

The production of Rhizobium, Bio-fertilizer in the treatment to enhance the growth of commercially important Padauk and Pyinkado seedlings

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ပိတောက်နှင့်ပျဉ်းကတိုးပျိုးပင်များကြီးထွားမှုမြန်စေရန်
ရိုက်ဇိုဘီယမ်အကျိုးပြုဘက်တီးရီးယားကိုခါတ်ခွဲခန်းတွင်မွေးမြူ၍
သဘာဝမြေဩဇာအဖြစ်ထုတ်လုပ်အသုံးပြုနိုင်ရန်စမ်းသပ်ခြင်း

စောရန်အောင်စိခူး(ပါမောက္ခ-ငြိမ်း)(မဟာသိပ္ပံ၊ ဟာဝိုင်ယီတက္ကသိုလ်)
ဝေဝေသန်း၊ သုတေသနအရာရှိ(မဟာသိပ္ပံ၊ သစ်တောတက္ကသိုလ်၊ ရေဆင်း)
မိုင်လူလူဖြေ၊ (စိုက်ပျိုးရေးဘွဲ့၊ စိုက်ပျိုးရေးတက္ကသိုလ်၊ ရေဆင်း)

စာတမ်းအကျဉ်းချုပ်

သစ်တောသုတေသနဌာန၊ အပင်ရောဂါဗေဒခါတ်ခွဲခန်းတွင် ရိုက်ဇိုဘီယမ် ဘက်တီးရီးယားကို မွေးမြူပွားများကာ ပိတောက်နှင့်ပျဉ်းကတိုးမြေတွင် ထည့်သွင်း၍ ပျိုးပင်များကြီးထွားမှုနှုန်း မြန်စေရန် စမ်းသပ်ပါသည်။ (၄)လသားပျိုးပင်များတွင် နိုက်ထရိုဂျင်အမြစ်ဖု ဖြစ်ပွားမှုနည်းသော်လည်း အမြစ်ဖု အရေအတွက်မှာ ပျိုးမြေ၌ရိုက်ဇိုဘီယမ် ထည့်သွင်းက ပိုမိုများပြားကြောင်း တွေ့ရှိရပါသည်။ ခါတ်ခွဲခန်း လေ့လာချက်အရ ရိုက်ဇိုဘီယမ် ဘက်တီးရီးယားသည် တိုသောအချောင်းငယ်ဖြစ်၍ လှုပ်ရှားမှုရှိပါသည်။ ပျိုးပင်ကြီးထွားမှုမှာလည်းရိုက်ဇိုဘီယမ်ပါသောပျိုးပင်က ကြီးထွားမှုပိုမိုကောင်းမွန်ကြောင်း တွေ့ရှိရပါသည်။ ကြီးထွားမှုနှင့် နိုက်ထရိုဂျင်အမြစ်ဖုဖြစ်ပေါ်ခြင်း ဆက်စပ်မှုကိုလေ့လာရာ ဆက်စပ်မှုရှိကြောင်း လေ့လာ တွေ့ရှိရပါသည်။

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Abstract

A small scale production of rhizobium bio-fertilizer was produced in the laboratory to enhance the growth of padauk and pyinkado seedlings. Nodules oven dry weight from inoculated seedlings were more than non-inoculated seedlings. At the age of four months old seedlings were less in nodule formation than older seedlings. Rhizoid investigations on the slides were short rod, aerobic and motile. Comparisons of growth of the two species in rhizobium inoculated were highly significant. The nodulation and growth were highly correlated in pyinkado and padauk. However, nodulation in padauk was observed extreme value in 4 months old seedlings.

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1. Introduction

Myanmar has a total land area of 67,657,700 ha, on which about 50% are still forested. In the natural forests, the composition of Pyinkado (*Xylia xylocarpa*) is 5.66% and Padauk (*Pterocarpus macrocarpus*) is 0.58 % (FD,2006).

In plantations, the percent of Pyinkado plantation is 6.8 and the percent of Padauk is 1.9 respectively (MKKG & ZWM, 2008). They are in the list of group (I) according to the grouping schedule of Myanmar Timber Enterprise (MTE) and are economically important as like as teak.

They are environmentally friendly N₂ fixation tree species. It should be noted that the plantations of these two species should be established in a large scale. For this, it is necessary to get the large quantity and quality of seedlings in the nursery operations for the plantation establishment. Therefore, the primary goal of this research is to produce good healthy seedlings for the establishment of plantation.

In Myanmar, biological fertilizers are producing from the agricultural sector by using leguminous crops and widely used. In forestry sector, some leguminous tree species have N₂ fixation associated with root nodules bacteria to produce biological fertilizer. These bacteria live on the roots of the leguminous plants and transfer atmospheric nitrogen to be utilized by the plants, and thus reducing the legume's reliance on soil nitrogen and nitrogenous fertilizer. Although the bacteria can infect the legume roots, the effectiveness of the association is dependent on the N₂ fixing efficiency of the rhizobial strain, the N₂ fixing capacity of the legume host and the environment (Burton, 1981).

These N₂ fixing bacteria can be cultured in the laboratory. In the other hand, chemical fertilizers are very expensive, economically inefficient in long term and the fertility of the soil can be gradually declined. Therefore, in forestry sector the investigation of biological fertilizer to enhance the growth of commercially important leguminous species is needed.

The main objective is the investigation of biological fertilizer to enhance the growth of commercially important leguminous species. The specific objectives are (1) to apply at the forest nurseries (2) to obtain healthy plants. (3) to substitute rhizobium biofertilizer in the forestry sector instead of using chemical fertilizer.

2. Literature Review

2.1 Characteristics of Rhizobia

Rhizobia are soil bacteria characterized by their unique ability to infect root hairs of legumes and include effective nitrogen-fixing nodules to form on the roots. (NifTAL,1984). Rhizoid are short to medium Gram-negative rods. Rhizobia are living one-celled microorganisms and will die if the temperature is too high or if there is insufficient moisture. They are aerobic and motile. They multiply by simple cell division (NifTAL, 1984).

2.2 Growth rate

Fast growing forms produce medium or large colonies (1 to 5 mm) after three to five days at 25°-28°C. Colonies of slow-growing rhizobia will be barely detectable at three to five days and even after ten, will commonly produce colonies not exceeding 1 mm (Vincent, 1982). The optimal growth temperature is 28 to 30°C (NifTAL, 1984)

2.3. Measuring the effectiveness of nodules

Measurements have been based on pattern of nodulations, nodules dry weight, colour and plant N content (Erdman and Means, 1952; Halliday, 1984).

2.4 Formation and distribution of nodules

Effective nodules are generally large and are clustered on the primary and upper lateral roots (NifTAL, 1984). Generally, effective strain nodules both tap and lateral roots, but ineffective strain primarily nodulate the lateral roots (Allen and Allen, 1940; Saric, 1963). Nodulation, especially large nodules, on the tap root is desirable since it indicates early infection and onset of N₂ fixation (Allen and Allen, 1940). Total nodule mass formed by effective rhizobia and the quantity of N₂ fixed is linearly related (Zary et al., 1978; Wadisirisuk and Weaver, 1985).

2.5 Nodule color

An effective nodule has a relatively large red inner region due to the presence of leghaemoglobin, which is positively correlated with the quantity of N₂ being fixed (Vincent, 1974; Graham and Parker, 1961). The red haemoglobin in effective nodules breaks down to green legcholeglobin as the nodules become older and senescent (NifTAL, 1984). Ineffective nodules are white to pale green on the inside and they do not change colour as they age (NifTAL, 1984).

2.6 Inoculants and Inoculation

In many soils, the nodules bacteria are not adequate in either number or quality. Under these conditions, it is necessary to inoculate the seed or soil with highly effective rhizobia cultures.

Nodules bacteria (*Rhizobium* spp.) are cultured in the laboratory and mixed with a suitable carrier material, such as peat to make an inoculant, nodule development at four to five weeks should reveal adequacy of the inoculation treatment. The process of adding this inoculant to seed on soil is called inoculation (NifTAL, 1984).

2.7 Types of inoculants

NifTAL, 1984 mentioned that legume inoculants are of two general types: those designed for application to seeds and those designated for application directly to soil. Seed inoculants are the most common because they are easy to apply and are generally effective under normal conditions. Application of inoculants directly to the soil may be necessary to obtain effective nodulation when sowing legume seeds in hot, dry or highly acidic soil or under adverse weather conditions.

2.8 Carriers

Preparation of high quality legume inoculants is dependent on selection of suitable carrier materials and their pre-treatment. Peat soil has organic matters highly and is widely used as carrier material for rhizobia (Vincent, 1982). Peat has been the most commonly used basis for commercial inoculants, but some peat does not meet the requirements of a good carrier. Carriers must have a high water holding capacity, provide a nutritive medium for growth of rhizobia and enhance survival during distribution and when inoculated onto seed.

The type of peat affects the number of developing rhizobia and their survival during storage. The qualities of good carrier materials are; (1) highly absorptive and easy to process (2) non-toxic to rhizobia (3) easy to sterilize (4) available in adequate amounts (5) inexpensive (6) good adhesion to seeds.

3. Materials and Methods

Experiments were carried out at the laboratory of Forest Protection, Forest Research Institute, Yezin from April 2005 to January 2007.

3.1 Collection of Rhizobial trees and Seeds

Two kinds of family Fabaceae tree species Padauk (*Pterocarpus macrocarpus*) and Mimosaceae tree species Pyinkado (*Xylia xylocarpa*) were selected randomly from the nursery of FRI. Each 3 years old healthy seedling of padauk and pyinkado were used to collect their nodules among the ten seedlings of the two species. With a spade, describe a circle with a radius of approximately 15 cm around the plant and cut out the section to a depth of at least 20-20cm or deeper. Carefully removed the soil and washed the clump gently. Collected the nodules from their roots to examine bacteroides and isolate in the Forest Pathology laboratory.

The seeds of *P. macrocarpus* and *X. xylocarpa* were bought from the seed collector of Pyinmana to plant as seedlings to make an inoculation.

3.2 Slides examination

Smear of Rhizobium from Pyinkado and Padauk were spread in thin layers slides to examine under the light microscope. The characteristic of rhizoid presence in the nodules were noted.

3.3 Isolation of Rhizoid Nodule

The effective of nodules (2 or 3) from collected plants of Pyinkado and Padauk were cut and sterilized in Hg C₁₂ (1:1000) for 1 minute and alcohol for 1 minute. Then the disinfectants were washed off by transferring into sterilized water for 4 - 5 times at 1 minute in each time. The nodules were teased with glass rod in a petridish to get smear. The smear was diluted into (-7) suspensions and isolation on the Yeast Manitol Agar Media (Appendix). After 3-7 days, the pure colonies were transferred into the test- tube which contained slant agar media.

3.4 Preparation of Pure Culture/Fermentation of Bacteria

After the test-tube preparation, a bit of growth from the bacterial culture was mixed with YM broth (Vincent, 1970) in 125 ml conical flask. Shaker was used with 1.5 RPM x 100 for rhizobium. One milliliter bacterial liquid from the 125 ml conical flask was transferred into the YM broth of 1000 ml conical flask. It was shaken for 14 days.

3.5 Germinating of Seeds

Seeds of Padauk and Pyinkado were germinated enough to obtain seedlings in the seedbed for 1 week or until the true leaf emerged.

3.6 Preparation of Seedling Bags

Peat soil from Pyn Oo Lwin was used as a carrier for growing of rhizoid. Remove debris and sifted the soil using a 0.5 cm mesh screen. Nursery soil (sand 1: manure 2: soil 3) used in the experiment was sterilized at 120 °C with pressure 15 lb for 20 minutes. Peat soil and soil mixture were tested the ratio of OM and p^H at soil lab, FRI.

3.7 Inoculums Preparation

Peat soil was kept in 6" x 8" plastic bag and sterilized them. 50 ml of fermented bacterial broth was injected into each of 100gm of sterilized peat packet. The inoculated packets were stored at 25-29°C until it was used in the experiment. It can be stored even up to six months before using.

The sterilized soil was mixed with the inoculated peat soil packet at the ratio of about 2800 gm (1 pyi) per 1 packet. The seedlings of Padauk and Pyinkado from the seedbed were transferred into the 3 x 7 inches seedling bags. They were placed in the mist chamber of the FRI. It had 25-30 °C and seedlings were watered daily.

Seedlings heights, , nodules color and nodules oven dry weight (80°C, 24 hours) were recorded starting at the stage of four months old seedlings and continually measured to seven months old in monthly.

3.8 Statistical Analysis

Statistical Analyses were performed by GENSTAT computer software program and calculated by Two x Two Factorial Experiment, CRD design with three replications. Correlations were evaluated by r² value in regression line.

4. Results

4.1 Nodule examination of Padauk and Pyinkado

Nodules of Pyinkado were found on the lateral roots and tap root. Nodulation of Pyinkado was medium size, red color (Plate 1) and isolation period was observed in only 5 days at room temperature. Bacteria colonies were found fast growing and color is white or cream.

Nodules of Padauk were observed on the tap root and lateral roots. Its nodules sizes were larger than Pyinkado nodules and nodules color was yellow-brown (Plate 2). Isolation period was found in one week at room temperature and growing bacteria color was white or cream.

4.2 Rhizoid investigation

Rhizoid investigations on the slides were short rod, aerobic and motile. Rhizobium nodulation was found after 5 weeks inoculation. in pyinkado, however, the nodulation was late in padauk. (Plate 5) (Plate 6)

4.3 Soil testing

Sterilized peat soil had P^H 7.5 and 0.28% and organic matter 10.4 %. Nursery soil and peat soil mixture contained P^H 7.5 and organic matter 9.5%.

4.4 Growth of seedling

Inoculated seedlings were gradually higher than non-inoculated seedlings in the monthly measurement. (Plate 3&4). Comparisons of high were highly significant (0.001 level) in inoculated seedlings of pyinkado and padauk (Figure 1.), (Figure 2.) (Appendix) The high correlation between of nodulation and growth was observed in Pyinkado (Figure 5) and Padauk seedlings (Figure 6).

4.5 Nodules dry weight

Nodules oven dry weight of seedlings were significant between inoculated and non-inoculated seedlings (Appendix). However, it was noted that the nodule weight was very small in 4 months seedlings of padauk. It had extreme value, therefore some of variation were higher than normal variation.

5. Conclusion and Discussion

N₂ fixation depends on the strain of the host plants, the associated rhizobial strain and the environmental conditions such as soil type, soil P^H, organic matters, temperature, light, moisture stress, and, planting density influence nodulation (NFTA, 1987). Both root nodules bacteria are currently classified into only genera which cannot be studied to know their strains. Additional methods (molecular biology, serology, etc.) are not available at the FRI. Using soil type, soil p^H, organic matters and temperature of mist chamber only could be recorded and applied to obtain favorable nodulation as NFTA, 1987 mentioned. Vincent (1982) stated peat soil has organic matters highly and is widely used as carrier material for rhizobia. In the soil testing, P^H and organic matters of using soil were suitable carrier materials for rhizobia growing.

Total nodule mass formed by effective rhizoid and the quantity of N₂ fixed was linearly related (Zary et al., 1978; Wadisirisuk and Weaver, 1985). According to their descriptions, nodule formations were followed by inoculation of rhizoid, but the quantity of N₂ fixation should be studied in further.

Nodules formation could be found in non-inoculated seedlings, however, it was less than inoculated seedlings and also growth rate was differ from inoculated seedlings. In the plate 3 and 4, root hairs could be seen obviously and more in the inoculated seedlings, thus, it is easy to conclude that nutrient absorption of inoculated seedlings can be better than non-inoculated seedlings. The nodules could be found a few numbers in 4 months seedlings that mentioned the age is concerned with the nodulation.

Figure (5, 6) showed that the more nodulation, the higher growth of seedlings. highly significant and correlated (Appendix). However, padauk was observed extreme value in 4 months old seedlings, therefore it had high variation. If extreme values data to be omitted, variation can be less in analysis.

In Forestry, application of inoculation directly to the soil is commonly using method than seeds dressing with rhizobium. Therefore, which method is more effective should be compared between them.

This study is preliminary investigation of using biological fertilizer. Further investigation should be carried out rhizobium characteristics in detail. The objective of using bio-fertilizer is to substitute the chemical fertilizer for ensuring adequate nitrogen nutrition of legumes. Not only Forest Department will get the benefits of new technology for the production of quality seedlings but also next generation of the researchers can refer to study more from the research.

Plate 1. Nodules from 3 years old pyinkado seedling, FRI nursery

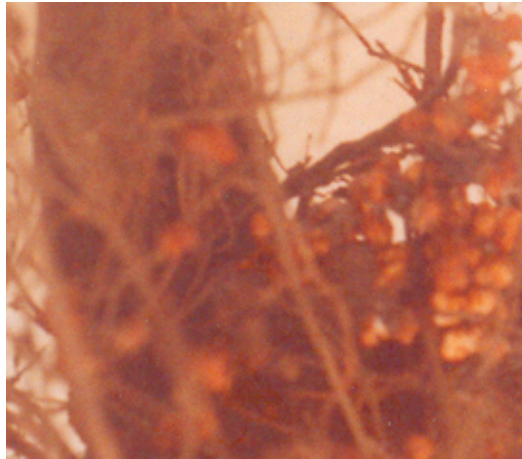


Plate 2. Nodules from 3 years padauk seedling, FRI



Plate 3. Comparison of height and nodulation of inoculated (t) and non-inoculated (c) with rhizobium of Pyinkado seedlings



Plate 4. Comparison of height and nodulation of inoculated (t) and non-inoculated (c) with rhizobium of Padauk seedlings



Plate 5. Rhizoid of pyinkado (400X)

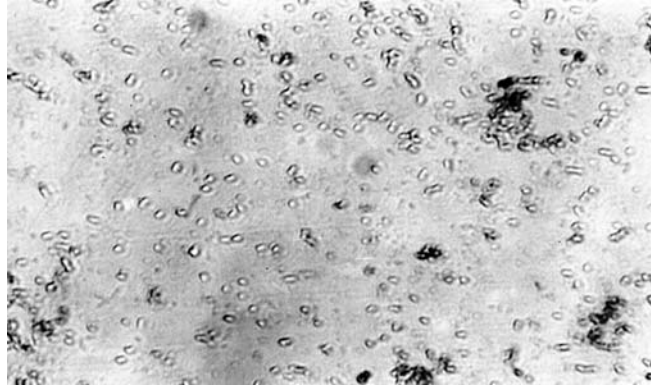


Plate 6. Rhizoid of padauk (400X)

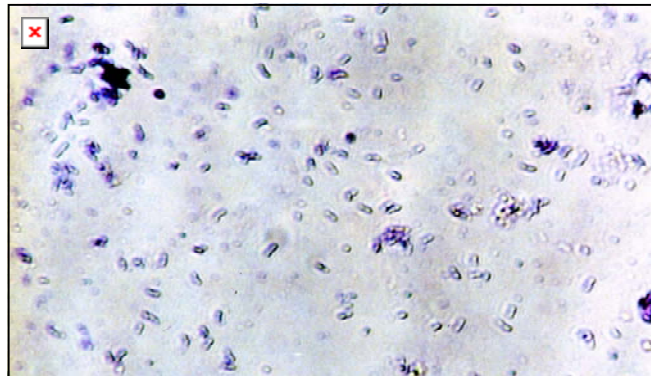


Figure 1. Comparison in growth with rhizobium treated and non-treated seedlings, pyinkado

Figure 3. Comparison in nodule oven dry weight with rhizobium treated and non-treated seedlings, pyinkado

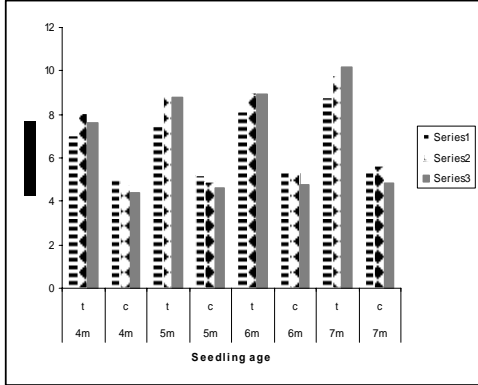


Fig. 1

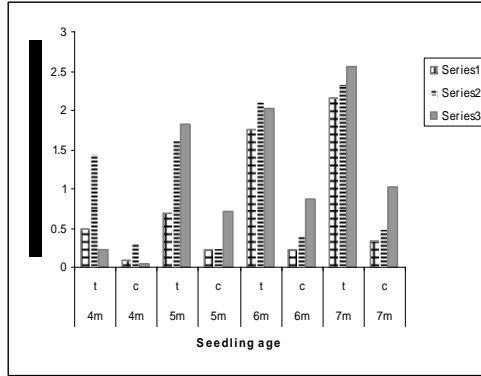


Fig 3.

Figure 2. Comparison in growth with rhizobium treated and non-treated seedlings, padauk

Figure 4. Comparison in nodule oven dry weight with rhizobium treated and non-treated seedlings, padauk

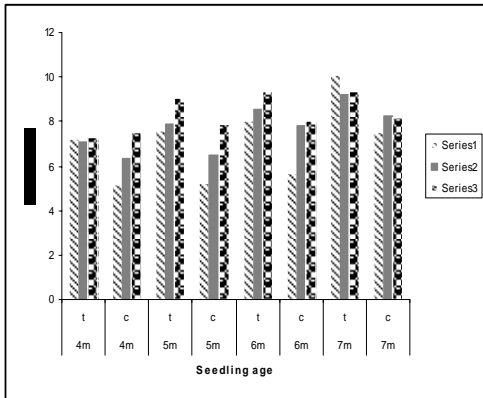


Fig 2.

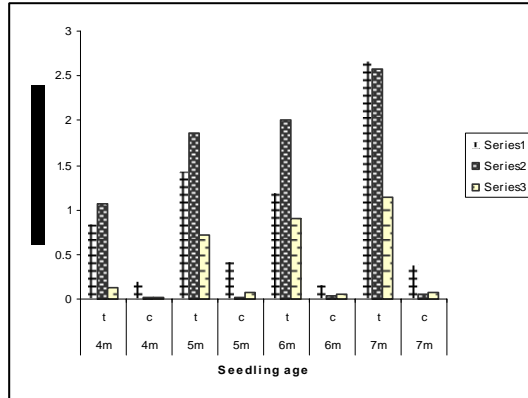


Fig.4

Figure 5. Corellation with nodulation and growth of pyinkado seedlings

Figure 6. Corellation with nodulation and growth of padauk seedlings

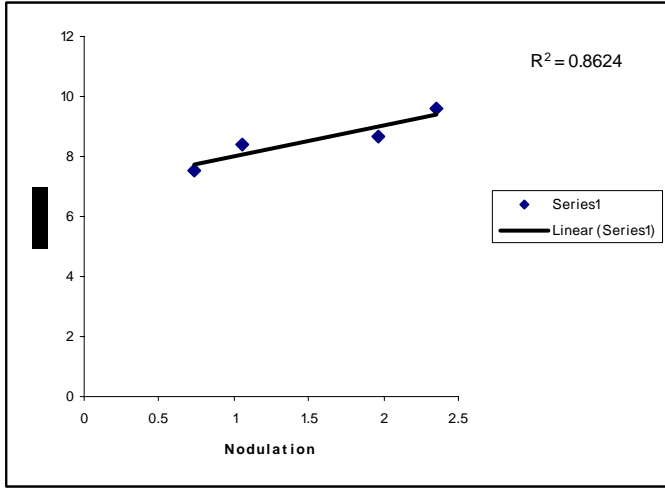


Fig 5.

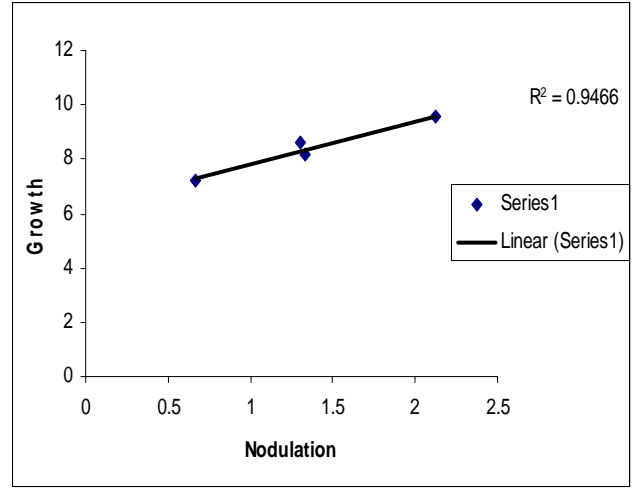


Fig.6

Appendix

Yeast Manitol Agar

Manitol	10 (gram)
K ₂ HPO ₄	0.5
MgSO ₄ .7H ₂ O	0.2
NaCl	0.1
Yeast	0.5
Agar	15
Congo red	0.25
Distilled water	1 liter

ANOVA

H4

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	1.1756	1.1756	2.69	0.140
B	1	10.3119	10.3119	23.59	0.001**
A.B	1	2.8188	2.8188	6.45	0.035
Residual	8	3.4976	0.4372		
Total	11	17.8040			

CV 10.2%

H4 height of 4 months old seedling

** highly significant

H5

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	1.4630	1.4630	1.94	0.202
B	1	19.5841	19.5841	25.92	<0.001**
A.B	1	2.6040	2.6040	3.45	0.100
Residual	8	6.0441	0.7555		
Total	11	29.6952			

CV 12.4%

H5 height of 5 months old seedling

** highly significant

H6

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	2.9701	2.9701	4.67	0.063
B	1	18.8752	18.8752	29.70	<0.001**
A.B	1	3.3391	3.3391	5.25	0.051
Residual	8	5.0849	0.6356		
Total	11	30.2692			

CV 10.8%

H6 height of 6 months old seedling
** highly significant

H7

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	5.2140	5.2140	19.79	0.002
B	1	26.1370	26.1370	99.22	<0.001
A.B	1	5.5897	5.5897	21.22	0.002
Residual	8	2.1074	0.2634		
Total	11	30.2692			

CV 6.3%

H7 height of 7 months old seedling
** highly significant

N4

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	0.0167	0.0167	0.10	0.765
B	1	1.0017	1.0017	5.74	0.043*<0.05
A.B	1	0.0018	0.0018	0.01	0.922
Residual	8	1.3956	0.1745		
Total	11	2.4158			

CV 101.2%

N4 oven dry weight of 4 months old seedling
* significant

N5

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	0.0528	0.0528	0.26	0.623
B	1	3.4949	3.4949	17.27	0.003**
A.B	1	0.0239	0.0239	0.12	0.740
Residual	8	1.6187	0.2023		
Total	11	5.1903			

CV 54.9%

N6 oven dry weight of 6 months old seedling

** highly significant

N6

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	0.7626	0.7626	6.48	0.034
B	1	5.6925	5.6925	48.38	<0.001**
A.B	1	0.0257	0.0257	0.22	0.653
Residual	8	0.9414	0.1177		
Total	11	7.4221			

CV 35%

N6 oven dry weight of 6 months old seedling

** highly significant

N7

Source of variation	d.f	s.s	m.s	v.r	F pr
A	1	0.3407	0.3407	1.46	0.261
B	1	10.2342	10.2342	43.89	<0.001**
A.B	1	0.0365	0.0365	0.16	0.703
Residual	8	1.8655	0.2332		
Total	11	12.4769			

CV 36.8%

N7 oven dry weight of 7 months old seedling

** highly significant

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