Khulna University
Life Science School
Forestry and Wood Technology Discipline

Author(s): Chanchal Kumar Biswas

Title: Seed germination and seedling growth performance of *Phyllanthus emblica* L. at nursery stage

Supervisor(s): Md. Abdul Matin, Professor, Forestry and Wood Technology Discipline, Khulna University

Programme: Masters of Science in Forestry

Seed Germination and Seedling Growth Performance of *Phyllanthus emblica L.* at Nursery Stage

MS THESIS

BY

Chanchal Kumar Biswas

FORESTRY AND WOOD TECHNOLOGY DISCIPLINE
KHULNA UNIVERSITY
KHULNA

2014

SEED GERMINATION AND SEEDLING GROWTH PERFORMANCE OF *Phyllanthus Emblica L.* AT NURSERY STAGE

MS Thesis

By

Chanchal Kumar Biswas

Student No.: MS-110506

Session: 2010-2011

Forestry and Wood Technology Discipline

School of Life Science

Khulna University, Khulna-9208

Bangladesh

SEED GERMINATION AND SEEDLING GROWTH PERFORMANCE OF *Phyllanthus emblica L.* AT NURSERY STAGE

Course title: Project Thesis
Course No: FWT-5112

This project thesis has been prepared and submitted for the partial fulfillment of professional degree of MS in Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh.

Supervisor

29/8/28

Md. Abdul Matin
Professor
Forestry & Wood Technology
Discipline
Khulna University
Khulna-9208

Prepared by
Chanchal Kumar Biswas
29/08/28

Chanchal Kumar Biswas
Student No: MS-110506
Forestry & Wood Technology
Discipline
Khulna University
Khulna-9208

Dedicated

To

Shril Prabhupada

&

My Beloved Parents

ACKNOWLEDGEMENT

All praises to Lord Krishna, whose mercy keeps us alive and enables us to pursue my education in Forestry and Wood Technology Discipline, Khulna University, Khulna and to complete my project thesis for the degree of Masters of Science in Forestry. Then I would like to express my gratitude to all of my family members specially my parents. Without their continuous inspiration, the present achievement would not be possible.

I also gratefully acknowledge my deepest sense of gratitude and sincere appreciation to my respectable supervisor professor Md. Abdul Matin and also grateful to Md. Salim Azad, Associate professor, Forestry and Wood Technology Discipline , Khulna University, Khulna for their scholastic guidance and advice, constructive criticism, cordial encouragement untiring help and their kind supervision.

I especially thank the nursery staff of FWT discipline for their assistance. I would like to thank Shohel, Biplob, Swapon, Uzzal and Hori for their cooperation.

Finally, I wish to offer especial compliments to all of my friends and well wishers, who gave me mental and materialistic support during completing this project thesis.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

ABSTRACT

Phyllanthus emblica L. is an important tree species for its multipurpose uses. It is native in Bangladesh. The objective of the present study is to find out the best pre-sowing treatment for getting highest germination percentage and to determine the seedling growth performance (height, diameter and leaf number) at nursery stage. The experiment was conducted in the nursery of Forestry and Wood Technology Discipline, Khulna University, Khulna and treated with four pre-sowing treatments (Control, Immersion in normal water for 24 hours, Immersion in normal water for 48 hours and Immersion in sun heated water for 10 hours). The germination was done in poly bags at the nursery. External morphology of fruits was studied in the nursery. The average weight per fruit was 14 ±0.79 gm. The average length and diameter per fruit were 14 ±1.4 mm and 20.35 ±0.35 mm respectively. On the other hand, each fruit contained 4-6 seeds and weight per 100g seeds containing 4000-4500 seeds. In case of germination, the highest success 75% was observed in soaking in normal water treatment for 24 hours followed by sun heated treatment for 10 hours 63.33% which was significantly different than the lowest 51.33% in control. In all the treatments, germination was completed within 7-13 days after sowing the seed. On the other hand, there is no significant difference in the seedling growth performance (height, diameter and leaf number) at nursery stage. Growth performance (height and diameter) showed strong positive correlation (r=0.99). Leaf number also showed a strong positive correlation with height (r=0.96) and diameter (r=0.97). Finally, normal water treatment for 24 h is suggested to obtain good germination percentage in rural Bangladesh. This finding provides important information for genetic resource conservation for future research.

CHAPTER ONE: INTRODUCTION

1.1 Botanical Description

Scientific classification
Kingdom: Plantae
Division: Flowering plant
Class: Magnoliopsida
Order: Malpighiales
Family: Phyllanthaceae
Tribe: Phyllantheae
Subtribe: Flueggeinae
Genus: Phyllanthus
Species: *P. emblica*

The tree is a graceful ornamental, normally reaching a height of 60 ft (18 m) and, in rare instances, 100 ft (30 m). Its fairly smooth bark is a pale grayish-brown and peels off in thin flakes like that of the guava. While actually deciduous, shedding its branchlets as well as its leaves, it is seldom entirely bare and is therefore often cited as an evergreen. The miniature, oblong leaves, only 1/8 in (3 mm) wide and 1/2 to 3/4 in (1.25-2 cm) long, distichously disposed on very slender branchlets, give a misleading impression of finely pinnate foliage. Small, inconspicuous, greenish-yellow flowers are borne in compact clusters in the axils of the lower leaves. Usually, male flowers occur at the lower end of a growing branchlet, with the female flowers above them, but occasional trees are dioecious. The nearly stemless fruit is round or oblate, indented at the base, and smooth, though 6 to 8 pale lines, sometimes faintly evident as ridges, extending from the base to the apex, give it the appearance of being divided into segments or lobes. Light-green at first, the fruit becomes whitish or a dull, greenish-yellow, or, more rarely, brick-red as it matures. It is hard and unyielding to the touch. The skin is thin, translucent and adherent to the very crisp, juicy, concolorous flesh. Tightly embedded in the center of the flesh is a slightly hexagonal stone containing 6 small seeds. Fruits collected in South Florida vary from 1 to 1 1/4 in (2.5-3.2 cm) in diameter but choice types in India approach 2 in (5 cm) in width. Ripe fruits are astringent, extremely acid, and some are distinctly bitter (Morton, 1987).

In India, it is common to eat gooseberries steeped in salt water and turmeric to make the sour fruits palatable. There are two varieties of Amla - cultivated (gramya) and wild (vanya). The wild amla is small, while cultivated amla is big, smooth and juicy. Chemical composition of the amla fruit contains more than 80% of water. It also has protein, carbohydrate, fiber and mineral and also contains gallic acid which is a potent poly-phenol. Vitamin C is important for human beings. The tree is small to medium sized, reaching 8 to 18 m in height, with a crooked trunk and spreading branches. The branch lets are glabrous or finely pubescent, 10-20 cm long, usually deciduous. The leaves are simple, sub sessile and closely set along branch lets, light green, resembling pinnate leaves. The flowers are greenish-yellow. The fruit is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with 6 vertical stripes or furrows. Ripening in autumn, the berries are harvested by hand after climbing to upper branches bearing the fruits. The taste of Indian gooseberry is sour, bitter and astringent, and is quite fibrous (Brun, 1987).

1.2 Geographic distribution

Amlaki tree is native to tropical southeastern Asia, particularly in central and southern India, Pakistan, Bangladesh, Ceylon, Malaya, southern China and the Mascarene Islands. It is commonly cultivated in home gardens throughout India and grown commercially in Uttar Pradesh. Many trees have been planted in southern Malaya, Singapore, and throughout Malaysia. In India, and to a lesser extent in Malaya, the emblic is important and esteemed, raw as well as preserved, and it is prominent in folk medicine. Fruits from both wild and dooryard trees and from orchards are gathered for home use and for market. In southern Thailand, fruits from wild trees are gathered for marketing. In 1901, the United States Department of Agriculture received seeds from the Reasoner Brothers, noted nurserymen and plant importers of Oneco, Florida. Seeds were distributed to early settlers in Florida and to public gardens and experimental stations in Bermuda, Cuba, Puerto Rico, Trinidad, Panama, Hawaii and the Philippines. The fruits of these seedlings aroused no enthusiasm until 1945 when Mr. Claud Horn of the Office of Foreign Agricultural Relations in Washington, D.C., inspired by Indian ratings of the emblic as the "richest known natural source of vitamin C", asked that analyses be made in Puerto Rico. A high level of ascorbic acid was found and confirmed in Florida but interest quickly switched to the Barbados cherry (q.v.) which was casually assayed and found to be as rich or richer when under ripe. The emblic was soon forgotten. Some old trees still exist in southern Florida; others have been removed in favor of

housing or other developments. In 1954, the Campbell Soup Company in Camden, New Jersey, requested 5 lbs (2.25 kg) of the fruits for study. They were sent, but no further interest was evidenced. In 1982, several individuals asked for and were given seeds for planting in Australia. They did not reveal whether the tree was desired for its own sake or for its fruit (Morton, 1987).

1.3Background of the study

Species selection`

The genus Phyllanthus (Euphorbiaceae) is widely distributed in most tropical and subtropical countries. (Summanen, 1999). *Phyllanthus emblica* is a native species. The species has a great food, fodder, fuel and medicinal value. If we do not take necessary steps to conserve it, we will lose this native species. Conservation and sustainable use of genetic resource depends upon the knowledge of the extent and pattern of intra-specific variation, seed germination and clonal propagation. Characterization of natural resource is important for a better understanding of genetic resource conservation. Germination percentage is low in fresh seeds (4-20%). Dry storage for one year at ambient condition with 4-7% moisture content has increased the germination percentage up to 85%; during cold storage this germination was only reached after more than 4 years. Pretreatment with plant growth regulators has very little effect on this type of dormancy (Kundu, 2012). Under natural condition, the growth is probably slower (Troup, 1921).That is why, *Phyllanthus emblica* has been chosen to determine the germination percentage and growth performance at nursery stage.

Treatment selection

Pretreatment is a kind of treatment applied to seed to overcome seed dormancy and hasten germination (Bonner, 1985). Pretreatment of seeds are intended to improve the survival or germination of seed after sowing. The term is often shortened to "seed pretreatment", which is really a misnomer, since what we are concerned with here is treatment before seed sowing, not some action before seed treatment (Wilan,1990).

In pre-soaking treatment seeds soaked in the minimum amount of water and afterward slowly dried at ordinary temperatures imbibe water and develop more quickly when allowed to take up water and germinate more than untreated seeds.

a. Seeds, which are rapidly dried after the initial soaking, germinate more slowly than untreated seeds.

b. Seeds swollen in water and sown in moist condition germinate more quickly than untreated seeds (Copland, 1988).

In the past, in case of *Phyllanthus emblica* various treatment such as Gibberlic acid, KNO_3, hot water were used for germination and growth performance. But this type of treatment is costly to be used on a large scale plantation. On the other hand, chemical treatment is not available for the farmers. So, the present investigation will be done by water treatment by water treatment under different time variation. Water is available to the farmer which is very cheap. It has no hazardous effect when the farmer uses it. That is why, water has been chosen as treatment for germination and growth performance of *Phyllanthus emblica.*

Economic importance

Amlaki is a gift of nature to mankind. It is an indispensable part of the ayurvedic and unani-ystem with amazing remedial qualities. Various types of use of amlaki are given bellow:

Food Uses

Rural folk in India claim that the highly acid, fresh, raw fruit, followed by water, produces a sweet and refreshing after taste. Wood-cutters in Southeast Asia eat the amlaki to avoid thirst, as the fruit stimulates the flow of saliva. This is the one tree left standing when forests are clear-cut in Thailand, and busses stop along highways to let thirsty travelers run to the tree to get the fruits. The amlaki is regarded as sacred by many Hindus and the Hindu religion prescribes that ripe fruits are eaten for 40 days after a fast in order to restore health and vitality. It is a common practice in Indian homes to cook the fruits whole with sugar and saffron and give one or two to a child every morning. Fresh emblics are baked in tarts, added to other foods as seasoning during cooking, and the juice is used to flavor vinegar. Both ripe and half-ripe fruits are candied whole and also made into jam and other preserves, sweetmeats, pickles and relishes. They are combined with other fruits in making chutney. In Indonesia, emblics; are added to impart acidity to many dishes, often as a substitute for tamarinds (Morton, 1987).

When necessary, bitterness is overcome by soaking the fruits in a salt solution or by adding citrus fruit, unripe mango or tamarind. In preserving emblics; whole, the fruit is first brined, washed and pricked, blanched in an alum solution, layered with sugar until a sirup is formed, and then boiled. It is finally packed in enameled cans or crystallized as a confection. In India,

This, also, is commonly eaten after fasting. During World War II, emblic powder, tablets and candies were issued to Indian military personnel as vitamin C rations. Drs. Rama Rao, Balakushnan and Rajagopalan, of the Institute of Science at Bangalore, describe a method of spray-drying emblic juice to produce a special powder for fortifying salt as a means of increasing vitamin C intake. In Thailand, where the tree is common in the forests, the fruits are favored by deer, especially the tiny barking deer (Morton, 1987).

Other Uses

Fruit: The dried fruit yields ink and hair-dye and, having detergent properties, is sometimes used as a shampoo. A fixed oil derived from the fruit allegedly acts as a hair-restorer and is used in shampoos in India. This oil is the main ingredient in an "Amla Conditioner" currently sold by Shikai Products of Santa Rosa, California, by mail and through "health food" stores and other "natural" product outlets. A most curious custom is the making of simulated pottery jars from a paste of the boiled fruit, the surface being decorated with impressed colored seeds. Dyes from the fruit and leaves impart an appealing light-brown or yellow-brown hue to silk and wool. When sulfate of iron is added as a mordant, the color becomes black (Morton, 1987).

Bark: The tannin-rich bark, as well as the fruit and leaves, is highly valued and widely employed in conjunction with other so-called myrobalans, especially fruits of various species of *Terminalia.* The twig bark is particularly esteemed for tanning leather and is often used with leaves of *Carissa spinarum* and *Anogeissus latifolia* (Morton, 1987).

Leaves: The foliage furnishes fodder for cattle and branches are lopped for green manure. They are said to correct excessively alkaline soils (Morton, 1987).

Wood: The hard but flexible red wood, though highly subject to warping and splitting, is used for minor construction, furniture, implements, gunstocks, hooks and ordinary pipes. Durable when submerged and believed to clarify water, it is utilized for crude aqueducts and inner braces for wells, and branches and chips of the wood are thrown into muddy streams for clarification and to impart a pleasant flavor. The wood serves also as fuel and a source of charcoal (Morton, 1987).

Soil improver: The branches are lopped for green manure. They are said to correct excessively alkaline soils (Anon, 2014).

Pest and disease: The chief pest of this tree in India is the bark-eating caterpillar, Indarbela sp., which tunnels into the branches and trunk. A secondary enemy produces shoot galls. A non-pathogenic problem, especially in India in 'Francis', is called "fruit necrosis", characterized by internal browning which gradually extends to the surface where dark spots become corky and gummy evidences it. Bi-monthly sprays of borax can overcome it in September and October. There are few serious diseases but the fungi, *Bestonea stylophora, Phakospora phyllanthi* and *Ravenelia emblicae*, cause ring rust, leaf rust and fruit rot. Fresh amlaki on the market or in storage are subject to blue mold and rotting caused by *Penicillium islandicum*. Rinsing with very dilute borax or sodium chloride solutions helps retard such spoilage (Anon, 2014).

Medicinal use

Antibacterial, antifungal, antiviral: Medical studies conducted on fruit suggest that it has antiviral properties and also functions as an antibacterial and anti-fungal agent (Treadway & Linda, 1934).

Antioxidant:The use of amlaki as an antioxidant has been examined by a number of authors (Bhattacharya et al., 1999). Experiments conducted at the Niwa Institute of Immunology in Japan have shown Amlaki to be a potent scavenger of free radicals. The studies showed that Amlaki preparations contained high levels of the free-radical scavenger, superoxide dimutase (SOD), in the experimental subjects (Treadway & Linda, 1934).

Boils and spots: The pericarp of the fruit is often used in decoctions along with other ingredients and also applied externally on boils with cow ghee to promote suppuration (Jayaweera, 1980).

Chelating agent: Photoaging of the skin is a complex biologic process affecting various layers of the skin with major changes seen in the connective tissue within the dermis. Amlaki was shown to reduce UV induced erythema and had excellent free-radical quenching ability, chelating ability to iron and copper as well as MMP-1 and MMP-3 inhibitory activity (Chaudhuri et al., 2003).

Constipation: The fruit is occasionally pickled or preserved in sugar. When dry it is said to be gently laxative (Drury, 1873), according to some sources the fresh fruit is also laxative (Nadkarni & Nadkarni, 1999). The fresh ripe fruits are used extensively in India as a laxative, one or two fruits being sufficient for a dose. They have been exported to Europe, preserved in sugar, and are valued as a pleasant laxative for children and made into a confection consisting of the pulp of the de-seeded fruit .Fruits along with those of *Terminalia bellirica* and *T. chebula* are the constituents of "Triphala" which are used as a laxative (Thakur et al., 1989).

Dental problems: The roots of Amlaki (10 g) are ground and taken twice daily for one day only after taking food. Alternatively, the leaves are squeezed and the juice extracted. This juice is put in the ear (a few drops) to find relief from toothache. A final alternative is to grind the node of amlaki and mix it with water. After vigorous stirring it is filtered through a cloth. This water is put drop by drop in the right ear if the teeth on the left hand side are in pain and vice versa. The remedy is continuing for three days.

Diabetes: The fruits are used in the treatment of diabetes (Drury, 1873) and in other references an infusion of the seeds are also used (Nadkarni & Nadkarni, 1999). Decoctions of the leaves and seeds are used in the treatment of diabetes mellitus (Treadway & Linda, 1934).

Diarrhoea: It is used medicinally for the treatment of diarrhoea. As a fruit decoction it is mixed with sour milk and given by the natives in cases of dysentery (Drury, 1873). The bark partakes of the astringency of the fruit. A decoction and evaporation of the root solution produces an astringent extract equal to catechu (Nadkarni & Nadkarni, 1999). An infusion of the leaves with fenugreek seed is given for chronic diarrhoea (Jayaweera, 1980).

Fevers: Malays use a decoction of its leaves to treat fever (Burkill, 1966). The fresh fruit is refrigerant .The seeds are given internally as a cooling remedy in bilious affections and nausea, and in infusion make a good drink in fevers (Nadkarni & Nadkarni, 1999).

Hair growth: A fixed oil is obtained from the berries that are used to strengthen and promote the growth of hair. The dried fruits have a good effect on hair hygiene and have long been respected as an ingredient of shampoo and hair oil (Thakur et al., 1989).

Headache: A paste of the fruit is a useful application to the forehead in cases of cephalalgia (headache). The name "Itrifal" of Unani medicine is the same as "Triphala" in the Ayurvedic system and represents a group of preparations used for the care of all manner of cranial conditions (Thakur et al., 1989).

Indigestion: Fruit is carminative and stomachic (Nadkarni & Nadkarni, 1999). The tender shoots given in butter-milk cure indigestion and it are known that green fresh leaves combined with curds have similar effect.

Inflammation: *P. emblica* has been used for anti-inflammatory and antipyretic treatments by rural populations in its growing areas (Burkill, 1966).

Mouth ulcers: A decoction of the leaves is used as a chemical-free bactericidal mouthwash (Treadway & Linda, 1934). Bark of the root mixed with honey is applied to aphthous inflammations of the mouth (Drury, 1873 and a decoction of the leaves is also useful as a mouth wash in the treatment of aphthae. Another remedy suggests root bark rubbed with honey is used in aphthous stomatitis (an inflammation of the mouth) (Nadkarni & Nadkarni, 1999).

Nose bleed: The seed are fried in ghee and ground in conjee (the liquid from boiled rice) is applied to the forehead to stop bleeding from the nose.

Perfumery: An essential oil is distilled from the leaf that is used in perfumery.

Respiratory problems: The fresh fruit is used in Turkeystan in inflammations of the lungs. The juice or extract of the fruit is mixed with honey and pipit added is given to stop hiccough and also in painful respiration. The expressed juice of the fruit along with other ingredients is used to cure cough, hiccough, asthma and other diseases (Jayaweera, 1980).

Skin sores and wounds: The milky juice of the leaves is a good application to sores. Grind the bark of amlaki (10g) into a paste and apply to the cut or wound area once daily for 2 to 3 days. Alternatively, squeeze leaves and extract the juice to the cut once daily for3 to 4 days. Healing occurs when the dynamic harmony of the doshas is restored (Treadway & Linda, 1934).

Sore eyes: Infusion of the leaves is applied to sore eyes (Drury, 1873). The dried fruit immersed in water in a new earthen vessel a whole night yields a decoction which is used as a collyrium (a medical lotion applied to the eye as a eyewash) in ophthalmia. It may be applied cold or warm (Nadkarni & Nadkarni, 1999).

Miscellaneous: The bark of the tree itself is astringent, and is used for tanning purposes.

Table 1: Food Value per 100 g Edible Portion of *Phyllanthus emblica* Fruit

Ingredient	Amount
Moisture	77.1 g
Protein	0.07 g
Fat	0.2 g
Carbohydrates	21.8 g
Fiber	1.9 g
Ash	0.5 g
Calcium	12.5 mg
Phosphorus	26.0 mg
Iron	0.48 mg
Carotene	0.01 mg
Thiamine	0.03 mg
Riboflavin	0.05 mg
Niacin	0.18 mg
Tryptophan	3.0 mg
Methionine	2.1 mg
Lysine	17.0 mg
Ascorbic Acid**	625 mg

**The ascorbic acid ratings vary immensely (Morton.1987).

1.4 Objective of the study

The objective of the study was to:

- find out the suitable pre-sowing treatment for getting better germination percentage.
- determine the seedling growth performance (height, diameter and leaf number) at nursery stage.

CHAPTER TWO: REVIEW OF LITERATURE

2.1 Germination

Germination is the development of a young embryo into a young seedling. We need germination for plants to grow. With no plants there would be less oxygen and less food for animals. We need water to begin germination. When water soaks the seed coat, it splits open. The seed coat is the outer layer of the seed coat that protects it. Water softens the seed coat, making it easier to split.

2.2 Factors affecting the seed germination

Seeds are living organisms held in a state of suspended animation or dormancy. There are many factors that can affect the viability of seeds, including moisture, air, temperature, and light. In an ideal situation and environment, every single seed we planted would grow into a seedling, but as we all know, that doesn't normally happen. Although dormant, seeds are still slowly respiring and using food reserves within. When the right environmental cues wake the seeds up they begin to germinate and emerge from their hard seed coat. There are four major factors that are affect germination:

Moisture: A dormant seed only contains 10-15% of water and is essentially dehydrated. The seed has to absorb water in order to become active. It is imbibed by the seed coat and enzymes within the seed become active and functional, metabolizing stored food reserves. The embryo then begins to swell. The softened seed coat ruptures as the seed grows too big for its encasement and germination has commenced. The seed leaves or cotyledons are now apparent. Photosynthesis does not begin until the true leaves are developed and at this point in development the seedling is still surviving on its own food reserves (Anon, 14).

Air: In the dormant condition the seeds respiratory rate is very low and so oxygen is required in very small quantities. But for germination, oxygen is needed in large quantities. The seeds obtain oxygen that is dissolved in water and from the air contained in the soil. If soil conditions are too wet, an anaerobic condition persists, and seeds may not be able to germinate (Anon, 14).

Temperature: Germination can take place over a wide range of temperature and is specific to individual crop types, and can be specific to varieties. The optimum for most crops is between 65-75°F, but exceptions do apply. For example lettuce germinates best at 65°F and can be inhibited at temperatures over 68°F while peppers and eggplants prefer warmer temperatures around 80°F and will not germinate well at cooler temperatures. If the soil is too cold or too hot, the seeds may not sprout (Anon, 14).

Light: Light has varied effects on germinating seeds of different plants. Some seeds need light for germination, while in some seeds germination is hindered by light. Most wild species of flowers and herbs prefer darkness for germination and should be planted deep in the soil while most modern vegetable crops prefer light or are not affected by it, and are planted shallowly to allow small amounts of light to filter through the soil (Anon, 14).

2.3 Phenology of *Phyllanthus emblica*

The leaves commence at failing about falling about November to December and the trees are leafless from about February or March to March or April, when the new shoots appear. The minute yellowish flowers, densely fascicled in the axiles of the young leaves, appear from March to May, and visited by swarms of bees. The fruits which ripen from November to February or sometimes later, are 0.5-0.8 in. in diameter , globose, yellowish green, smooth, fleshy and very astringent, with a 6-riged bony endocarp containing about four to six dark brown smooth 3 gonous seeds of which about 1800-1900 weigh 1 oz (Troup, 1921).

2.4 Seed Morphology of *Phyllanthus emblica*

The endocarp is a slightly hexagonal 3-celled stone containing 6 trigo-nous small seeds. Seeds are kidney-shaped, shining and reddish brown. Seed weight 50-70,000 seeds per kg or 1000 psw 15-20 g (Kundu, 2012).

2.5 Seed collection and Storage of *Phyllanthus emblica*

Fresh fruits are collected from the trees. The optimum period of collection is when the color of the fruit turns white or greenish yellow. The collection method is to spread a tarpaulin under the tree and collect the fruits by shaking the trees or lopping the branches or plucking. Seeds are of orthodox type and tolerate desiccation to 4-5% moisture content. The seeds can be stored for long periods (more than six years) if stored at low temperature or below freezing

(-20°C to 15°C) even with wide range of moisture contents (4-12%). But seed viability will decline after two years of storage at ambient condition at any moisture content (Kundu, 2012).

2.6 Soaking in water

Seeds of different species of Pinus, Picea, Larix, Cunninghamia, Ptatychladus, Hippophas and Vitex germinated better when they were previously soaked in water for 15 to 24 hours. Soaking for more than 24 hours was detrimental to the germination of all the species (Ma and Liu, 1986). Seeds of teak soaked in running water for 96 hours before sowing in the nursery showed better germination (Nayaka, 2006).

2.7 Pre-sowing reatment of *Phyllanthus emblica*

Soaking the seeds in water for about 12 h before sowing improves germination (Singh, 1998; Chacko et al., 2002). Seeds are soaked in Gibberellic Acid 500 ppm solution for 24 hrs. Fresh seeds treated with 0.5% KNO3 and one-year-old seeds treated with 200 ppm GA3 for 8 h give germination percentage of 69.33 and 46.00%, respectively. Seeds treated with 250 ppm GA3 have 75.98% germination in the laboratory. Seeds of *Emblica officinalis* soaked with 400 ppm GA3 give 87.25% germination. Seeds soaked for 12 h in 400 ppm gibberellic acid give 87.25% germination, and those soaked in water give 56% (Dhankhar et al., 1997; Dhankhar, Santosh Kumar 1996; Rajamanickam et al., 2002; Wagh et al., 1998; Pawshe et al., 1997). Hot water soaking at 60°C for 5 minutes was found to be beneficial (Pawshe et al., 1997). Seeds treated with 1% KNO3 for 18 h give seed germination of 93.33% (Purbey and Meghwal, 2005).

An investigation entitled "Propagation studies in aonla (*Phyllanthus emblica L.*) was conducted at Department of Horticulture and Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Dharwad during 2005-06. The experiments were carried out to find out suitable seed treatments to break the seed dormancy, identify ideal age of rootstock and best season for softwood grafting to obtain maximum graft success and development. The micro propagation technique was also attempted. Scarification of seeds with concentrated sulphuric acid for 30 seconds significantly increased germination per cent to 80.39, taking least number of days for 50 per cent germination (12.27) and highest vigor index (1223.20) as compared to the rest of treatments. GA_3 at 500 ppm for 24 hours resulted in vigorous seedling growth (Nayaka, 2006).

2.8 Growth Performance of *Phyllanthus emblica*

Growth is the biological phenomenon of size increase over time, and is measured in diameter, height, basal area, and volume (Kyaw, 2003). Pre-treated or pre-germinated seeds are sown in polythene bags filled with potting mixture (Kumar and Bhanja, 1992) or in open beds provided with overhead shade (Troup, 1921) during March. Germination takes place in 24 to 27 days. The plants become ready for planting in four to five months (Kumar and Bhanja, 1992; Chacko et al., 2002). Seeds are soaked in Gibberllic acid 500 ppm solution for 24 h giving good growth performance. Seeds sown in the middle of July have higher germination and seedling survival compared to those sown on other dates. Percentage budding success and growth of buds are maximum when budding is on the last week of June (Srivastava et al., 2002). The percentage of seedling survival is maximum in seeds sown in July, followed by those sown in August and June (Singh et al., 2002). Spraying of seedling rootstocks raised in polyethylene bags once or twice (with the second spray applied one month after the first spray) with GA3 (100 ppm) increase plant height and diameter and length of the primary and tap root. Application of GA3 at 50 ppm + urea at 0.5% also significantly increase various vegetative and root growth characteristics. Second spray further increase the effectiveness (Virendra Singh and Shafaat Mohammed, 1996). Treating seeds with 200 ppm GA3 have effect on shoot elongation irrespective of age of seeds. Treating fresh seeds with 0.5% KNO3 have a positive influence on dry matter production and also give good vigour index (Rajamanickam et al., 2004). Treatment with 750 ppm thiourea gives good root development (Dhankhar et al., 1997; Dhankhar, and Santosh Kumar, 1996).

Seedling development in terms of plant height, number of leaves/plant and root development is also good with 400 ppm GA3 (Wagh et al., 1998). Fresh seeds treated with Azospirillum+Phosphobacteria+0.5% KNO3 for 8 h give germination percentage of 52.08, and one-year-old seeds treated with Azospirillum+Phosphobacteria+200 ppm GA3 for 8 h give 49.17% germination (Rajamanickam and Anbu, 2001). The applications of AM fungi and PSB (phosphate-solubilizing bacteria) in combination produce maximum plant height, maximum diameter of seedlings. The application of AM fungi along with companion fungus or Azospirillum and companion fungus boost the growth of Aonla in nursery (Verma et al., 2008).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was conducted in the nursery of Forestry and Wood Technology Discipline, Khulna University, Bangladesh. The study area is situated in the south-western part of Bangladesh and it is the part of the Sundarbans delta. The elevation is four meter above sea level. The geographic distribution of this area lies between 22°48′N and 89°30′E. The study area is situated in subtropical region like other part of our country. There are mainly three seasons winter, summer, monsoon which affects the climatic condition of my study area. The winter season start from November and this season is ended in February when the temperature is fluctuated but this fluctuation is very low and it is limited up to 7-12° C. The summer is continuing during March to June when the general temperature is 25-32° C but sometimes the temperature is raised up to 36-40° C. The monsoon season is continuing from July to October (BBS 1993, Alam et al 2005). The recorded average air temperature was 27-32° C and average soil temperature was 23-27° C. The average relative humidity was 75% that is suitable for the experiment.

3.2 Plant materials and design of the experiment

The seeds of *Phyllanthus emblica* were collected from Dalutpur upazilla under Khulna district, Bangladesh. The ripe fruits were collected by manually from standing trees during the month of April.The mother tree was 15-20 years old and it was healthy, straight bole, and spreading crown. Measurement of the fruits and seeds were taken in unopened condition and before putting them into the germination test respectively. The seeds were extracted from the fruit manually. After collection the seeds were dried for two days in open sun to reduce the moisture .The extracted seeds were tested to eliminate the stained, discolored and damaged seed. For the germination test poly bag (4cm × 6cm) was used. The poly bag was filled with the mixture of topsoil and decomposed cow dung in the ratio of 3:1. The present investigation will be done by water treatment under different time variation. Water is available to the farmer which is very cheap. It has no hazardous effect when the farmer uses it. The experiment was conducted under four pre-sowing treatment as follows-

T1: Control.

T2: Immersion in normal water for 24 hours.

T3: Immersion in normal water for 48 hours.

T4: Immersion in sun heated water for 10 hours.

There were three replications of each treatment. One seed was used for each polybag. The polybags were kept in shade until the germination occurs. The seeds were sown manually and water was given every alternative day. Completely Randomized Design (CRD) was used for the experiment. Three hundred (3×100) seeds in polybag were used for each treatment. So, a total 1200 seeds i.e. 1200 (4×3×100) seeds in polybag was used for germination test. The number of seed germinated in each treatment was recorded every day. The starting and finishing dates of germination were also recorded.

After one month later completing germination, 30 strong and healthy seedlings were separated from every treatment. Then every treatment was divided into tree replications (design one, design two and design three) according to germination design. Height growth was measured by a wooden scale keeping just above the polybag at the base of seedlings. Diameters were measured by an Electronic Digital Caliper at the collar zone and numbers of leaf were counted manually for each seedling individually. The duration of the growth performance was six months. Data were collected on the same date of each for each treatment.

3.3 Seed germination

The number of seeds germinated on every day was recorded for each treatment in polybag. The starting and finishing dates of germination were also recorded. At the end of the germination period, the germination percentage and rates of germination were obtained using the following equations (Azad et al., 2012) which was modified from Maguire (1962):

$$G_p = N_g / N_t \times 100 \quad (1)$$

$$G_r = \sum \frac{Ng}{\text{Days of count}} \quad (2)$$

i.e.,

$$G_r = \sum \frac{Ng}{\text{Days to first count}} + \ldots\ldots + \sum \frac{Ng}{\text{Days to final count}} \quad (3)$$

where G_p is the germination percentage, N_g the number of germinated seeds, N_t the total number of seeds planted and G_r the rate of germination.

3.4 Data analysis

Analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) were carried out to analyze the data. Data were analyzed using MS Excel and SAS software to explore possible treatment variation. Analysis of variance was carried out to determine the treatment effect on germination starting and closing date after seed sowing, length of germination period, seed germination percentage, germination rate and growth performance (height, diameter and leaf number) of *Phyllanthus emblica*. Data transformation was done by using Arcsine transformation. Many biological variables do not meet the assumptions of parametric statistical tests: they are not normally distributed, the variances are not homogeneous, or both. Using a parametric statistical test (such as an anova or linear regression) on such data may give a misleading result. Transforming the data will make it fit the assumptions better. So, data transformations are an important tool for the proper statistical analysis of biological data. DMRT was conducted to compare mean germination starting and closing date after seed sowing, length of germination period, seed germination percentage , germination rate and growth performance (height, diameter and leaf number) of different pre-sowing effect of *Phyllanthus emblica.*

CHAPTER FOUR: RESULT

4.1 Morphological characteristics of the fruits

The colors of fruit and seed were yellowish green and dark brown respectively and average weight per fruit was 14 ±0.79 gm. The average length and diameter per fruit were 14 ±1.4 mm and 20.35 ±0.35 mm respectively. On the other hand, each fruit contained 4-6 seeds and weight per 100g seeds containing 4000-4500 seeds.

4.2 Effect of treatment on seed germination in polybag

In all treatments, germination was completed within 7-13 days after sowing the seed in polybag. The highest germination success 75% was observed in normal water treatment for 24 hours (T2) and lowest 51.33% in control (T1). The second and third germination success was 63.33% in sun heated water for 10 hours (T4) and 60.66% in normal water treatment for 24 hours (T3) respectively. Analysis of variance (ANOVA) showed significant difference ($P<0.05$) in germination starting day, germination closing days, germination period, germination percentage and germination rate among the treatment.

DMRT showed significant difference on seed germination in normal water treatment for 24 hours with other treatment but no significant difference was found between normal water for 48 hours (T3) and sun heated water for 10 hours (T4).

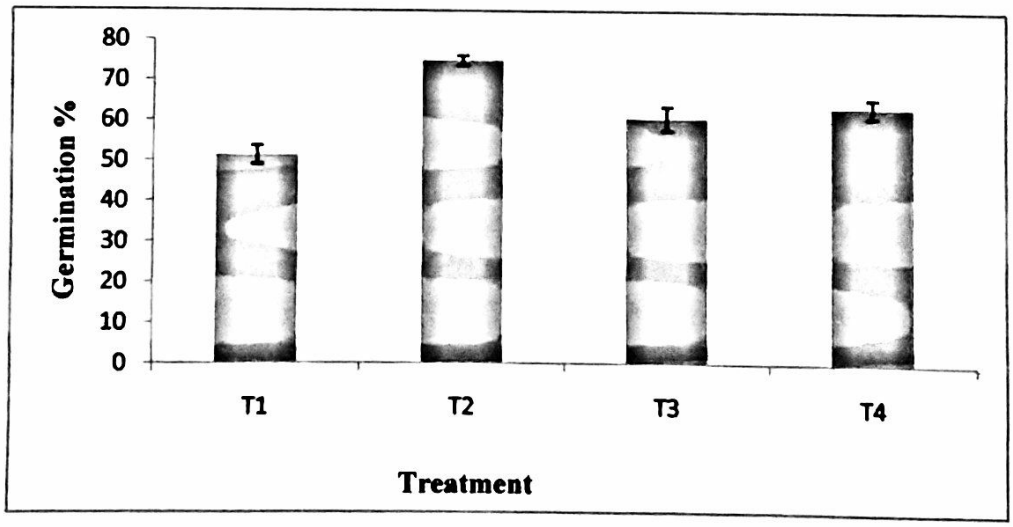

Fig-1: Effect of pre-sowing treatments on seed germination of *Phyllanthus emblica*. Bars show 95% confidence limit.

Table 2: Summary of different pre-sowing effect on germination starting days, closing days, germination period, germination percentage of *Phyllanthus emblica* in polybag at nursery stages. Same letter in the same row show no significance different and ± indicates 95% confidence limit.

Particulates	Treatment (T1)	Treatment (T2)	Treatment (T3)	Treatment (T4)
Germination starting days	8.67 ±1.15a	6.33 ±0.58b	8±1a	5.33 ±0.58b
Germination closing days	21 ±0.58a	17 ±0.58b	17.33±0.89b	12.33±0.89c
Germination period	12.33 ±0.33a	10.66±0.67ab	9.33 ±0.89b	7 ±0.58c
Germination percentage	51.33±2.33c	75 ±1.15a	60.66 ±2.96b	63.33 ±2.33b
Germination rate	3.54±0.031d	5.75±0.023a	3.90±0.017c	4.61±0.027b

Note: **T1**: Control, **T2**: Immersion in normal water for 24 hours, **T3**: Immersion in normal water for 48 hours, **T4**: Immersion in sun heated water for 10 hours.

4.3 Effect of treatment on seedling growth performance

4.3.1Height

At the end of 6 months, the highest height growth of seedlings 54.68% was found in sun heated water for 10 hours (T4) and lowest 51.95% in normal water treatment for 24 hours (T2). The second and third positions in height growth were found 53.40% in control (T1) and 52.87% in normal water treatment for 48 hours (T3) respectively. Analysis of variance (ANOVA) did not show any significant difference ($P<0.05$) in height growth among the four treatments.

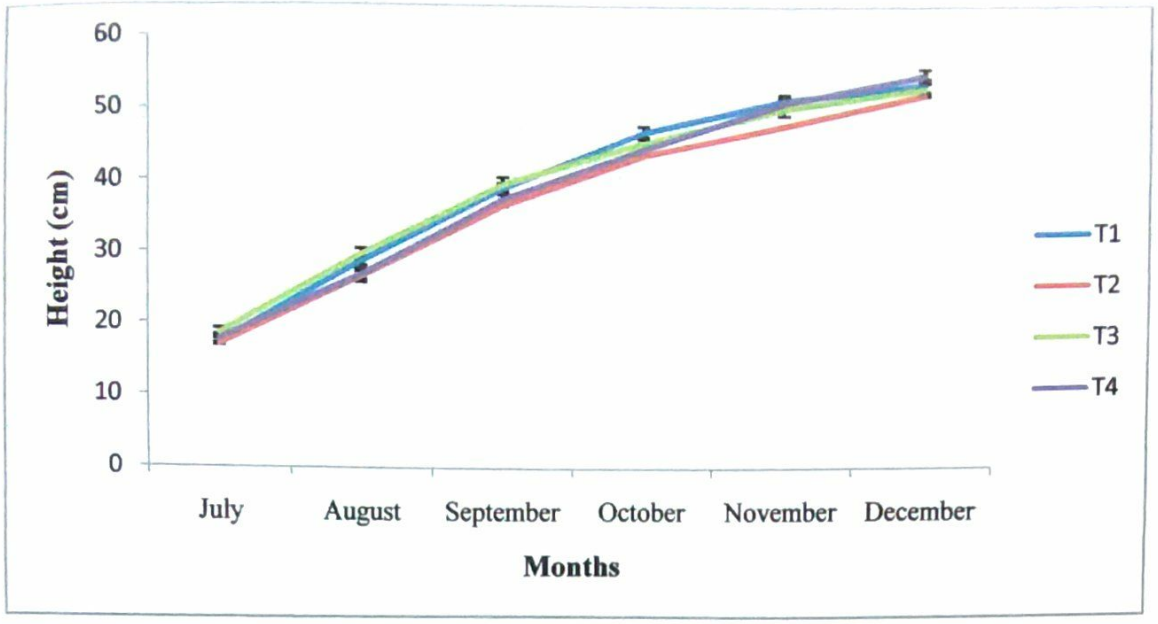

Fig-2: Effect of pre-sowing treatments on height growth of *Phyllanthus emblica* at nursery stage. Bars show 95% confidence limit.

4.3.2 Diameter

At the end of 6 months, the highest diameter growth of seedlings 5.43% was found in sun heated water for 10 hours (T4) and lowest 5.25% in normal water treatment for 24 hours (T2). The second and third diameter positions in growth were found 5.36% in control (T1) and 5.26% in normal water treatment for 48 hours (T3) respectively. Analysis of variance (ANOVA) did not show any significant difference ($P<0.05$) in diameter growth among the four treatments.

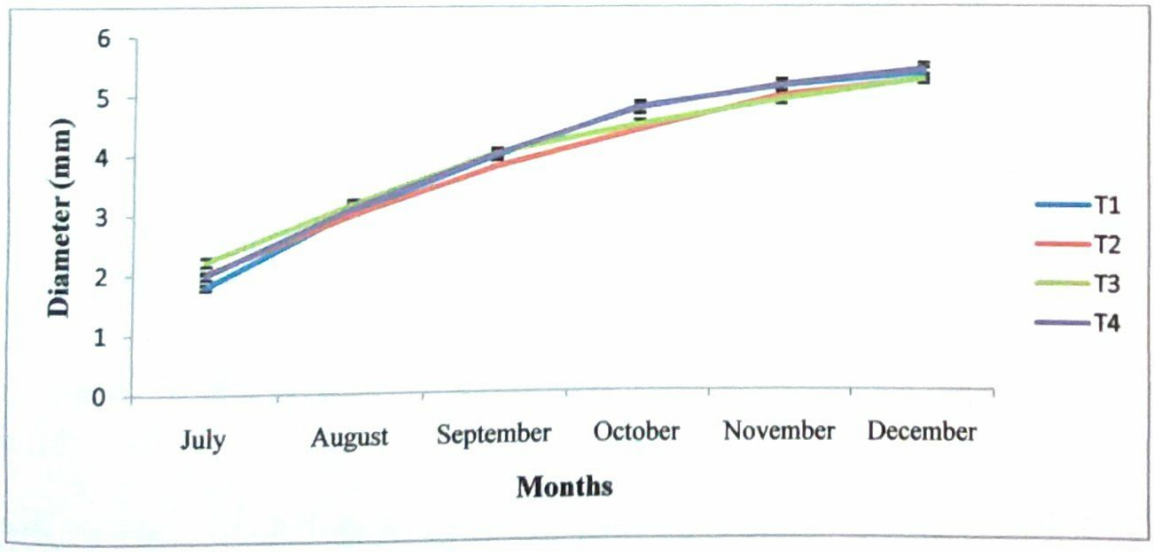

Fig-3: Effect of pre-sowing treatments on collar diameter growth of *Phyllanthus emblica* at nursery stage. Bars show 95% confidence limit.

4.3.3 Leaf Number

At the end of 6 months, highest average leaf number of seedlings 30.60% was found in sun heated water for 10 hours (T4) and lowest 29.90% in normal water treatment for 24 hours (T2). The second and third positions in average leaf number were found 30.10% in control (T1) and 29.46% in normal water treatment for 48 hours (T3) respectively. Analysis of variance (ANOVA) did not show any significant difference ($P<0.05$) in leaf number among the four treatments.

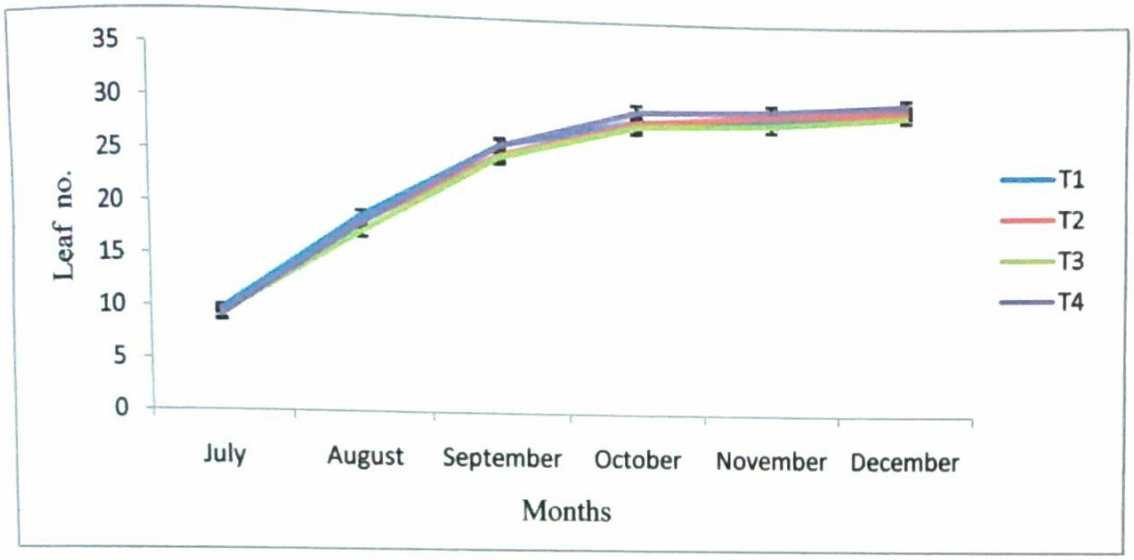

Fig-4: Effect of pre-sowing treatments on leaf production of *Phyllanthus emblica* at nursery stage. Bars show 95% confidence limit.

Table 3: Summary of different pre-sowing effect on seedlings growth performance (height, diameter and leaf number) of *Phyllanthus emblica* in polybag at nursery stages. Same letter in the same row show no significance different and ± indicates 95% confidence limit.

Particulates	Treatment (T1)	Treatment (T2)	Treatment (T3)	Treatment (T4)
Height (cm)	53.40 ±.848a	51.95 ±.708a	52.87±.829a	54.68±0.899a
Diameter (mm)	5.36 ±0.096a	5.25±0.079a	5.26±0.0.08a	5.43±0.101a
Leaf number	30.10 ±0.487a	29.90±0.422a	29.46 ±0.584a	30.60±0.475

Note: T1: Control, **T2**: Immersion in normal water for 24 hours, **T3**: Immersion in normal water for 48 hours, **T4**: Immersion in sun heated water for 10 hours.

Table 4: P-values among treatment in polybag of *Phyllanthus emblica* at 95% level of confidence.

Particulates	P-value
Germination starting day	0.005*
Germination closing day	0.0003*
Germination period	0.002*
Germination percentage	0.0006*
Height	0.351
Diameter	0.516
Leaf number	0.317

Note: (*) indicates significant difference.

4.3.4 Relationship between Height and Diameter growth

After experiment, it was observed that there was highly strong positive relationship between height and diameter growth of *Phyllanthus emblica* seedlings. The height of the seedlings was increased with increasing diameter

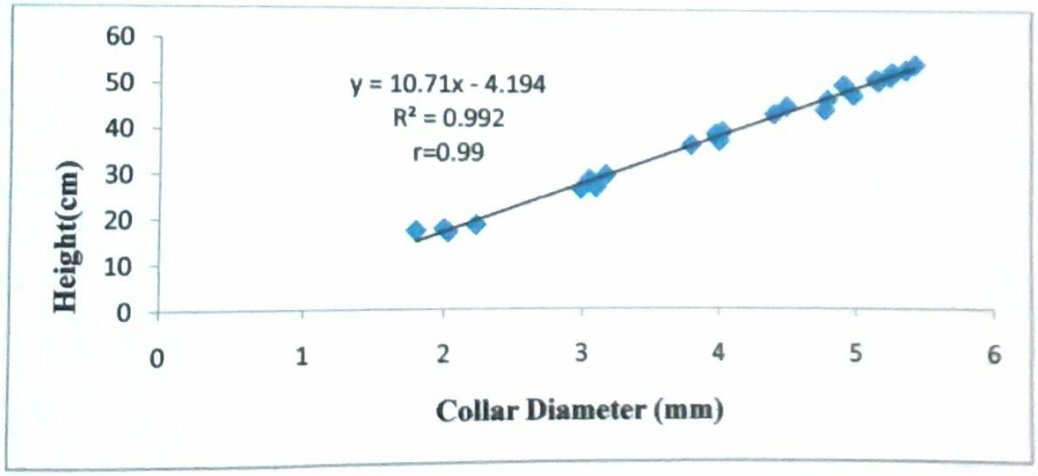

Fig-5: Relationship between height and diameter growth at nursery stage

4.3.5 Relationship between Height and Leaf number

After experiment, it was observed that there was highly strong positive relationship between height and leaf number of *Phyllanthus emblica* seedlings. The leaf number of the seedlings was increased with increasing the height.

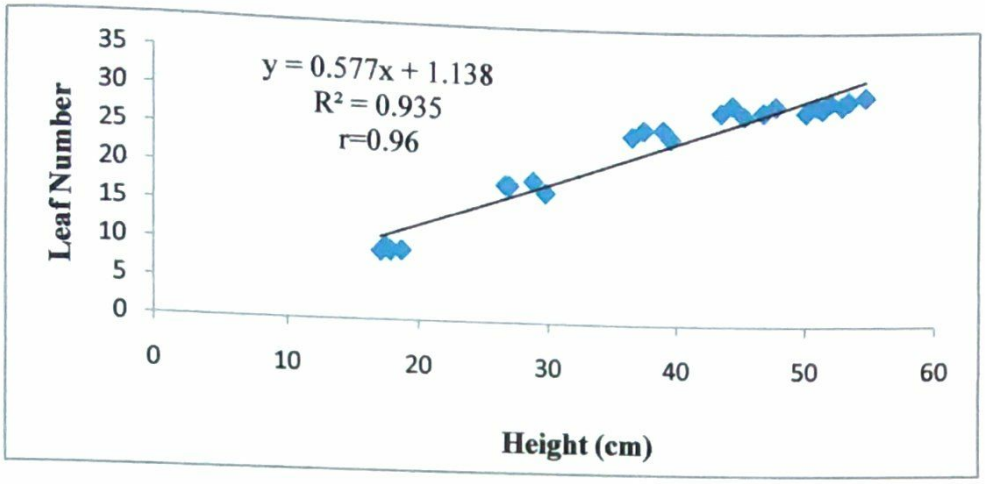

Fig-6: Relationship between height and leaf number at nursery stage

4.3.6 Relationship between Diameter and Leaf number

After experiment, it was observed that there was highly strong positive relationship between diameter and leaf number of *Phyllanthus emblica* seedlings. The leaf number of the seedlings was increased with increasing the diameter.

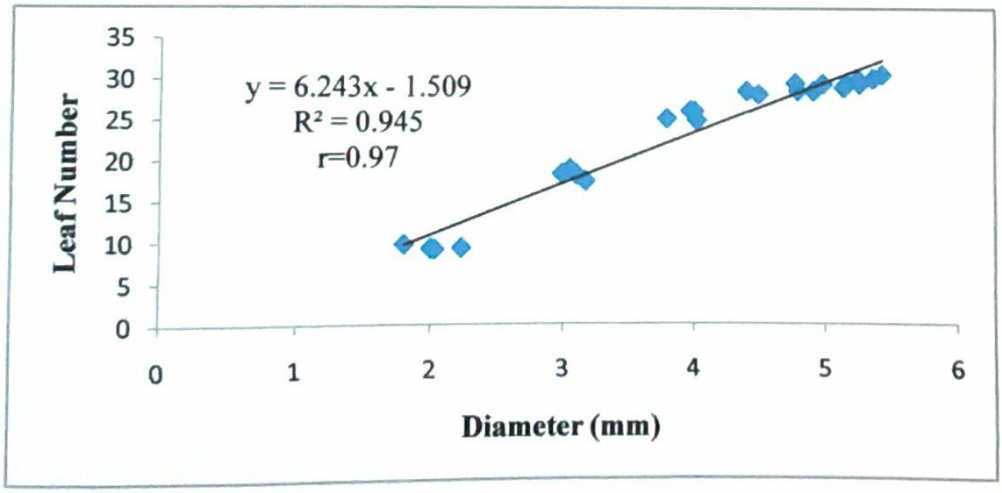

Fig-7: Relationship between diameter and leaf number at nursery stage

4.3.6 Relationship between Diameter and Leaf number

After experiment, it was observed that there was highly strong positive relationship between diameter and leaf number of *Phyllanthus emblica* seedlings. The leaf number of the seedlings was increased with increasing the diameter.

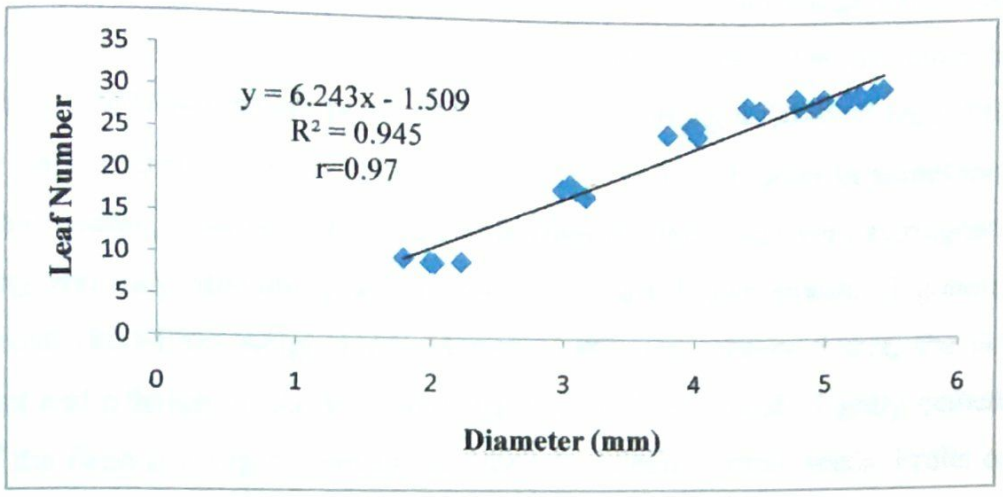

Fig-7: Relationship between diameter and leaf number at nursery stage

CHAPTER FIVE: DISCUSSION

5.1 Morphological characteristics of the fruit

It was observed that the color of the fruit and seed was yellowish green and dark brown respectively and average weight per fruit was 14 ±0.79 gm. The average length and diameter per fruit were 14 ±1.4 mm and 20.35 ±0.35 mm respectively. On the other hand, Fruit contained 4-6 seeds and weight per 100g seed was containing 4000-4500 seed. Troup (1921) observed that, the fruits which are ripen from November to Februrary or sometimes later, are 0.5-0.8 in diameter, globose, yellowish green, smooth, fleshy and very astringent, with a 6-riged bony endocarp containing about four to six dark brown smooth 3 gonous seeds of which about 1800-1900 weigh 1 oz. Morton (1987) also observed that, the skin is thin, translucent and adherent to the very crisp, juicy, concolorous flesh. Tightly embedded in the center of the flesh is a slightly hexagonal stone containing 6 small seeds. Fruits collected in South Florida vary from 1 to 1 1/4 in 2.5-3.2 cm in diameter but choice types in India approach 2 in (5 cm) in width.

5.2 Seed germination in polybag

Different methods of pre-sowing treatments of seed germination to break down the seed dormancy and thereby increase the germination rate and speed up germination process were argued by several authors Seed dormancy can vary from species to species, stage of maturity of seed, degree of drought etc. Therefore, pretreatment should be adjusted accordingly. Furthermore, the findings of the present study show that seeds of *Phyllanthus emblica* under different treatments improved seed germination success significantly ($P<0.05$) higher than the control. Immersion in normal water treatment for 24 h showed the best germination success (75%) followed by immersion in sun heated water for 10 hours (63.33%) than control (51.33%) in polybag. DMRT showed significant differences among the treatments. The difference of germination percentages may be due to the difference of immersion time. As the seed is very small and the seed coat is slightly hard, low immersion time may increase the germination percentage and high immersion time may damage the seed resulting poor germination. However soaking the seeds of *Phyllanthus emblica* in water for about 12 h before sowing improve germination (Singh, 1988; Chacko et al., 2002) .Seeds soaked for 12h in 400 ppm gibberelic acid give 87.25% germination and those soaked in water give 56%

(Dhankhar et al., 1997; Dhankhar, Santosh Kumar, 1996; Rajamanickam et al., 2002; Wagh et al., 1998; Pawshe et al., 1997). Hot water soaking at 60°c for 5 minutes was found to be beneficial. Seeds treated with 1% KNO3 for 18 h give seed germination of 93.33% (Purbey and Meghwal, 2005).

5.3 Height growth

The highest height growth of seedlings was observed in sun heated water for 10 hours. After six months, the mean height growth under four treatments was close to each other. Analysis of variance (ANOVA) did not show any significant difference in height growth among the four treatments. During the month of (July-November) there was a sharp rise in height growth in the species. This increase in height growth might be due to adequate rainfall in this period that increased soil moisture in the polybags of the seedlings. Similar observations were also found during the growth study of some forest tree seedlings by Matin and Banik (1993). On the other hand, during December there was less height growth than previous period. The causes of this tendency of poor height growth might be due to water stress (Loomis, 1934: Matin and Banik, 1993). Leaf formation was found to increase throughout the periods. This reduction of photosynthetic surface decreased the relative amount of carbohydrates available for growth, as compared with unstressed plants (Kramer, 1969, Matin and Khan, 2000).

5.4 Diameter growth

The highest diameter growth of seedlings was observed in sun heated water for 10 hours. After six months, the mean diameter growth under four treatments was close to each other. Analysis of variance (ANOVA) did not show any significant difference in diameter growth among the four treatments. During the month of (July-November) diameter growth began to rise. The causes of the increase might be due to formation of new leaves and roots which ultimately increased the total carbohydrate concentrations to the seedlings. Similar instances were mentioned by Matin (1989) in *Nauclea diderrichii* cuttings. On the other hand, during December there was less diameter growth than previous period. It might be cause that water stress reduced photosynthesis and decreased translocation of carbohydrates and growth regulators, all these add to reduced turgor in reducing growth (Kramer, 1969).

5.5 Leaf Number

The highest average leaf number of seedlings was observed in sun heated water for 10 hours. After six months, the average leaf number under four treatments was close to each other. Analysis of variance (ANOVA) did not show any significant difference in leaf number among the four treatments. The species showed increased leaf number during first four months whereas the number slightly increases during last two months. It might be the fact that leaf shedding was influenced by water stress. Similar result also found in seedling growth study of different albizia species in different seasons of the year (Matin and Khan, 2000).

CHAPTER SIX: CONCLUSION & RECOMMENDATION

Conclusion

Among the pre-sowing pre-treatment, normal water immersion for 24h performed significantly well than others, though the performance of seed germination by the treatments of sun heated water for 10 hours was not bad. Nevertheless, the use of water for pre-soaking treatment of *Phyllanthus emblica* is quite simple and inexpensive for small scale farmers. On the other hand, growth performance (height and diameter) showed strong positive correlation. Leaf number also showed a good correlation with height and diameter growth. Therefore, growth in plants is not always controlled by their physiological or environmental factors rate is controlled by genetic factor.

Recommendation

- Normal water treatment for 24 h is suggested to obtain good germination percentage.
- Rainy season play an important role to reduce germination period and increase germination percentage. So it is suggested to sown in rainy season.

References

Alam MA, Matin MA, Hoque MM, Hoque ATMR.2005. Seed morphology and germination of Ipil-ipil (*Leocaena leococephala* Lam.)De Wit)under different condition at nursery stage in Bangladesh. Asian Journal of Plant Science, 4(2):98-101.

Anon.2014. Access on 27 February, 2014. http://www.highmowingseeds.com/sb-factors-affecting-germination-of-organic-seeds.html.

Azad MS, Biswas RK and Matin MA. 2012. Seed germination of *Albizia procera* (Roxb.) Benth. in Bangladesh: a basis for seed source variation and pre-sowing treatment effect. For Stud China, 12(2): 124–130.

Burkill IH. 1966. A Dictionary of the Economic Products of the Malay Peninsula, Vol. 1, Ministry of Agriculture and Co-operatives, Kuala Lump.

Calixto JB, Santos ARS, Filho VC and Tunes RA.1998. A review of the plants of the genus Phyllanthus: Their chemistry, pharmacology, and therapeutic potential. J. Med. Biol. Res.31: 225–258.

Chacko KC, Pandalai RC, Seethalakshmi KK, Mohanan C, George Mathew and Sasidharan N. 2002. Manual of Seeds of Forest Trees, Bamboos and Rattans. Kerala Forest Research Institute, Peechi, Kerala. 192-193.

Chaudhuri, Ratan K, Guttierez, Gilles and Serrar, Mustafa. 2003: Low Molecular-Weight Tannins of *Phyllanthus emblica*: A New Class of Anti-Aging Ingredients. Proceedings Active Ingredients Conference, Paris.

Copeland LO.1988. Principles of seed Science and Technology. S.S. Chabra for Surjeet publications, 7-k, Kolhapur Road, Kamla Nagar, Delhi- 110007.

Dhankhar DS and Santosh Kumar. 1996. Effect of bio-regulators on seed germination and seedling growth in aonla (Phyllanthus emblica Linn.) cv. Anand-2. Recent Horticulture. 3(1): 45-48.

Dhankhar DS, Santosh Kumar and Bhalla R. 1997. Effects of GA3 and thiourea on protein, peroxidase activity and chlorophyll status in seedlings of anola. Madras Agricultural Journal. 84(9): 529-531.

Drury, Colonel Heber1873.The useful plants of India; with notices of their chief medicinal value in commerce, medicine and the arts. Higginbotham and Co. Madras. ISBN No. not available.Ethnopharmacol.45: 1–18.

Govinda Nayaka D. 2006. Propagation of Anola (*Phyllanthus emblica L.*). Department of Horticulture College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad - 580 005. Chaptre no –ii.

Hartmann HT, Kester DE, Davis FT and Geneve RL. 1997. Plant Propagation Hensleigh TE and Holway, BK.1988. Agroforestry species for the Philippines. Washinton DC: US peace Crops, 404.

Jackson JK. 1987. Manual of afforestation in Nepal. Department of Forestry, Kathmandu.

Jari Olavi Summanen. 1999. A Chemical and Ethnopharmalogical Study on *Phyllanthus emblica.*

Jayaweera, DMA. Medicinal Plants used in Ceylon Part 2. National Science Council of Sri Lanka. Colombo 1980.

Kramer PJ. 1969. Water and its role in plants. In:Plant and soil water relationships: A modern synthesis.McGraw-Hill.New York:483

Kumar SV and Bhanja M. 1992. Forestry Seed Manual of Andhra Pradesh. Andhra Pradesh Forest Department, Hyderabad, India.

Kyaw N N. 2003. Site Influence on Growth and Phenotype of Teak (*Tectona grandis* Linn.f.) in Natural Forest of Myanmar. Göttingen, Cuvillier Verlag Göttingen.

Luna RK. 1996. Plantation Trees. International Book Distributors, Dhera-Dun, India.120-125.

Maguire JD.1962.Speed of germination in selection and evalution for seedling vigor.Crop Sci, 2:176-177.

Maitreyee Kundu. 2012. Seed Leaflet- *Emblica officinalis Gaertn.* Director, Tropical Forest Research Institute, P.O. R.F.R.C., Mandla Road, Jabalpur 482021, India.No-154.

Matin MA, Banik RL.1993. Effect of Polybag size on groth of some forest tree seedlings of Bangladesh. Bangladesh Journal of Forest Science, 22(1&2):37-43.

Matin MA, Khan MNI. 2000. Growth Performance of seven species of *Albizia* seedlings at nursery stages in Bangladesh. Khulna University studies, 1(2):277-282.

Matin MA. 1989. Carbon economy during rooting of cuttings of *Nauclea diderrichii(*de, wild and th. Dur.)Merill. M. Phil Thesis,Department of Forestry and Natural

Morton J. 1987. Emblic. p. 213–217. In: Fruits of warm climates. Julia F. Morton, Miami, FL.

Nadkarni KM, Nadkarni AK. 1999: Indian Materia Medica - with Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies. Vol1. Popular Prakashan Private Ltd., Bombay, India. ISBN No. 81-7154-142-9.

Onions, Alan: Siddha Medicinal Herbs As Cosmetics Ingredients. SPC, March 1994. Perry, L.M. (1980): Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. 149–150. MIT Press, Cambridge.

Pancel L. 1993. Tropical Forestry Handbook. Hamburg Germany, springer - Verlag.

Pawshe YH, Patil, BN and Patil LP.1997. Effect of pre-germination seed treatments on germination and vigour of seedlings in Aonla (*Emblica officinalis Gaertn.*). PKV Research Journal. 21(2): 152-154.

Purbey SK and Meghwal PR. 2005. Effect of pre-sowing seed treatment on seed germination and vigour of aonla seedlings. Research on Crops. 6(3): 560-561.

Rajamanickam C, Anbu S and Balakrishnan K. 2002. Effect of chemicals and growth regulators on seed germination in Aonla (*Emblica officinalis G.*). South Indian Horticulture. 50(1/3): 211-214.

Silva RP, Santos J dos, Tribuzy ES, Chambers JQ, Nakamura S and Higuchi N .2002. "Diameter increment and growth patterns for individual tree growing in Central Amazon, Brazil." Forest Ecology and Management 166(1-3): 295-301.

Singh IS. 1998. Anola: Production technology. Department of Horticulture, N.D. University of Agriculture and Technology, Kumarganj, Faizabad, India.

Srivastava AL, Singh HK and Singh IS. 2002. Studies on propagation of aonla (*Emblica officinalis*). Haryana Journal of Horticultural Sciences. 31(3/4): 156-158.

Thakur RS, Puri HS, Husain, Akhtar.1989. Major Medicinal Plants of India.. Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

Treadway, Linda. 1994. Amla Traditional food and medicine. HerbalGram.; 31:26. HerbalGram: The Journal of the American Botanical Council. Issue: 31. 26

Troup RS. 1921. The Silviculture of Indian Trees Vol. III. Clarendon Press, Oxford.

Unander DW, Webster GL and Blumberg BS 1995. Usage and bioassays in (Euphorbiaceae). University of Helsinki Department of Pharmacy, Division of Pharmacognosy.Chapter-3. 15.

Verma RK, Khatri PK, Kunhikannan C, Verma RKand Totey NG. 1998. Advantageous effects of the tree plantation on the rehabilitation of bhata land ecosystem. Indian Journal of Forestry. 21(3): 197-203.

Virendra Singh and Shafaat Mohammed. 1996. Effect of gibberellic acid and urea on growth and development of seedling rootstock of aonla (*Emblica officinalis Gaertn*). Journal of Applied Horticulture Navsari. 2(1/2): 116-121.

Vu Van Hung. 2013. Gradual transformation of Forest Plantations into Close-to-Nature Forests in NE Vietnam. Faculty of Forest Sciences and Forest Ecology, Georg-August-University Goettingen, Germany.p-92.

Wagh AP, Choudhary MH, Kulwal LV, Jadhav, BJ and Joshi PS. 1998. Effect of seed treatment on germination of seed and initial growth of aonla seedling in polybag. PKV Research Journal. 22(2): 176-177.

Appendices

Germination %

The SAS System 1

10:17 Sunday, June 4, 2000

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
TRTMENT	4	T1 T2 T3 T4

Number of observations in data set = 12

The SAS System 2

10:17 Sunday, June 4, 2000

Analysis of Variance Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	854.91666667	18.09	0.0006
Error	8	126.00000000		
Corrected Total	11	980.91666667		

R-Square	C.V.	GERM Mean
0.871549	6.341348	62.5833333

Source	DF	Anova SS	F Value	Pr > F
TRTMENT	3	854.91666667	18.09	0.0006

Analysis of Variance Procedure

T tests (LSD) for variable: GERM

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 8 MSE= 15.75
Critical Value of T= 2.31
Least Significant Difference= 7.4723

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRTMENT
A	75.000	3	T2
B	63.333	3	T4
B	60.667	3	T3
C	51.333	3	T1

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: GERM

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 8 MSE= 15.75

Number of Means	2	3	4
Critical Range	7.472	7.787	7.963

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRTMENT
A	75.000	3	T2
B	63.333	3	T4
B	60.667	3	T3
B			
C	51.333	3	T1

Height

The SAS System

1
23:34 Friday, June 2, 2000

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TRTMENT	4	T1 T2 T3 T4

Number of observations in data set = 12

The SAS System

2
23:34 Friday, June 2, 2000

Analysis of Variance Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	11.71993333	1.26	0.3511
Error	8	24.79073333		
Corrected Total	11	36.51066667		

R-Square	C.V.	GERM Mean
0.321000	3.307276	53.2266667

Source	DF	Anova SS	F Value	Pr > F
TRTMENT	3	11.71993333	1.26	0.3511

Analysis of Variance Procedure

T tests (LSD) for variable: GERM

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 8 MSE= 3.098842
Critical Value of T= 2.31
Least Significant Difference= 3.3145

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRTMENT
A	54.683	3	T4
A			
A	53.400	3	T1
A			
A	52.873	3	T3
A			
A	51.950	3	T2

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: GERM

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 8 MSE= 3.098842

Number of Means	2	3	4
Critical Range	3.314	3.454	3.532

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRTMENT
A	54.683	3	T4
A			
A	53.400	3	T1
A			
A	52.873	3	T3
A			
A	51.950	3	T2.
A			

Diameter

The SAS System 1
23:28 Friday, June 2, 2000

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TRTMENT	4	T1 T2 T3 T4

Number of observations in data set = 12

The SAS System 2
23:28 Friday, June 2, 2000

Analysis of Variance Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	0.06154233	0.83	0.5156
Error	8	0.19867933		
Corrected Total	11	0.26022167		

R-Square	C.V.	GERM Mean
0.236500	2.956771	5.32983333

Source	DF	Anova SS	F Value	Pr > F
TRTMENT	3	0.06154233	0.83	0.5156

Analysis of Variance Procedure

T tests (LSD) for variable: GERM

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 8 MSE= 0.024835
Critical Value of T= 2.31
Least Significant Difference= 0.2967

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRTMENT
A	5.4310	3	T4
A			
A	5.3637	3	T1
A			
A	5.2657	3	T3
A			
A	5.2590	3	T2

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: GERM

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 8 MSE= 0.024835

Number of Means	2	3	4
Critical Range	.2967	.3092	.3162

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRTMENT
A	5.4310	3	T4
A			
A	5.3637	3	T1
A			
A	5.2657	3	T3
A			
A	5.2590	3	T2

Leaf number

The SAS System 1

10:21 Sunday, June 4, 2000

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
TRTMENT	4	T1 T2 T3 T4

Number of observations in data set = 12

The SAS System 2

10:21 Sunday, June 4, 2000

Analysis of Variance Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	1.99000000	1.38	0.3173
Error	8	3.84666667		
Corrected Total	11	5.83666667		

R-Square	C.V.	GERM Mean
0.340948	2.310121	30.0166667

Source	DF	Anova SS	F Value	Pr > F
TRTMENT	3	1.99000000	1.38	0.3173

Analysis of Variance Procedure

T tests (LSD) for variable: GERM

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 8 MSE= 0.480833
Critical Value of T= 2.31
Least Significant Difference= 1.3056

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRTMENT
A	30.6000	3	T4
A A			
A	30.1000	3	T1
A A			
A	29.9000	3	T2
A A			
A	29.4667	3	T3

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: GERM

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 8 MSE= 0.480833

Number of Means	2	3	4
Critical Range	1.306	1.361	1.391

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRTMENT
A	30.6000	3	T4
A A			
A	30.1000	3	T1
A A			
A	29.9000	3	T2
A A			
A	29.4667	3	T3