedang* in Meghalaya) when translated into English it means the inner side of a blanket. This is possibly because the bark of the tree yields a beautiful silky fiber, like that of hemp. Root bark of *Ulat Kambal* is a valuable emmenagogue and uterine tonic, chiefly used in intra-uterine diseases and other gynecological disorders mostly related to menstrual disorders such as dysmenorrhoea, amenorrhoea and gonorrhea. Powdered root of this medicinal plant is an abortifacient and anti-fertility agent. The leaves and stem are demulcent. Infusion of fresh leaves and stems is effective in treatment of gonorrhea [1].

Abroma augusta L. is an evergreen small tree and native of Asia. In India, it is found in eastern and western Himalayan region. Plant reaches 10 feet (2.5 m) in height with very little spread. The leaves are heterophyllic, reach 8 inches (20 cm) across and are 3–5 lobed with very distinct palmate veins. The leaves and stems are covered with soft bristly hairs that are very irritating to the touch. The bark yields a jute-like fiber. The plants bloom from late spring to early summer. Dark maroon flowers are formed in terminal panicles. Individual flowers are up to 3 inches (7.5 cm) across. *A. augusta* is also featured as "Plant of the Week" May 28–June 4, 2004 [2]. This plant species is present in Assam, Meghalaya, Arunachal Pradesh and Nagaland states of north eastern Himalayas. The plant species is declining in number due to exploitation and lack of awareness and also facing severe threat due to biotic pressure [3]. According to one of report [4], there are only 2000 medicinal plants known in Brahmaputra valley and Assam hills in North East India in which important ones are *A. augusta*, *Smilax glabra, Aquilaria malaccensis* and *Hydnocarpus kurzii*. The root and stem bark of this plant is in great demand in herbal market (800–1000/kg) as per a report by Singh [5]. This plant also needs protection according to one of report by Botanical Survey of India at Itanagar, Arunachal Pradesh [4]. 'Monofil', an herbal formulation containing *A. augusta* can be used as an alternate in the treatment of post menopause syndrome [6]. Also, *A. augusta* harbors a good population of microbes in its rhizosphere.

A narrow zone of soil affected by the presence of plant roots is defined as rhizosphere [7]. The rhizosphere is an environment that the plant itself helps to create and where pathogenic and beneficial microorganisms constitute a major influential force on plant growth and health [8]. Microbial groups and other bioagents found in the rhizosphere include bacteria, fungi, nematodes, protozoa, algae and microarthropods [8, 9]. Microorganisms that adversely affect plant growth and health are the pathogenic fungi, oomycetes, bacteria and nematodes, whereas microorganisms that are beneficial include nitrogen-fixing bacteria, endo- and ectomycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR) and fungi [10]. *Trichoderma* is the most common and easily culturable soil fungus present in almost all types of soil. *Trichoderma* has been reported to possess biocontrol activities against different pathogens and also possess plant growth promoting characteristics [11]. Similarly, some bacterial species are also known to help in plant growth promotion. One such Plant growth promoting rhizobacteria is *Pseudomonas putida* [12]. It is a gram negative bacterium which is widespread in nature and can be easily cultured. Arbuscular mycorrhizal (AM) fungi are known to increase phosphate solubilization and help increase in nutrient uptake thus, helping in improving overall growth of a plant. Also, interaction of AM fungi with *Trichoderma* sp. has been reported to have better effects in improving the overall growth of *Eucalyptus saligna* Sm. [13].

So keeping in mind all these biological and chemical processes, the study was undertaken on biodiversity of rhizospheric microbes and their effect on the seedlings of *Abroma augusta* which is a threatened important plant species in Brahmaputra Valley of Assam, India.

2. Materials and methods

2.1 Survey and collection of soil samples

As *Abroma augusta* L. is scattered in its distribution the survey of different study sites (Assam) were done for the collection of rhizospheric soil samples. The plant specimen was preserved in herbarium sheets for identification. Rhizosphere soil

samples (at least three samples) were taken by digging out a small amount of soil (500 g) close to plant roots up to the depth of 15–30 cm and these samples were kept in sterilized polythene bags at 10°C for further processing in the laboratory.

2.2 Isolation of useful microbes

For qualitative studies of soil mycoflora, Warcup soil plate method [14] and Waksman soil dilution method [15] were used.

2.3 Isolation of *Trichoderma* inoculum

Fungal species *Trichoderma harzianum* was isolated from the soil samples by using serial soil dilution method [15] on potato dextrose agar (PDA) medium. The inoculated plates were incubated at 30°C for 4 days. The pure fungal colonies were picked up and purified by streaking on agar slants and incubated at 30°C for 7–8 days. Green conidia forming fungal bodies were selected and microscopic observation was done and the fungus was identified to be *T. harzianum* (Isolate/accession no. MSML/RFRI/TH-13). The preserved fungal isolate/culture maintained on PDA slants are retained with Mycology and Soil microbiology Laboratory, Rain Forest Research Institute, Jorhat, Assam, India for further study.

2.4 Preparation of solid substrate media

In this experiment, different saw dusts like *Pinus kesiya* Royle ex. Gordon, *Shorea robusta* Gaertn. and *Callicarpa arborea* Roxb. were taken for evaluation. The different saw dusts were shade dried. The dried saw dusts were mixed with wheat bran by adding sterilized water in the ratio (wheat bran: saw-dust: water; 3:1:4 w/w) as explained above. The moisture level of the mixture was maintained up to 50–60%. The substrate was sterilized through Autoclave (Labotech, BDI-81 make, India) at 120°C and 15lbs [16].

2.5 Mass multiplication of fungal inoculum

The inoculum of *Trichoderma harzianum* was grown on synthetic PDA (Potato Dextrose Agar) medium (SRL, India) for 7–8 days and incubated at 27–30° ± 1°C. The inoculum was kept in B.O.D. incubator (Labotech, BDI-55 make, India) for 10–12 days for maximum growth and sporulation. Then the inoculum containing medium was cut into small discs and were put in flasks containing wheat bran and different saw-dust medium in the ratio (3,1,4 w/w) for mass production of *T. harzianum*. Approximate 50 g of substrate was taken in 500 ml conical flasks, inoculated with 5 mm mycelial mat incubated at 28°C incubator for 7–10 days earlier. In control set, no saw dust component was added to the substrate. Six replicates of each treatment were taken. The colony forming units were calculated with help of the following formula through serial dilution of 1 g of substrate and results are expressed as cfu g^{-1} ml^{-1} of suspension of each substrate [16].

$$\text{CFU/g/ml} = \frac{\text{Number of colonies per ml plated}}{\text{Total dilution factor}} \quad (1)$$

2.6 Mass multiplication of bacterial inoculum

The *Pseudomonas putida* (MSML/RFRI/Ps-1) multiplication was carried out through bacterial cultivation technique by using growth curve after specific time

intervals of 1 h [17]. The inoculum of Bacteria was taken in stationary phase (10–11 h) for inoculation experiment.

2.7 Isolation of VAM = AM spores

Isolation of VAM spores was done by using wet sieving and decanting technique of Gerdemann and Nicolson [18] and Singh and Tiwari [19]. Sieves of different sizes, i.e. 150, 120, 90, 63 and 45 μm were used. About 150 μm sieve were used for collecting plant debris in the soil. About 100 g of soil were mixed in water in a small plastic container having the capacity of about 1500 ml. The soil was thoroughly mixed with water and allowed to settle for overnight. The water was decanted on a series of sieves in the following order 150, 120, 90, 63 and 45 μm from top to bottom on which spores were trapped. The trapped spores were then transferred to Whatman filter paper No. 1 by repeated washing with water. The spores were picked by hypodermic needle under stereo-binocular microscope and mounted in polyvinyl lactic acid.

2.8 Mass multiplication of endomycorrhizal inoculum

The mycorrhizal inoculum production was done by using '*soil funnel technique*'. Single dominant and efficient AM spore/s of *Glomus* sp. (MSML/RFRI/M_1) and (*Acaulospora* sp. (MSML/RFRI/M_2) was/were mass produced in this technique.

The best host were selected for starter culture of inoculum production, i.e. sorghum (*Sorghum vulgare*), wheat (*Triticum aestivum*) and onion (*Allium sativum*). In this technique, glass funnels/earthen funnels were taken and germination of seeds was made in such a way that roots of the seedlings must touch the inoculum of AM fungi. The seedlings were raised up to 30 days in the glass/earthen funnels containing sterilized sand: soil (40:120 g.). The experiment was repeated again and AM spores were collected by wet sieving and decanting technique of Gerdemann and Nicolson [18] and Singh and Tiwari [19] after 45–60 days. These final spores were used for mass multiplication by using different hosts in bigger earthen pots for further study.

To have mass inoculum in bulk quantity, the test inocula were multiplied in field conditions by preparing standard size beds on thin polyethylene sheet (0.5 mm) so that no contamination will occur to the inocula. The experiment was repeated for maintaining the inocula cultures viable for further experiments.

2.9 Cultivation, conservation and growth studies

The plantlets of target plant species after survey were raised from seeds/propagules with the help of inoculation in root trainers in laboratory conditions. These inoculated seedlings were transplanted in bigger pots and then in field conditions again with bioagents inoculation for primary establishment and better growth of quality seedlings. The experiment was designed in different treatments like single and double inoculations. Three replications of each treatment were taken. In control sets, no bioagent (inoculum) was given or added. The following design of experiment was adopted.

T_0 = Control (no inoculation).

T_1 = Treatment/inoculation of A endomycorrhizal strain (AM fungus).

T_2 = Treatment/inoculation of B another bio-agent (fungus).

T_3 = Treatment/inoculation of C another bio-agent (bacteria).

T_4 = Treatment/inoculation of A+B, A+C, B+C and A+B+C (endomycorrhizal strain + another bio-agent/s) (bioagents consortium).

Observations were recorded to see the inoculation effect on plant seedlings for the following parameters after specific time intervals up to 180 days after inoculation/DAI in sterilized soil conditions.

- Shoot length in cm (increase in height).
- Diameter in mm.
- Percentage root colonization.
- Mycorrhizal (AM) spore number.

3. Results

The data on the effect of bio-agents inoculation on height of *Abroma augusta* after inoculation (DAI)* in sterilized soil condition was tabulated and further analyzed (**Table 1**). The data on the increase in height (cm) was recorded after 30, 60,

Treatments	Initial height (cm)	Increase in height (cm)				
		30 days	60 days	90 days	120 days	150 days
Control	3.67 ± 0.76	5 ± 0.54	14.66 ± 2.0	16.33 ± 1.7	18.16 ± 0.95	19.33 ± 0.71
T_M	6.16 ± 1.06	2.84 ± 0.94	19.64 ± 1.3	21.51 ± 1.44	23.17 ± 1.34	24.17 ± 1.65
T_B	5.67 ± 0.14	4.16 ± 0.14	22.33 ± 5.3	23.33 ± 1.89	25.33 ± 1.93	26.33 ± 1.69
T_F	3 ± 0.41	5.5 ± 0.85	29.67 ± 0.54	31 ± 0.47	34 ± 0.47	35.33 ± 0.72
T_{M+B}	8.5 ± 0.62	3 ± 1.55	14 ± 4.55	15.17 ± 4.75	16.27 ± 4.75	18 ± 4.63
T_{M+F}	6.17 ± 1.6	4.83 ± 0.82	17.83 ± 1.25	18.5 ± 1.44	20.83 ± 1.78	21.83 ± 1.7
T_{F+B}	2.3 ± 0.29	4.53 ± 1.34	11.2 ± 4.94	12.87 ± 5.14	14.2 ± 5.4	15.27 ± 5.43
T_{M+B+F}	3.17 ± 0.14	5.76 ± 1.08	19.5 ± 2.33	21.5 ± 2.43	23.16 ± 1.33	23.83 ± 2.62
CV (%)	0.13	0.22	0.17	0.15	0.13	0.12

**Average of three replications (Days after inoculation).*
M, mycorrhiza (consortium); B, bacteria *(Pseudomonas putida);* F, fungi *(Trichoderma harzianum);*
CV, coefficient of variance; ±SEm, standard error of mean.

Table 1.
Effect of bio-agents inoculation on height of Abroma augusta *L. after inoculation (DAI)* in sterilized soil condition.*

Treatments	Initial diameter (mm)	Increase in diameter (mm)				
		30 days	60 days	90 days	120 days	150 days
Control	1.2 ± 0.094	0.63 ± 0.136	1.2 ± 0.17	1.57 ± 0.12	1.87 ± 0.24	1.97 ± 0.31
T_M	0.8 ± 0.047	0.33 ± 0.072	1.37 ± 0.22	2.13 ± 0.19	2.53 ± 0.17	2.66 ± 0.17
T_B	0.9 ± 0.045	1.1 ± 0	1.27 ± 0.25	2.3 ± 0.08	2.67 ± 0.03	2.93 ± 0.07
T_F	1.03 ± 0.072	0.5 ± 0.119	1.8 ± 0.14	2.27 ± 0.14	2.47 ± 0	2.6 ± 0.18
T_{M+B}	1.27 ± 0.098	0.3 ± 0.191	0.46 ± 0.3	1.16 ± 0.18	1.66 ± 0.19	1.79 ± 0.29
T_{M+F}	0.90 ± 0.043	0.93 ± 0.071	2.17 ± 0.19	2.77 ± 0.07	3.2 ± 0.08	3.4 ± 0.12

Treatments	Initial diameter (mm)	Increase in diameter (mm)				
		30 days	60 days	90 days	120 days	150 days
T_{F+B}	0.87 ± 0.075	0.6 ± 0.196	1.13 ± 0.43	1.46 ± 0.59	1.83 ± 0.67	2.29 ± 0.64
T_{M+B+F}	0.90 ± 0.091	0.87 ± 0.129	1.97 ± 0.11	3.0 ± 0.08	3.2 ± 0.14	3.43 ± 0.14
CV (%)	0.07	0.23	0.22	0.11	0.09	0.10

**Average of three replications (Days after inoculation).*
M, mycorrhiza (consortium); B, bacteria *(Pseudomonas putida);* F, fungi *(Trichoderma harzianum);*
CV, coefficient of variance; ±SEm, standard error of mean.

Table 2.
Effect of bio-agents inoculation on diameter of Abroma augusta *L. after inoculation (DAI)* in sterilized soil condition.*

Treatments	Abiotic parameters		Biotic variables				
	Temperature (°C)	*p*H	Increase in height (cm)	Spore count/ 50 g	Hyphal infection (%)	Arbuscular infection (%)	Vesicular infection (%)
Control	34 ± 0	5.62 ± 0.25	29 ± 0.82	19 ± 0.30	35 ± 1.24	0	10 ± 0.37
T_M	34 ± 0	5.4 ± 0.47	35.33 ± 0.29	81 ± 0.072	100 ± 0	40 ± 0.19	50 ± 1.24
T_B	34 ± 0	5.43 ± 0.37	35.67 ± 0.16	4 ± 0.16	0 ± 0	0 ± 0	0 ± 0
T_F	34 ± 0	5.47 ± 0.19	41.33 ± 0.22	30 ± 0.44	30 ± 1.24	10 ± 0.072	20 ± 1.63
T_{M+B}	34 ± 0	5.62 ± 1.63	30.67 ± 1.63	28 ± 0.25	80 ± 0.22	40 ± 0.10	10 ± 0.29
T_{M+F}	34 ± 0	5.65 ± 0.22	37.67 ± 0.30	34 ± 1.24	70 ± 0.44	30 ± 0.25	20 ± 0.47
T_{F+B}	34 ± 0	5.6 ± 0.16	24.33 ± 0.072	10 ± 0.10	20 ± 0.29	0 ± 0	0 ± 0
T_{M+B+F}	34 ± 0	5.55 ± 0.82	29.67 ± 1.24	117 ± 0.82	100 ± 0	30 ± 0.37	60 ± 0.19
CV (%)	0	0.092	0.019	0.010	0.008	0.013	0.026

**Average of three replications (Days after inoculation).*
M, mycorrhiza (consortium); B, bacteria *(Pseudomonas putida);* F, fungi *(Trichoderma harzianum);* CV, coefficient of variance; ±SEm, standard error of mean.

Table 3.
Effect of bio-agents inoculation on Abroma augusta *L. after 180 days of inoculation (DAI)* in sterilized soil condition.*

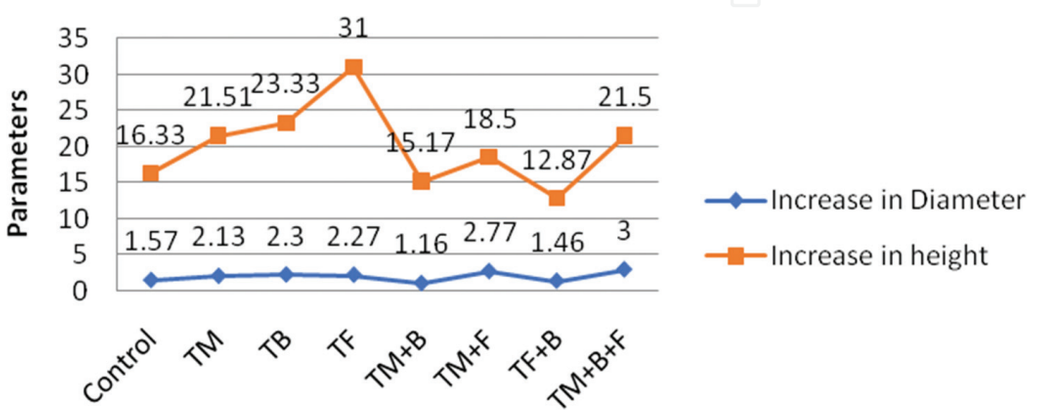

Figure 1.
Effect of bio-agents inoculation on increase in diameter and height of Abroma augusta *L. after 90 days (DAI)*.*

90, 120 and 150 days after inoculation. The increase in height after 30 days showed maximum height increase (5.76 ± 1.08) in $T_{M+B+F,}$ whereas, minimum increase in height (2.84 ± 0.94) was recorded in T_M treatment. The coefficient of variance was found to be 0.22 after analysis. After 60 days of inoculation, the increase in height (29.67 ± 0.54) was maximum in $T_{F,}$ treatment, whereas, minimum increase in height (11.2 ± 4.94) was recorded in T_{F+B} treatment. The coefficient of variance was 0.17. After 90 days of inoculation, $T_{F,}$ treatment showed maximum increase in

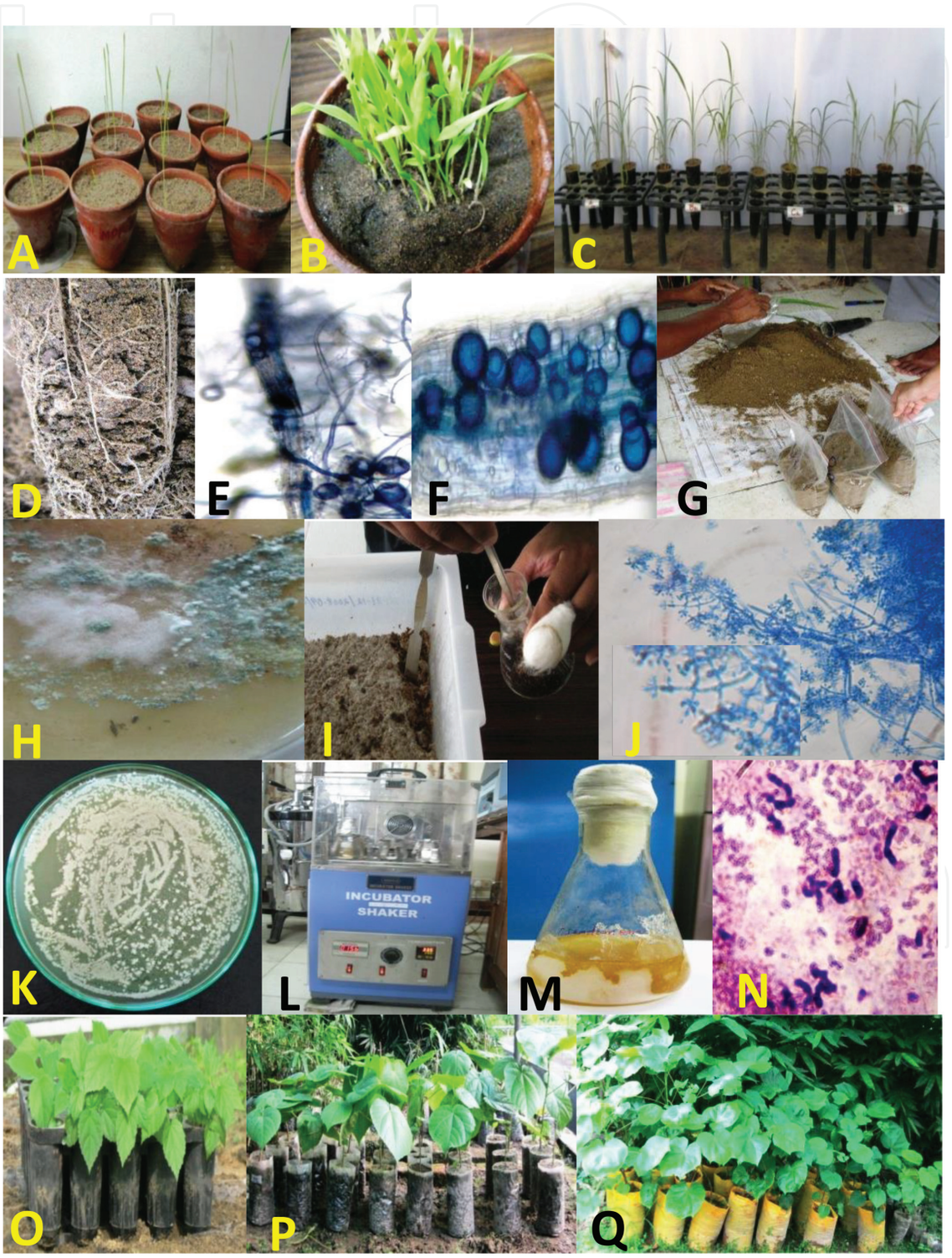

Figure 2.
(A–C) Trap and starter cultures of AM fungi inoculum. (D–G) Roots showing trapping of AM spores and harvesting of inoculums. (H–J) Mass production of Trichoderma harzianum *inoculums. (K–N) Mass production of* Pseudomonas putida *inoculums. (O–Q) Bioinoculation growth effect on seedlings of* Abroma augusta.

height (31 ± 0.47), whereas, minimum increase in height (12.87 ± 5.14) was found in T_{F+B} treatment. The coefficient of variance was found 0.15 in this case. The data after 120 days of inoculation revealed that $T_{F,}$ showed maximum increase in height (34 ± 0.47), whereas, T_{F+B} showed minimum increase in height (14.2 ± 5.4). The coefficient of variance was 0.13. After 150 days of inoculation, maximum increase in height (35.33 ± 0.72) was recorded in T_F treatment, whereas, minimum increase in height (15.27 ± 5.43) was recorded in $T_{F+B,}$ treatment. The coefficient of variance was 0.12 in this analysis (see **Table 1**).

The study of the effect of bio-agents inoculation on diameter of *Abroma augusta* after inoculation (DAI)* in sterilized soil condition in **Table 2**. The data on the increase in diameter (cm) was recorded after 30, 60, 90, 120 and 150 days after inoculation. The increase in diameter after 30 days showed maximum increase of (1.1 ± 0) in T_B treatment, whereas, minimum increase in diameter (0.3 ± 0.191) was recorded in T_{M+B} treatment. The coefficient of variance was found 0.23. After 60 days of inoculation, the increase in diameter was maximum (2.17 ± 0.19) in T_{M+F} treatment, whereas, minimum increase in diameter (0.46 ± 0.3) was recorded in T_{M+B} treatment. The coefficient of variance was 0.22. After 90 days of inoculation, $T_{M+B+F,}$ treatment showed maximum increase in diameter (3.0 ± 0.08), whereas, minimum increase in diameter (1.16 ± 0.18) was found in $T_{M+B,}$ treatment. The coefficient of variance was 0.11. The data after 120 days of inoculation revealed that, $T_{M+B+F,}$ showed maximum increase in diameter (3.2 ± 0.14), whereas, T_{M+B} treatment showed minimum increase in diameter (1.66 ± 0.19). The coefficient of variance was 0.09. After 150 days of inoculation, maximum increase in diameter (3.43 ± 0.14) was recorded in T_{M+B+F} treatment, whereas, minimum increase in diameter (1.79 ± 0.29) was recorded in $T_{M+B,}$ treatment. The coefficient of variance was 0.10 (see **Table 2**).

The data on the effect of bio-agents inoculation on *Abroma augusta* after 180 days of first stage of inoculation (DAI)* (before second stage inoculation) was tabulated in **Table 3**. The analysis of the data revealed that among the various treatments the pH varies between 5.4 ± 0.47 to 5.65 ± 0.22 respectively. The increase in height (cm) was recorded and it was found that, T_F treatment showed maximum increase in height (41.33 ± 0.22), whereas, minimum increase in height (24.33 ± 0.072) was recorded in T_{F+B} treatment (**Figures 1** and **2**).

4. Discussion

According to Jha et al. [20], as they have already reported that the ecology of microorganisms, however, cannot be considered solely in terms of their relationships with the abiotic environment, as their ability to co-exist with other microorganisms. However, the link between rhizospheric microbial biota and abiotic soil properties can be exclusively advocated for a broader utilization as an ecological parameter for any plant species. Therefore, the present study was an attempt to determine the association ecology of an important plant species like *Abroma augusta* under threat due to anthropogenic pressure in the Brahmaputra valley of Assam, India. The synergistic effect of dual inoculation of AM and *Rhizobium* on chickpea was found on nodulation, plant growth, dry matter production and N-fixation by Jalali and Thareja [21]. Similarly in cowpea, inoculation with AM and *Rhizobium* under field conditions increased shoot dry matter and yield over the alone AM or *Rhizobium* inoculation [22]. Similar synergistic effect of dual inoculation was reported in *Leucaena leucocephala* and *Cajanus cajan*. The combined inoculations of

symbionts showed significant increased N-fixations growth and nutrient uptake in *Leucaena leucocephala* and *Cajanus cajan* [23, 24]. Similarly in this case, the combined synergistic effect of tripartite inoculation treatment showed positive growth effect on *Abroma augusta* seedlings.

According to Chang [25] and Rani et al. [26], the above ground part, i.e. height of plant and fresh shoot weight were more in *Trichoderma* sp. treatment in *Acacia nilotica*. In the present study also, above ground part, i.e. shoot length were more in *Trichoderma* treatment alone in *Abroma augusta* seedlings. It is may be due to *Trichoderma* fungus which is secreting some substances in the rhizosphere which are responsible for better growth of above ground parts.

In the present investigation, results were similar to the findings of Gill and Singh [27], Parkash and Aggarwal [28], Parkash et al. [29]. 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. The dual inoculation of AM fungi and *Rhizobium* had synergistic effect on nodulation, plant growth, dry matter, production and nitrogen fixation [30, 31]. Similarly, Singh and Singh [32] also recorded higher yield in dual inoculation of VAM and *Rhizobium* in lentil. Increased N-fixation due to increased mycorrhizal colonization and nodulation may contribute towards growth and yield of plants [27]. Kaushish et al. [33] also found mycorrhizal inoculation effect on growth and physical parameters on *Rauwolfia serpentina* Benth. Ex. Kurtz. Similar results on physical parameters were found by synergistic inoculation on *Abroma augusta* [34].

Similar results were also obtained by Kumar et al. [35]. They reported that the inoculation with all the tested VAM fungi, e.g., *Glomus mosseae, G. fasciculatum, G. constrictum, Acaulospora laevis* and *Gigaspora gilmorei* resulted in significant increase in plant height of chickpea because in present investigation *G. mosseae* in combination showed better growth response in *Abroma augusta* after 90 days.

The root colonization by mycorrhiza was directly related to nutrient uptake by plants. Lowest nutrient uptake was observed in non-mycorrhizal plants and highest nutrient in mycorrhizal plants grown in sterilized soil. Numbers of mycorrhizal spores were also higher in mycorrhizal inoculated soil and directly related to mycorrhizal root colonization. The plants with highest root colonization showed greater number of mycorrhizal spores in soil [36]. In the present investigation, same trend was observed in the plant seedlings as far as mycorrhizal spore number and root colonization were concerned.

5. Conclusion

All the bio-inoculation treatments were significant than control treatment in terms of increase in height and girth. The combined treatments T_{F+B} and T_{M+B+F} are the best and significant bio-inoculation treatments for this plant species. If all parameters are considered together then consortium of endomycorrhizae, fungus, bacterium (T_{M+B+F}) treatment is better in inoculating the target species although, all alone bioagents had good growth and development effect on the seedlings than control treatment where no inoculum was added. Although, bacterium (*Pseudomonas putida*) alone had no significant effect but if it mixed with other bioagents like *Trichoderma harzianum* and AM fungi consortium (*Glomus* species + *Acaulospora* species) then this treatment has significant effect on this target plant species.

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Conflict of interest

We confirm that there are no conflicts of interest.

Notes/thanks/other declarations

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