



STUDY ON THE DEVELOPMENT OF MIYAWAKI FOREST AND ITS SOIL PHYSICO-CHEMICAL CHARACTERISTIC ANALYSIS

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Abstract : Living organisms depends on healthy environment for their sustainability. As an initiation to develop and maintain a healthy environment miyawaki forest was developed in our college premises. About 109 saplings including 16 varieties of species were planted in the prescribed way in the miyawaki forest. Control was maintained with the same measurement of area as miyawaki forest with 10 saplings that are grown conventionally. The growth of the saplings was observed periodically and compared with the control. The saplings in the miyawaki area showed good growth than control. The soil samples were analyzed for their physico-chemical parameters, nutrient content and microflora. The results of soil parameter analysis showed that, significant difference was observed in the soil samples that is collected before and after sapling growth. The nutrient content was found to be more after the growth of the saplings. Microorganism like *Paenibacillus jamilae* and *Paenibacillus polymyxa* were isolated and identified from the soil samples. The synergistic interaction of soil microbes and soil nutrients have potentiated the growth of saplings in the miyawaki forest. The study proved that, development of miyawaki forest will definitely pave way for a healthy environment.

IndexTerms - Miyawaki forest, tree saplings, soil parameters, *Paenibacillus jamilae*, *Paenibacillus polymyxa*

I. INTRODUCTION

Forests are the dominant terrestrial ecosystem of the earth. It accounts 75% of gross primary production of earth's biosphere and contains 80% of plant biomass. Flora of India has diverse vegetation and historically experienced a wide variety of management practices. Forest and human society influences each other in both ways.

Soil is a vital component of forest ecosystems and is responsible for biomass production. Forest cannot be understood without the knowledge of soil. The soil and vegetation have a complex inter relation as they emerge and grow together over a long period of time. The vegetation influences the physical, chemical and biological properties of soil to a great extent. Soil nutrients are the key factors that affect plant growth and species distribution. Its organic matter determines the physico-chemical characteristics of soil such as pH, water holding capacity, texture, nutrient availability, etc., All these together contribute to the healthy ecosystem.

Humans beings for their need and greed have decreased the amount of forest and soil fertility worldwide for various reasons and by various means. This lead to deforestation, which sowed seeds for many environmental issues like, global warming, greenhouse effect, climate change, pollution, soil erosion, biodiversity loss, economy fall etc., To overcome all these seroius environmental issues forest ecology must be protected and afforestation should be promoted. Supporting this concept, many techniques of afforestation have emerged; one of those is Miyawaki forest.

Miyawaki technique was developed by Japanese Botanist *Akira miyawaki* in 1978. He applied the method of Potential Natural Vegetation (PNR). This technique helps to build dense native forest. In this method, the plants grow 10 times faster and 30 times denser than usual plantation. It involves planting of native species in the same area and becomes maintenance free after three years. About 20,000 to 30,000 saplings per hectare were planted, monitored, watered and weeded for the development of a healthy Miyawaki forest. It is reported that, many organizations like 'Afforest' and 'Say trees', Bengaluru has planted 45 lakhs and 70,000 trees respectively in 2008. Organization like thuvakkam in Chennai has planted 4.5 lakhs trees out of their 108 projects.

As it is reported to be a successful method and practiced throughout the world, this technique is selected for the study to check the potency in our locality.

II. RESEARCH METHODOLOGY

2.1 Study area

The present study was carried out in the premises of The Standard Fireworks Rajaratnam College for Women, Sivakasi, Virudhunagar Dt, Tamil Nadu, India. This is a semi-arid tropical climatic dry land area with the annual temperature range of 20 °C to 37°C and receives scanty rainfall with an average of 812 mm annually, which is lesser than the state average of 1,008 mm.



Fig 1: Study area

2.2 Selection of land

For the development of miyawaki forest, a small land measuring 31×11 meter was selected. This area is located beside the indoor auditorium which remained unused for many years. The selected area was marked and weeded properly (Fig 1).

Water is the second most important component for development of Miyawaki forest. Saplings must be watered regularly for 2-3 years to maintain good growth, it is backed by a well and water pipelines. Availability of water in the well is very scarce and pipeline water will also be not surplus. So, it is to be noted that, the miyawaki forest is developed in a water scarce area.

Parallel to the miyawaki forest area, control area measuring the same 31×11 meter was maintained. This area is also marked and weeded properly as miyawaki forest area.

2.3 Selection of species for planting

Study was done to identify the species that grow well in this geographical and climatic condition.

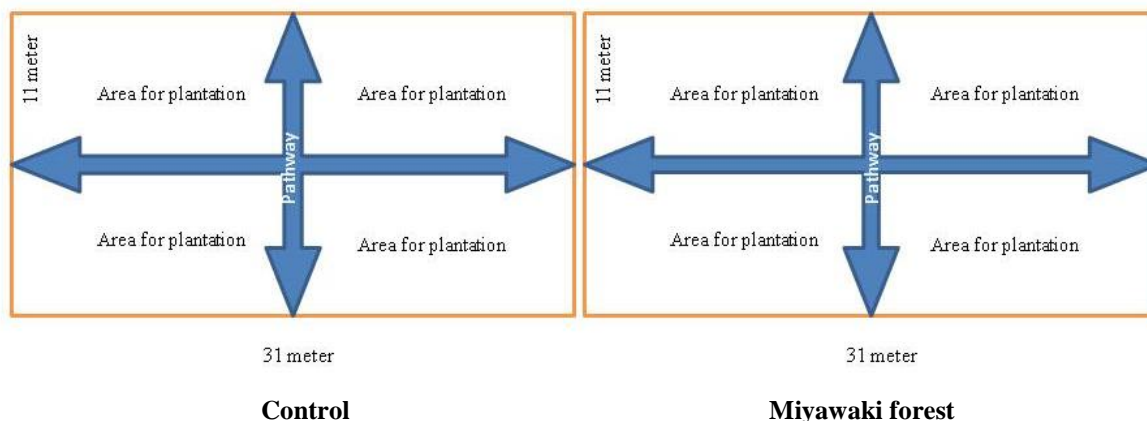
The plant species were collected from local nurseries, Vijaya nursery, Sivakasi and Sivasakthi Nursery, Srivilliputhur, Virudhunagar (Dt) Tamilnadu, India. Few species are collected from local areas of Sivakasi and Sattur, Virudhunagar (Dt) Tamilnadu, India.

Saplings selected to plant in miyawaki forest are *Pongamia pinnata*, *Terminalia catappa*, *Azadirachta indica*, *Albizia lebbeck*, *Ficus religiosa*, *Thespesia populnea*, *Syzygium cumini*, *Ricinus communis*, *Mangifera indica*, *Madhuca longifolia*, *Mimusops elengi*, *Pterocarpus santalinus*, *Caesalpinia pulcherrima*, *Tectona grandis*, *Tecoma stans* and *Bauhinia variegata*. Totally 109 saplings were collected.

For the control area, the saplings like *Azadirachta indica*, *Albizia lebbeck*, *Ficus religiosa*, *Thespesia populnea*, *Mimusops elengi*, *Pterocarpus santalinus*, *Caesalpinia pulcherrima*, *Tectona grandis*, *Tecoma stans* and *Bauhinia variegata* were selected. Totally 10 saplings were collected.

2.4 Preparation of land for planting

The area was first cleaned, ploughed, weeds removed and disposed. The cleared area was marked with wooden pegs for demarcation. In the selected area, pathway is created from the four sides of the area as shown in the Fig 2.



Control Miyawaki forest

Fig 2: Layout of control and miyawaki forest area

In the control area, pits were dug at a distance of 12 feet in all directions whereas in miyawaki forest, it was 2 feet in all directions. Perforator, organic fertilizer and water retainer per mixed in equal proportion and added to the pits. Rice husk, bio compost and coco peat are used as perforator, organic fertilizer and water retainer respectively.

2.5 Planting of saplings

After the filling of necessary materials in the pits, the sapling are planted and covered with soil. These materials help to loosen and aerated the soil after planting. The saplings are watered well twice a day at the rate of 3 liters approximately to each sapling. In the later days, the plants are watered regularly once a day through pipes.

The planted saplings are serially numbered and their growth is recorded periodically on 30th, 90th and 120th day from the day of planting. Weeding is done to the extent possible in the earlier days and in later days, growth of saplings naturally controlled the weed growth.

The forest area is kept clean, free from paper, plastics, chemical fertilizers, pesticides and insecticides. If insects or pest are noticed they are left undisturbed to provide a natural environment. Supporting sticks are placed to the needed plants. Proper draining is done to avoid water accumulation. No other special care is taken for the tree saplings of Miyawaki forest.

Control is maintained with 10 saplings parallel to the Miyawaki forest. Same procedure of planting and maintenance was adopted.

2.6 Soil parameters analysis

The soil parameters like pH, moisture, acid insoluble, water soluble, texture, electrical conductivity, specific gravity, organic content, nutrient content were determined following the methods of Subash chandrabose *et al.*, (1988). Aluminium, magnesium, calcium, total nitrogen, phosphorus, potassium was also estimated following the methods of Terry J. Hanson and Kerla M. Smetana (1975).

2.7 Isolation of microbes from the Miyawaki forest soil

The soil samples were randomly collected from the selected area. Serial dilution was done to isolate the microbes present in the soil. 10⁶ to 10⁹ dilutions were used to isolate the microbes in the soil. Muller Hinton medium was used as the growth medium. Two morphologically varied organism were isolated and identified by isolating, quantifying and sequencing the DNA. Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystem) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystem, USA) following manufactures protocol. The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for 2 minutes followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for 4 minutes.

III. RESULTS AND DISCUSSION

In the miyawaki forest area, 16 different species of tree saplings were planted which includes flowering, timber yielding, ornamental and medicinal trees. Totally 109 saplings were planted very closely at a distance of 2 feet (Table 1; Fig 3).



Fig 3: Preparation and planting of saplings in Miyawaki forest

In the control area, totally 10 saplings were planted at a distance of 12 feet (Table 2; Fig 4).



Fig 4: Control area

Planting was done randomly without any categorization of tree saplings. The saplings after planting were individually given sapling code. The height of each sapling was measured and recorded.

Table 1: Details of the saplings planted in Miyawaki forest

S. No	Common name	Botanical name	Tamil name	No. of saplings planted
1	Pongamia	<i>Pongamia pinnata</i>	Pungai maram	42
2	Terminalia	<i>Terminalia catappa</i>	Vadam maram	7
3	Azadirachta	<i>Azadirachta indica</i>	Vembu	12
4	Albizia	<i>Albizia lebeck</i>	Vagai maram	5
5	Ficus	<i>Ficus religiosa</i>	Arasa maram	3
6	Thespesia	<i>Thespesia populnea</i>	Poovarasu	5
7	syzygium	<i>Syzygium cumini</i>	Naaval maram	11
8	Ricinus	<i>Ricinus communis</i>	Amanakku	1
9	Mangifera	<i>Mangifera indica</i>	Ma maram	1
10	Madhuca	<i>Madhuca longifolia</i>	Illuppei	2
11	Mimusops	<i>Mimusops elengi</i>	Magila maram	4
12	Pterocarpus	<i>Pterocarpus santalinus</i>	Chemaram	5
13	Caesalpinia	<i>Caesalpinia pulcherrima</i>	Mayilkonrai	5
14	Tectona	<i>Tectona grandis</i>	Tekku maram	1
15	Tecoma	<i>Tecoma stans</i>	Manjarali	1
16	Bauhinia	<i>Bauhinia variegata</i>	Mantharai	5

Table 2: Details of the saplings planted in control area

S.No	Common name	Botanical name	Tamil name	No. of Saplings Planted
1	Azadirachta	<i>Azadirachta indica</i>	Vembu	1
2	Albizia	<i>Albizia lebeck</i>	Vagai maram	1
3	Ficus	<i>Ficus religiosa</i>	Arasa maram	1
4	Thespesia	<i>Thespesia populnea</i>	Puvarasu maram	1
5	Mimusops	<i>Mimusops elengi</i>	Magila maram	1
6	Pterocarpus	<i>Pterocarpus santalinus</i>	Chemaram	1
7	Caesalpinia	<i>Caesalpinia pulcherrima</i>	Mayilkonrai	1
8	Tectona	<i>Tectona grandis</i>	Tekku maram	1
9	Tecoma	<i>Tecoma stans</i>	Manjarali	1
10	Bauhinia	<i>Bauhinia variegata</i>	Mantharai	1

The growth of the plants was measured accurately and recorded (Fig 4, 5, 6). Among the planted tree saplings *Albizia lebeck*, *Ricinus communis* followed by *Caesalpinia pulcherrima* showed good growth. These plants are found to be more suitable for the selected geographical location to develop miyawaki forest.

Moderate growth was observed in *Pongamia pinnata*, *Azadirachta indica*, *Ficus religiosa*, *Thespesia populnea*, *Syzygium cumini*, *Mangifera indica*, *Madhuca longifolia*, *Mimusops elengi*, *Tectona grandis* and *Tecoma stans*.

Terminalia catappa, *Pterocarpus santalinus*, *Caesalpinia pulcherrima* and *Bauhinia variegata* showed comparatively lower growth than the other plant species.

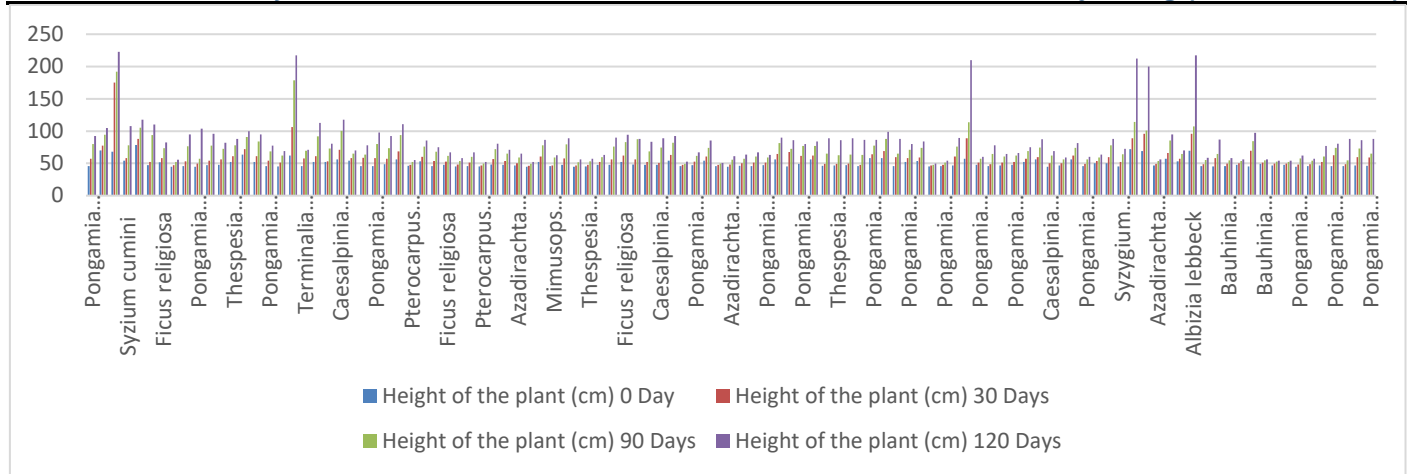


Fig 4: Plant growth analysis of saplings planted in miyawaki forest area

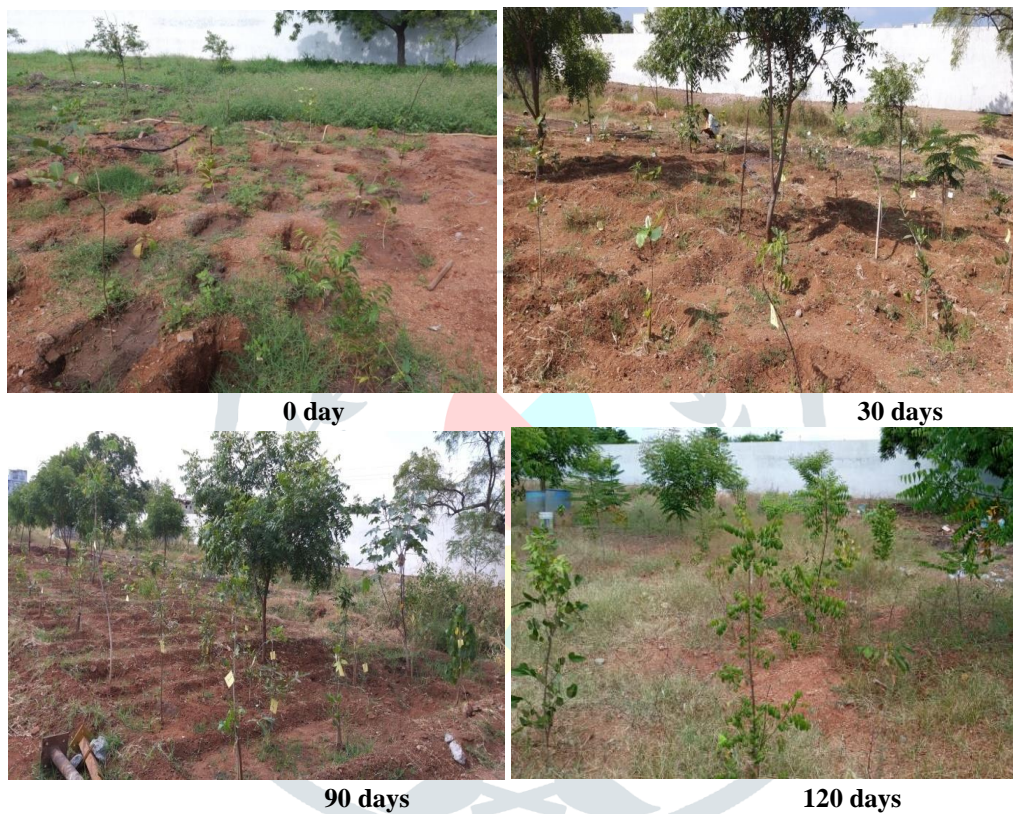


Fig 5: Plant growth in miyawaki forest



Fig 6: Visitors to Miyawaki forest

In control, *Albizia lebeck* showed good growth followed by *Caesalpinia pulcherrima*, *Thespesia populnea*, *Pterocarpus santalinus*, *Mimusops elengi*, *Azadirachta indica*, *Ficus religiosa*, *Tecoma stans*, *Tectona grandis* and *Bauhinia variegata* (Fig 7).

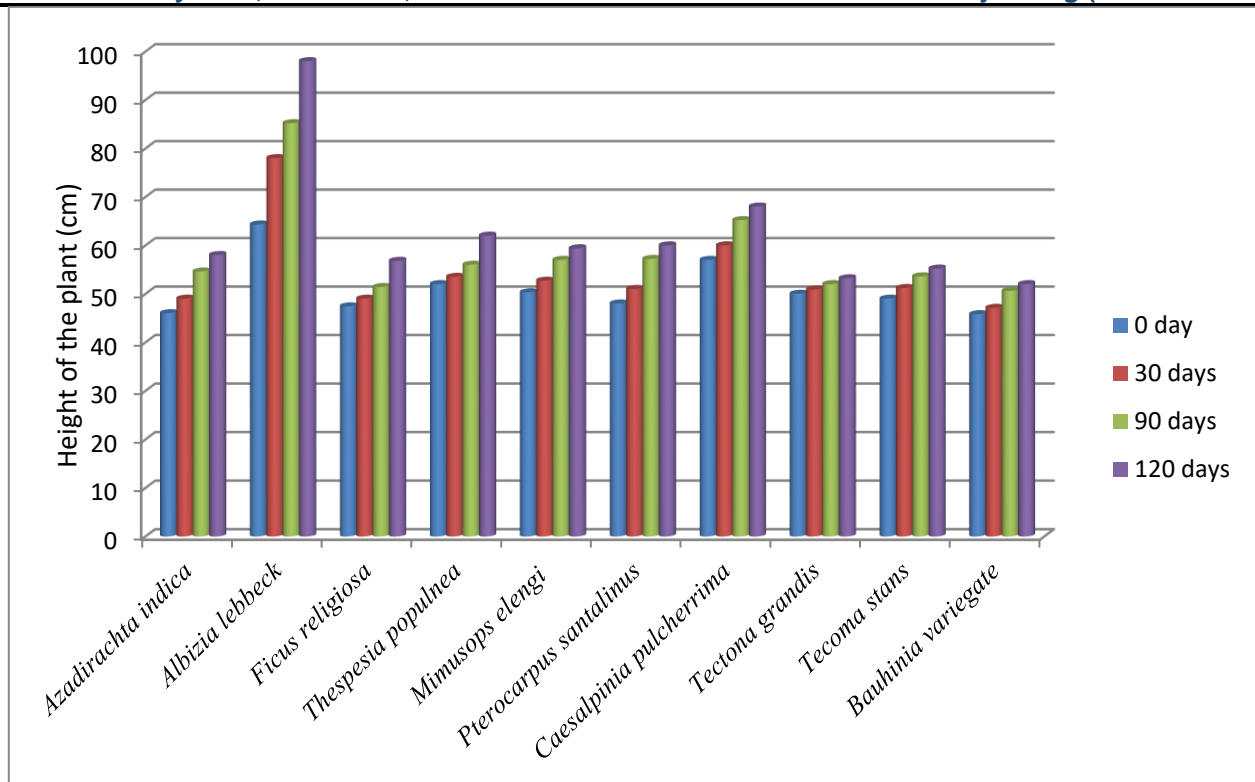


Fig 7: Plant growth analysis of saplings planted in control area
Compared to miyawaki forest saplings growth of the control area saplings showed low growth.

3.1 Soil parameters analysis

pH was found to be alkaline in both miyawaki and control soils. Comparatively, pH was higher in miyawaki forest soil than control. After the plant growth, pH was found to be reduced in both control and trial area. Soil pH reduction was found to be more in control than trial forest area. It is reported that, soil receiving less rainfall will have alkaline pH. Soil with alkaline pH will have more base than H. Nutrients like Calcium, magnesium and potassium will be more in alkali soils. These elements will split water molecules and help the plants to absorb nutrients (Jarrod Ottis Miller, 2016). Soil moisture content was found to be equal in almost all the soil samples studied. Soil moisture content is essential to determine the agronomic, hydrological and meteorological processes at all spatial scales. It also helps in water stress detection (Pavan *et al.*, 2018).

Acid insoluble content was found to increase more in Miyawaki forest soil than control. Miyawaki forest soil showed high percent of water soluble content (0.91%) after plantation. Presence of acid insoluble and water soluble content indicates the presence of mineral and nutrients (Singh *et al.*, 2017). Increase in the percentage of acid insoluble and water soluble content in the selected soil samples indicates the deposition of soil mineral and nutrient content. This on a long term improves the soil fertility leading to enriched ecosystem.

Bulk density was higher in Miyawaki forest area soil is reduced after plantation. Electrical conductivity influences the bulk density hence, as the electrical conductivity is more in miyawaki forest the bulk density is also high (Pavan kumar Sharma *et al.*, 2018).

The soil of the selected areas was found to be sandy clay. The Electrical conductivity was high in control than the trial area. Electrical conductivity was found to be lower in control after the growth of the saplings whereas in miyawaki forest, electrical conductivity was low in the soil before plantation but increased after sapling growth. This might be due to increase in bulk density of the soil.

Significant difference was not observed in the specific gravity of the selected soil samples.

Soil organic carbon content was found to be increased after the saplings growth in miyawaki forest whereas in control no change in the quantity of organic carbon was detected. It is reported that, soil organic carbon is the tool for maintaining fertility of the soil. Organic carbon holds the nitrogen, phosphorus and other nutrients of the soil. It also supports in increasing the water holding capacity, exchange of gases and root growth (Suzanne and Linda, 2018). This must have supported the appreciable growth of the saplings in miyawaki forest. In control, there is no increase of organic carbon content that might have delayed the growth of saplings in the control area.

Lime content was found to get reduced after the plant growth. It is reported that, reduction of lime in the soil makes the soil acidic and splits the nutrients in the soil (Ramachandran *et al.*, 2016). The lime content might have reduced due to the utilization of the plants for its growth (Table 3).

Table 3: Soil parameters analyzed

S.No.	Parameters studied	Control		Miyawaki forest	
		Before plantation	After plantation	Before plantation	After plantation
1	Soil pH	7.81	7.56	7.93	7.83
2	Soil moisture content (%)	8	8	8	8
3	Acid insoluble (%)	0.60	0.78	0.42	0.81
4	Water soluble (%)	0.82	0.86	0.76	0.91
5	Bulk density (%)	300	302	305	301
6	Soil texture	Sandy clay	Sandy clay	Sandy clay	Sandy clay
7	Electrical conductivity (μ S)	312	255	248	293
8	Specific gravity (G)	3.58	3.58	3.50	3.50
9	Organic carbon (%)	1.0	1.0	1.10	1.14
10	Lime status (%)	1.0	0.6	1.0	0.4

3.2 Nutrient Analysis

3.2.1 Qualitative nutrient analysis of the selected soil samples

In the selected areas, aluminum and calcium were found to be present (Table 4). It is stated in the earlier studies that, soil which is alkaline in nature will contain predominantly aluminum, calcium and phosphorus and is proved in this study. It is also reported that, presence of aluminum in acidic soil will cause plant toxicity (Jarrod Ottis Miller, 2016). Since it is alkaline soil, plant toxicity might not have occurred. Magnesium was found to be absent in both the selected soil samples.

Table 4: Qualitative nutrient analysis of the selected soil samples

S.No.	Element studied	Control		Miyawaki forest	
		Before plantation	After plantation	Before plantation	After plantation
1.	Aluminum	+	+	+	+
2.	Magnesium	-	-	-	-
3.	Calcium	+	+	+	+

3.2.2 Quantitative nutrient Analysis of selected soil samples

Quantitative estimation of nitrogen, phosphorus, potassium and calcium was done (Table 5). Soil nutrients like Nitrogen, phosphorus and potassium forms the basis for plant growth and development (Stromberger *et al.*, 2015). The plant growth has increased by the increase in soil nutrient content. Comparatively, miyawaki forest soil showed more amount of nutrients than the control.

Table 5: Quantitative nutrient analysis of selected soil samples

S.No	Nutrients present (per 10 g of soil)	Control		Miyawaki forest	
		Before plantation	After plantation	Before plantation	After plantation
1	Nitrogen (%)	1.4	1.5	1.4	1.9
2	Phosphorus (%)	0.5	0.6	0.5	0.8
3	Potassium (%)	0.06	0.10	0.06	0.13
4	Calcium (%)	0.76	0.78	0.75	0.8

3.2.3 Isolation and identification of soil microbes in the selected soil samples

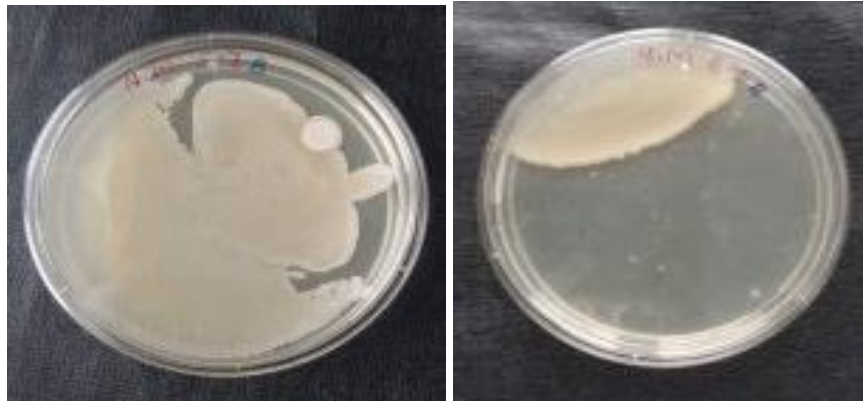
Two different species of microbes were isolated from the selected soil before and after the growth of the saplings. The isolated microbes were identified as *Paenibacillus jamilae* and *Paenibacillus polymyxa* (Fig 8). Molecular identification is done to identify the microbes. (Table 6).

It is reported that, *Paenibacillus* species can improve plant growth directly by nitrogen fixation, phosphate solubilization, phytohormone indole-3-acetic acid production and release of siderophores which enable iron absorption. They can also protect plants against insects, herbivores and phytopathogens like bacteria, fungi, nematodes, and viruses. They produce number of antimicrobials and insecticides, and stimulate hypersensitive defensive response of the plant which is known as induced systemic resistance (ISR) (Elliot Nicholas Grady *et al.*, 2016).

Paenibacillus jamilae has the capacity to biofloculate heavy metal ions and acid dyes so pollutants including heavy metal ions and dyes can be removed over a wide pH range (Li O *et al.*, 2013).

Xiaohui Wang *et al.*, 2019 reported that, *Paenibacillus polymyxa* has the ability to prevent plant diseases.

Activity of these microbes together has contributed to the good growth of the saplings and fertility of the soil.



Paenibacillus jamilae

Paenibacillus polymyxa

Fig 8: isolated and identified microorganisms

Table 6: Isolation of microbes from the selected soils

S.No	Soil microbes	Control	Miyawaki forest
1.	<i>Paenibacillus jamilae</i>	+	+
2.	<i>Paenibacillus polymyxa</i>	-	+

Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using MEGA 7.

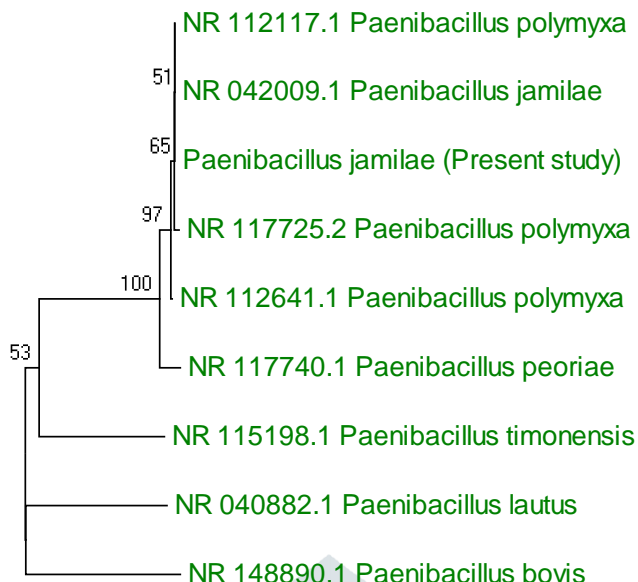
BLAST analysis

The amplified sequences belong to 16S rRNA were confirmed by similarity index built in the NCBI's BLAST program. Based on the higher percentage similarity against the reference species, the species utilized in this study was assigned as following species.

>S1,A,B – *Paenibacillus jamilae*

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GCCTAATACATGCAAGTCGAGCGGGGTTAGTTAGAAGCTTGCTTCTAACTAACCTAGCGGCGGACGGGTGAGTAA
CACGTAGGCAACCTGCCACAAGACAGGGATAACTACCGGAAACGGTAGCTAATACCCGATACATCCTTTTCCTG
CATGGGAGAAGGAGGAAAGGCCGAGCAATCTGTCACTTGTGGATGGGCCTGCGGCGCATTAGCTAGTTGGTGGG
GTAAAGGCCTACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC
CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCCGCTGA
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ACCG
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Phylogenetic tree

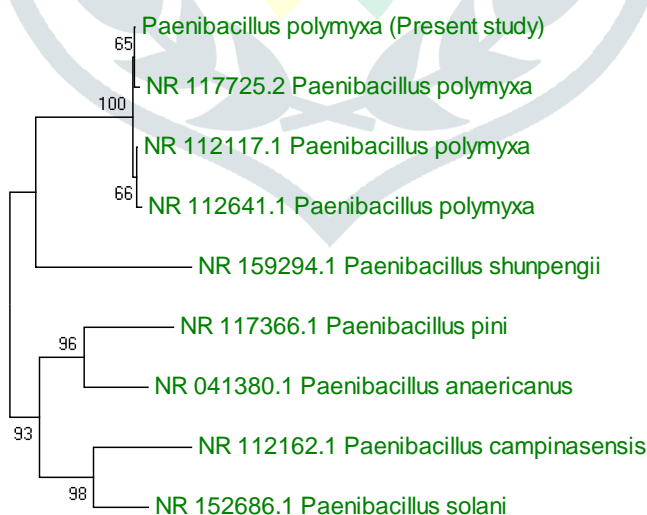


0.0050

>S2A,B – *Paenibacillus polymyxa*

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 ACCTGCCCACAAGACAGGGATAACTACCGGAAACGGTAGCTAATACCCGATACATCCTTTTCCTGCATGGGAGAAG
 GAGGAAAGGCGGAGCAATCTGTCACTTGTGGATGGCCTGCGGCGCATTAGCTAGTTGGTGGGGTAAAGGCCTAC
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 ATGTGGTTTAATTCTGAAGCAACGCG

Phylogenetic tree



0.0050

CONCLUSION

Miyawaki forest area saplings showed better growth compared to control area saplings. Soil physico-chemical parameters like pH, moisture content, acid insoluble, water soluble, bulk density, soil texture, electrical conductivity, specific gravity, organic carbon, lime status content were estimated which determines the quality of soil and in turn the plant growth. Soil nutrient analysis was carried out to identify the impact of plant growth on soil fertility. Nutrients like Aluminum, Magnesium, Calcium were qualitatively estimated and nutrient like Nitrogen, Phosphorus, Potassium, Calcium were quantitatively estimated. The results showed that, the soil in miyawaki forest area contained higher quantity of nutrients after plant growth than before plantation and control. This might have improved the growth of the saplings and viceversa. Microbial analysis of the soil samples showed the

presence of *Paenibacillus jamilae* and *Paenibacillus polymyxa* and is confirmed by molecular studies. These organisms play the vital role for nitrogen fixation and soil fertility. From the study, it is obvious that the soil fertility is enriched by plant growth and viceversa. So, development of such miyawaki forests definitely ensures the restoration of the deteriorating ecosystem.

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