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EFFECTS OF PRE- SOWING TREATMENT ON SEED GERMINATION OF *Diospyros Philippinensis*

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DECLARATION

I Md. Sohel Rana declare that the thesis is a result of my own works and that has not been submitted or accepted for a degree in any other university.

I, hereby, give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans, and for the title and summary to be made available to outside organizations.

Signature:

(Md. Sohel Rana)

DEDICATED TO

MY

BELOVED PARENTS

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Abstract

An experiment in seed germination techniques of *Diospyros philippinensis* was carried out in the nursery of the Forestry and Wood Technology Discipline, Khulna University, Bangladesh in order to discover the pre-sowing treatment effects on seed germination. Mature seeds of *Diospyros philippinensis* were collected from healthy trees of home garden and treated with four pre-sowing treatments, i.e., control, immersion in cold water (4°C for 24 h) and immersion in hot water (100°C for 1 minute) and scarification. Germination was conducted in poly-bags with a mixture of topsoil and cow dung in a ratio of 3:1. The results revealed that pre-sowing treatments affected the rate of germination of seeds. The highest germination success was 83% in the treatment of immersion in hot water (100°C) for 1 minute, followed by 72% in immersion in cold water for 24 hour and control condition. Germination started 12 to 21 days after seed sowing and completed in a period of 14 to 22 days in all treatments. ANOVAs showed statistically significant differences ($p < 0.05$) in seed germination starting dates, germination closing dates, germination period, germination percentages and rates of germination among the treatments, but no significant difference in rates of germination. The hot water (100°C for 1 minute) treatment is recommended for seed germination of *Diospyros philippinensis* in rural Bangladesh.

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CHAPTER ONE : INTRODUCTION

1.1 Background of the study

Diospyros philippinensis is a autotrophic, flowering tree. It is perennial. It is a small tree and its height ranges from 6m to 15m. *Diospyros philippinensis* is an evergreen tree that can grow from 18 - 33 metres tall. The straight, cylindrical bole can be up to 80cm in diameter. The plant has conical crown. It is endemic to the Philippines. It is terrestrial plant. It is mainly found in primary rainforest and secondary rain forest. It is a evergreen tree. But seed germination of this species is very poor due to seed dormancy. Seed treatment is to ensure both that seeds described for many species. Yet dormancy still causes problems of low germination rate several rainforest species. Partly of general knowledge of their seed physiology. Partly because of variation in dormancy rate (Babeley and Kandya, 1988). Pretreatment methods often have to be adjusted to individual species and seed lots based on experience and experiment (Schmidt, 2000) . knowledge of the biology and physiology of various types of dormancy and the occurrence in relation to regeneration biology may often suggest the nature of particular seed problems and possible pretreatment methods(Bhardwas and Chakraborty, 1994). There are several kinds of dormancy in which mechanical dormancy restricts to embryo development may be overcome either by gradual softening of the enclosing seed – coat pericarp (Bachelard ,1967). Physical dormancy is caused due to impermeable seed coat or fruit enclosure which prevent imbibition and sometimes also gaseous exchange. Physical dormancy differs between species. stage of maturity and degree of desiccation, pretreatment must be adjusted accordingly physical dormancy may be overcome either by manual scarification of the seed by piercing, nicking, chipping, filling or burning with the aid of knife, needle, hot wire burner, abrasion paper (Catalan and Macchiavelli, 1991),hot water treatment (Khasa, 1992, Kobomoo and Hellum, 1984).

In Bangladesh the species is widely used fruits, medicines and timber. The tree has been a commercial source of black ebony and has been overexploited to the point that the tree has become rare in the wild. Exploitation of the tree has caused the species to become rare; very little lowland forest remains. The plant is classifieds 'Endangered' in the IUCN Red List of Threatened Species. A strong, unpleasant, cheese-like odour is given off by the whole fruit. It is moist but not very juicy, more or less sweet flavored (IUCN, 2013).

The effects of pretreatment on the seed germination of some species are reported by (Ahmed et al., 1983), (Nagarajan and Christopher,1991), (Koirala et al., 2000), (Alamgir and Hossan 2005).

Information on the effects of *Diospyros philippinensis* seed pre- treatment is scant poor germination and delayed nursery establishment limits the wide cultivation of the species in both forestry and homestead plantation programs. A good planning and profitability of forest nurseries depends on the appropriate techniques that speed up germination and obtain a more reliable germination seeds sown (Koirala et al.,2000), (Alamgir and Hassan,2005). Thus the objective of the present study was to determine the optimum pretreatment methods that maximize germination percentage.

1.2 Objectives of the study

Diospyros philippinensis is a well known species. Its edible fruit has a skin covered in a fine. Before doing any experiment it is important to fix objectives. The overall objective of the work was to provide scientific knowledge about germination trail of the species.

However, the specific objectives were

To find out the effect of different treatments on seed germination.

CHAPTER TWO: GENERAL DESCRIPTION

2.1 General description of the family

The Ebenaceae is a family of flowering plants belonging to order Ericales. It includes a lot of species of trees and shrubs. The family is distributed across the tropical and warmer temperate regions of the world. The family is the most diverse in the rainforests of Malesia, India, tropical Africa and tropical America. Many species are valued for their wood, particularly ebony, for fruit, and as ornamental plants.The fruits contain tannins, a plant defense against herbivory, so they are often avoided by animals when unripe. The ripe fruits of many species are a food source for diverse animal taxa. The foliage is consumed by insects. The plants may have a strong scent. Some species have aromatic wood. They are important and conspicuous trees in many of their native ecosystems, such as lowland dry forests of the former Maui Nui in Hawaii Caspian Hyrcanian mixed forests, Kathiarbar-Gir dry deciduous forests, Louisiade Archipelago rain forests, Madagascar lowland forests, Narmada Valley dry deciduous forests, New Guinea mangroves, and South Western Ghats montane rain forests. Ebony is a dense black wood taken from several species in the genus Diospyros, including *Diospyros ebenum* (Ceylon ebony, Indian ebony), *Diospyros crassiflora* (West African ebony, Benin ebony), and *Diospyros celebica* (Makassar ebony). Diospyros tesselaria (Mauritius ebony) was heavily exploited by the Dutch in the 17th century. The family includes trees and shrubs. The leaves are usually alternately arranged, but some species have opposite or whorled leaves. The inflorescence is usually a cyme of flowers, sometimes a raceme or a panicle, and some plants produce solitary flowers. Most species are dioecious. The flower has 3 to 8 petals, which are joined at the bases. There are usually several single or paired stamens, which are often attached to the inner wall of the corolla. Female flowers have up to 8 stigmas. The calyx is persistent. The fruits are berry-like or capsular. Like the wood of some species, the roots and bark may be black in color. The family name Ebenaceae is based on the genus name Ebenus (Estrada et all., 2013). It is a later homonym of Ebenus L., a genus already named in the family Fabaceae, and is thus nomen illegitimum. The plant that Kuntze had named Ebenus was accordingly reassigned to the genus Maba, which in turn has since been included in the genus Diospyros. Because the name Ebenaceae had become well known, having been used in major botanical references such as Bentham and Hooker's Genera Plantarum, Engler and Prantl's Natürlichen Pflanzenfamilien, and Hutchinson's Families of Flowering Plants, it was conserved and is therefore legitimate. During the last century, seven genera have been included in the family at one time or another. One phylogenetic analysis reduced the family to four genera.

2.2 General description of the species

The white waxy flowers have 4 petals and are 0.5 inches (1 cm) in diameter when fully open. Male and female flowers are produced on separate trees. Both flowers are necessary for pollination and fruit set. Fruiting generally occurs during summertime with fruits ripening from 2-4 months after flowering. Usually grown from seeds. Very beautiful dark red to purple colored fruit with velvet-like skin. Fruit is about the size of an apple, with mildly sweet flavored, somewhat mealy, flesh. Fruits are highly esteemed in some areas, but barely known in most parts of the world. Usually eaten fresh out of hand or used in salads and desserts. Mabolo is a medium-sized tree growing to a height of 20 meters. Leaves are leathery, oblong, up to 20 centimeters long, with a round base and acute tip. The blade is glossy green, smooth above and softly hairy below. Female flowers are axillary and solitary, larger than the male. Fruits are fleshy, globose, up to 8-10 centimeters in diameter, densely covered with short brown hairs. The pulp is edible. The fruit hairs have to be rubbed off before eating as it can cause peri-oral itching and irritation (UADA, 2007). Ethyl acetate extract of air-dried leaves yielded isoarborinol methyl ether a mixture of a-amyrin palmitate, α-amyrin palmitoleate, ß-amyrin palmitate and ß-amyrin palmitoleate and squalene. Leaf extract yielded alkaloids, reducing sugar, gum, flavonoids, and tannins. Fruit is high in tannin. Analysis for phenolic compounds yielded predominant amounts of rosmarinic acid followed by luteolin and hispidulin Nutritional analysis per 100 g of edible fruit yields 113 kcal, 26.6 g carbohydrates, 1.5 g fiber, 0.1 g fat, 58 mg calcium, 2.8 g protein, 18 mg phosphorus, 0.6 mg iron, 35 IU vitamin A, 0.02 mg thiamine, 0.03 mg riboflavin, 0.03 mg niacin, and 18 mg vitamin C. It has an unpleasant, foul cheesy odor which can be dissipated by skin removal and processing (Lunar and Arcega , 2011).

2.3 Geographic distribution

Mabolo is indigenous to the Philippines. It is a plant of very common occurrence there and is widely distributed in primary and secondary forests at low and medium altitudes. Besides Philippines, it is also distributed in Taiwan. It is cultivated in Peninsular Malaysia, Sumatra and Java, and other tropical countries. It is usually cultivated as a backyard plant. It has been introduced into other tropical countries. Mabolo is a very beautiful dark red to purple colored fruit with velvet-like skin. In fact the word, mabolo in the Philippine language means "hairy". So the name Mabolo has been assigned to it because of its hairy surface. Mabolo is higly esteemed locally but is virtually unknown in other parts of the world. Leaves alternate, oblong, 8-30 cm x 2.5-12 cm, entire, base usually rounded, apex pointed, coriaceous; upper surface dark-green, shiny, glabrous, lower surface silvery hairy; young leaves pale-green to pinkish, silky-hairy; petiole up to 1.7 cm long (USDA,2007).

2.4 Habitat and climatic requirement

D. Philippines grows well in areas with a monsoon climate from sea level to 800 m elevation, and on almost any soil. It is very resistant to typhoons. It is usually propagated by seed taking up to 24 days to germinate. It can be propagated vegetatively by marcotting, budding and grafting, the latter method being commercially used in the Philippines. In cleft grafting, 1-year-old seedlings are used as a rootstock. The scions are obtained from mature, current season's growth with well developed terminal buds and cut 10-12 cm long. Grafted young trees may be planted in the field 8-10 m apart at the onset of the rainy season. Seedling trees are planted along avenues at a spacing of 10-15 m. Once established in the field, the trees hardly receive any care. Watersprouts and interlacing branches are occasionally pruned, so are branches that touch the ground. Some insects have been reported to feed on shoots and leaves: toy beetles, leaf rollers, slug and tussock caterpillars, bagworms, and red scales. These are minor pests, however. No serious disease has been reported. (Valencia et al., 2004). The fruits are considered mature when they turn from greenish-brown to dull red. After harvest they are usually wiped with a piece of cloth to remove the hairy bloom to make them look more attractive. In 3-4 days, the fruits soften and become aromatic. Seedling trees exhibit a high degree of variability in leaf and fruit characters. Fruits vary in shape, size, flesh colour, degree of seediness and taste. A seedless, white-fleshed, sweet cultivar is being clonally propagated in the *Philippines*. *D. Philippines* is a very productive fruit tree. The fruit, however, lacks sweetness and is rather dry, making it less popular than many other tropical fruits. Unless cultivars that bear sweet, juicy fruits are developed, it will remain a minor crop. Its potential for processing has to be studied (Staples et al., 2003).

2.5 Economic importance

Edible Uses

Fruit - raw or cooked. A white or cream-coloured flesh, the ripe fruit is mealy, aromatic and somewhat sweet The fruit emanates a strong, cheese-like odour, it can be rather dry and astringent. The aroma is contained mainly in the skin, which is normally removed before the fruit is eaten It should be stored for 3 - 4 days after harvest in order to ripen more fully. The flesh can also be diced and combined with that of other fruits in salads. Some people prefer eating the unripe fruit, being crisp like an apple but juicier and sweeter. The furry skin is unpalatable and can cause irritation in the mouth - it needs to be removed before the fruit is eaten. The velvety, brown-reddish fruit is a globose or depressed-globose berry, 5 - 12cm x 8 - 10cm (Lemmens RHMJ et al.,1996).

Medicinal Uses

A decoction of the young leaves is used as a treatment for hypertension, heart ailments and diabetes. The leaves are heated and squeezed with the leaves of Plectranthus amboinicus to make a preparation that is used to treat chest colds. The bark is astringent, A decoction is used as a treatment for coughs, fevers, dysentery and diarrhea.

The bark and the leaves are used as a wash to treat skin ailments such as itchy skin. They are also used as an eyewash. The juice of the bark and leaves is used to treat snakebites .The juice of unripe fruit is astringent. It is used as a wash for wounds. An infusion of the fruit is used as a gargle in treating aphthous stomatitis An oil expressed from the seeds is used as a treatment for diarrhoea and dysentery (Newman MF et al.,1996).

Health Benefits of the *Diospyros philippinensis*

Hypertension and Heart Health: this species content is rich with using potassium content means that the fruit can function as a vasodilator, which relaxes the blood vessels, reduces stress on the cardiovascular system, and lowers blood pressure. The dietary fiber content can also lower cholesterol, so these two elements combined can greatly reduce atherosclerosis, blood clots, heart attacks, and strokes.

Circulation: The significant iron content in *Diospyros philippinensis* boosts the red blood cell count in the body, increasing oxygenation of important tissues and muscle groups, stimulating the growth of hair, speeding up the healing process of cells, and boosting metabolic efficiency.

Respiratory Conditions: In traditional medicine, *Diospyros philippinensis* have been commonly used to relieve coughs, chest congestion, and asthma, possibly due to the high content of vitamins and minerals, which also act to improve the immune system.

Immune System Strength: the high levels of vitamin C and vitamin A boost the immune system of the body by acting as antioxidants, eliminating damaging free radicals that can mutate or kill healthy cells. These two vitamins prevent premature aging, chronic diseases, stimulate cellular development and growth, and increase the health and appearance of the skin.

Digestive Health: The dietary fiber in *Diospyros philippinensis* helps to ease the passage of food through the digestive tract, thereby eliminating constipation and other gastrointestinal issues. In traditional medicine of the Philippines, velvet apples were relied on to cure dysentery and diarrhea, perhaps its most common application in human health.

Skin Irritation: When topically applied or consumed, the pulp of *Diospyros philippinensis* has shown remarkable ability to reduce inflammation and irritation on the skin, and is often turned to in alternative medicine as the fastest way to heal skin conditions and burns. Furthermore, velvet apple pulp and juice are applied to snakebites and other toxic incursions in the body, neutralizing toxins and helping to reduce the effects of these issues.

Other Uses

Sometimes used as a rootstock for the persimmon. The fresh wood is said to act as an insect repellent. The heartwood is streaked and mottled, sometimes nearly dead black; it is clearly demarcated from the up to 20cm wide band of reddish or pinkish sapwood. The sapwood sometimes stains more or less to a dull gray. The wood is smooth and durable, and is much used in the Philippines in making handicrafts. The wood is also used as streaked ebony, especially for carvings and special furniture (Bohlman at all, 2008).

CHAPTER THREE: REVIEW LITERATURE

The activation of the metabolic machinery of the embryo leading to the emergence of a new seedling plant is known as germination. Germination or sprouting is the resumption of growth by the dormant embryo or young plant is the seed. It is complete when growth has ruptured the seed coats and the emerged. For germination to be initiated, three conditions must be fulfilled.

First: the seed must be viable, that is the embryo must be alive and capable of germination.

Second: the seed must be subject to the appropriate environmental conditions, available water, proper temperature regimes, a supply of oxygen, and sometimes light.

Third: any primary dormancy is collectively known as after ripening and results from the interaction of the environment with the specific primary dormancy condition (Hamann et al.,1999).

Rapid germination of seeds is very desirable because the shorter the time required the less opportunity there is for injury by insects, fungi or unfavorable weather condition or for seeds to be eaten by birds or redents. Many environmental factors can affect seed germination. Light intensity, day length light color, water quality, gravity crowding , temperature, chemical agents, genetics, oxygen availability, seed condition, seed age, seed coat condition , seed size and other environmental condition can have measurable effects on seed germination. The most important environment factors controlling seed germination are water, temperature, light and radiation, aeration, oxygen and various chemicals. Non dormant seeds must imbible a certain amount of water before they resume the physiological processes involved regions of the world. The family is most diverse in the rainforests of Malesia, India, tropical Africa and tropical America (Bawa et al.,1991).

Dormancy can be defined as the physiological condition of a viable seed that prevents germination. During dormancy, the seed's metabolic rate is extremely slow, and it uses very little energy to stay alive. Germination is the reactivation of metabolic activity in the seed, an awakening from dormancy. It culminates with the emergence of the radicle (embryonic root) and finally, the plummule (Kaye et al.,1997).

Many seeds undergo a period of dormancy, during which metabolic activity is minimal. In part, dormancy can be maintained by a hormone, abscisic acid (ABA), which permeates the seed coat. In order for germination to commence, this water-soluble ABA must be rinsed out of the seed coat. Hence, most seeds will not begin to germinate until they have been rinsed or soaked in water. Changes in oxygen, light, temperature, soil chemistry, and/or humidity each may play a role in triggering germination. The effect of any environmental stimulus on a seed depends on its own unique genes as determined by the evolutionary history of its ancestors and any new mutations it may carry. As you already know, there may be genetic variation not only among different species, but also among individuals of the same species. Don't forget this when you design your experiment. Following is a bit of background on some environmental factors that could affect dormancy and germination in various flowering plant species. Consider not only these factors, but also the anatomy and natural history of the species provided for you in the lab. Since multiple factors may affect germination, you must be aware of them all to properly interpret your experimental results Some seeds will not germinate until they have undergone a process of after-ripening, defined as metabolic changes that must take place in a seed in order for it to overcome dormancy. For example, the seeds of some temperate species will not germinate until after they have been exposed to very cold temperatures. Other seeds must pass through an animal's digestive tract and become scarified (physically damaged) before they can take up water and become metabolically active. Still others must pass through fire, which is common in ecosystems where annual fires are part of the normal climate. A pigment is a substance that absorbs and reflects light in a characteristic fashion. In living systems, organic pigments can trigger biological reactions. Plants have a variety of pigments that function in capacities other than photosynthesis. Even the seeds of some plant species may have special photosensitive pigments that "tell" the seed whether it is close to sunlight or not. Short-wavelength light (e.g., violet and blue) penetrates deeper into soil (and other substrates, such as water) than long-wavelength light (To detect light, any organism must have some sort of pigment, a light-absorbing substance. In this case, it must be a pigment that can trigger a biological reaction. Phytochromes Phytochromes are proteinaceous molecules with a sulfur-linked (covalently bonded) pigment active group (the chromophore) consisting of a linear tetrapyrrole. The pigment absorbs primarily in the red and far-red region of the spectrum, and so appears blue-green to our eyes. The functional phytochrome consists of two identical proteins, each with a chromophore. One part of the protein acts as the photoreceptor, and the other as a kinase, which triggers cellular responses. When the chromophore absorbs light, it isomerizes from one form to the other. This change in configuration results in a slight change in the kinase portion of the protein. The kinase is the biologically active region of the molecule, and its interaction with other biological molecules elicits a physiological response. Different plants have different responses to temperature depending on their evolutionary history and where they have evolved. Cool temperature tolerant plants are found primarily in temperate regions (between 30° – 60° latitude, north and south). Optimum temperature for growth ranges between 77° - 86°F, although various species can tolerate a range of 40° 104°F. Cool temperature tolerant species include domestic cultivars of Brassica oleracea, such as broccoli, cabbage, turnips, cauliflower, etc. as well as many temperate grasses. Cool temperature requiring species require temperatures lower than 77°F. Seeds from these species will generally not germinate if temperatures are too warm, but require afterripening triggered by a period of very cold temperatures (40° F or lower). Examples include lilacs, freesias, apples. Warm temperature requiring plants tend to occur in the tropics (between 0° – 23.5 ° latitude, north and south) and subtropics (between 23.5° – 30 ° latitude, north and south). These species require temperatures of at least 50° - 60° F in order to germinate. Examples include beans, eggplant, corn, tomatoes, and peppers. Alternating/ Fluctuating day- night temperature plants. Some seeds require a drastic temperature differential in order to break dormancy. Such species as conifers and wild roses need at least an 18° F (10°C) difference between day and night before they can germinate. Soil must be of adequate hydration, oxygen levels, proper particle size, and tolerable chemical environment in order for seeds in the soil to germinate. Depth of planting can have marked effects on seed germination success, as oxygen and light decrease with soil depth. Soil particle size can also affect these factors: the smaller the soil particle size, the less passage of light there will be through the soil. Acidity or alkalinity of soil can affect germination success or germination rate. Soil that is hypertonic also can inhibit germination, as high osmotic potential in the seed's environment will prevent water from moving into the seed (Jahn and et al., 1998). Too much fertilizer can thus inhibit seed germination, as can living in a very dry environment, such as a desert. Soil water must be present in the appropriate amount. Too little water will prevent proper metabolism, whereas too much can literally drown the seeds, which die of anoxia (lack of oxygen). You can probably think of other variables of soil condition that might affect germination rate or germination success. Consider every possible option before you decide on the question your team will answer (Cadiz et al., 2010).

CHAPTER FOUR : MATERIALS AND METHOD

4.1 Materials

Seed source and collection:

The fruits of *Diospyros philliphinensis* were collected from some healthy and well formed trees. The fruits were collected from Batiaghata of Khulna. The fruits were collected in the month of June 2016. The mother tree of the fruits was very young. Fruits were collected manually. Before seed collection the mother tree ground was cleared by sweeping for removing all dead branches, leaves and floating grass etc. This was done because freshly fallen fruits in fresh ground were needed where there was no possibility of mixing up with the previously fallen fruits. As a result, ripe, pure and healthy fruits were obtained from the tree ground. The fruit collection procedure was done with my own hands. The owner of the tree also helped us for the collection procedure of fruits.

Seed extraction and storage

After collecting the fruits, the fruits were taken to the Khan Jahan ali hall of Khulna university. The seeds were separated from the ripe fruits. It was done in the the next day. It was done manually. My friends Rabin and Ripon also help me for doing this. The seeds were dried in the open sun for a day and only the disease free, healthy seeds were packed with a paper box for the use in the experiment.

Rooting medium :

The waste cow dung was collected from a dairy farm of a farmer. The cow dung was collected Banorgati of Khulna. The fertile soil was collected from Khulna university campus mix it with the cow dung in 3:1 ratio. The mixture of soil and cow dung were filled in polybags collected from our nursery. The sizes of the polybags were 10×15 cm.

The polybags were kept in the nursery of FWT Discipline. The study was carried out with 4 treatments.

4.2 Methods:

A total of 400 seeds were used for the experiments. One seed is used in one poly bag. The treatments are listed below:

i) Control / no Treatment (T_0)
ii) Immersion in cold water for 24 hour.(T_1)
iii) Immersion in hot water of 100^0c (T_2)
iv) Scarification with rough and sand paper (T_3)

Normal condition

Freshly collected seeds were directly sown in polybags.

Immersion in cold water for 24 hour:

100 seeds were submersed in cold water for 24 hour for this treatment before sowing. After this procedure, the seeds were kept out from the water and placed on a moisture free paper.

Hot water (100^0c) immersion 1 minute:

The seeds were soaked in hot water for 1 minutes in this treatment. For this the outer portion of the seeds became soften. With the help of electric heater some water was heated for this treatment. After some times the heating process was completed. After that, the temperature was checked out with the help of a thermometer. And when the thermometer showed the 100^0c temperature of the water then the seeds were immersed for 1 minute in that hot water. Just after 1 minute the seeds were removed from the water. 100 seeds were immersed, in this procedure.

Scarification with rough and sand paper:

Scarification helps to remove the seed coat for allowing inhibition. The seeds were scarified with rough and sand paper for the experimental purpose. The roughness power of the sand paper was low because high roughness power of the sand paper can damage the embryo. At first stage of the procedure the sand paper was kept on a table, then a seeds was taken with two fingers and kept on the sand paper and then moving the seed on the sand paper. For this procedure the outer surface of the seed was degrading. The abrasion was stopped when the outer surface was very thin. With the help of this procedure, 100 seeds of *Diospyros philliphinensis* were scarified. This procedure was done very carefully because if there was any abnormality the main objective of the experiment could be damaged.

Seed germination:

The germination percentage was calculated by using the following equation:

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds planted}} \times 100$$

Germination rate

$$= \frac{\text{Number of germinated seeds in first week}}{\text{7 days}} + \frac{\text{Number of germinated seeds in second week}}{\text{14 days}} + \frac{\text{Number of germinated seeds in third week}}{\text{21 days}} .... + \frac{\text{Number of germinated seeds in last week}}{\text{Total days needed in germination}}$$

Data analysis:

Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) (Duncan, 1955) were carried out to analyze the data, using the MS Excel and Statistica software the SPSS software and SAS software to explore the effect of the treatments. Specifically, the ANOVA was carried out to determine the effect of treatment effect (T) on starting and closing dates of germination, on germination periods, germination percentages and rates of germination. DMRT was conducted to compare mean germination closing dates, germination periods and germination percentages of the effect of different pre -sowing treatments. DMRTs were also carried out to compare mean germination closing dates and germination percentages of the seed pre-treatments.

CHAPTER FIVE : RESULTS

Results

Germination starting days, germination closing days, germination period, germination percentage and germination rate are given below

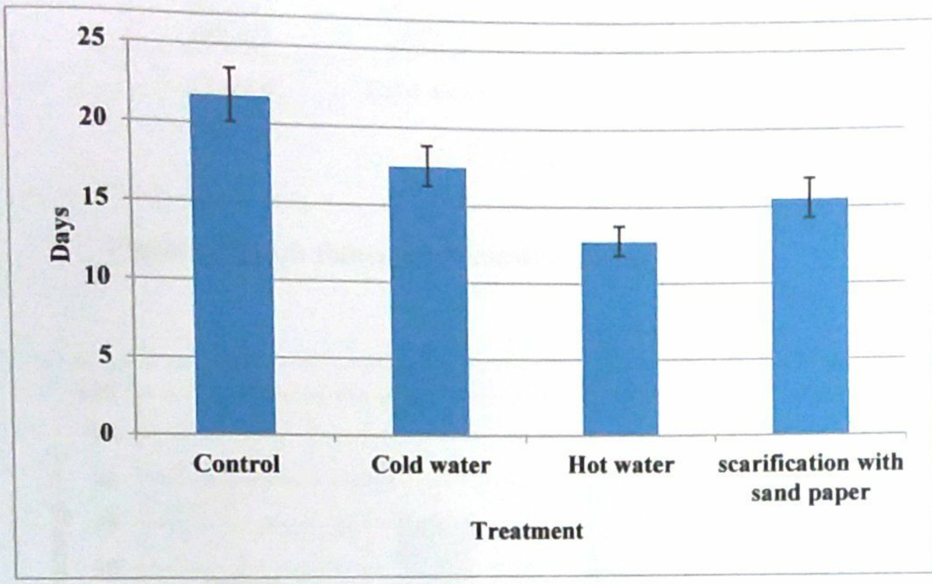

Figure 1: Figure showing germination starting days

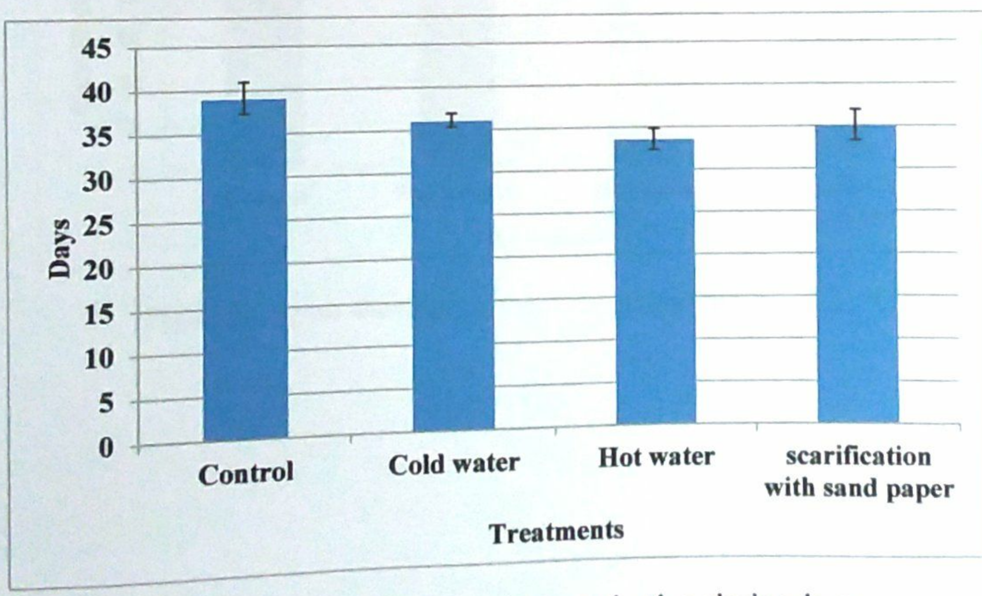

Figure 2: Figure showing germination closing days.

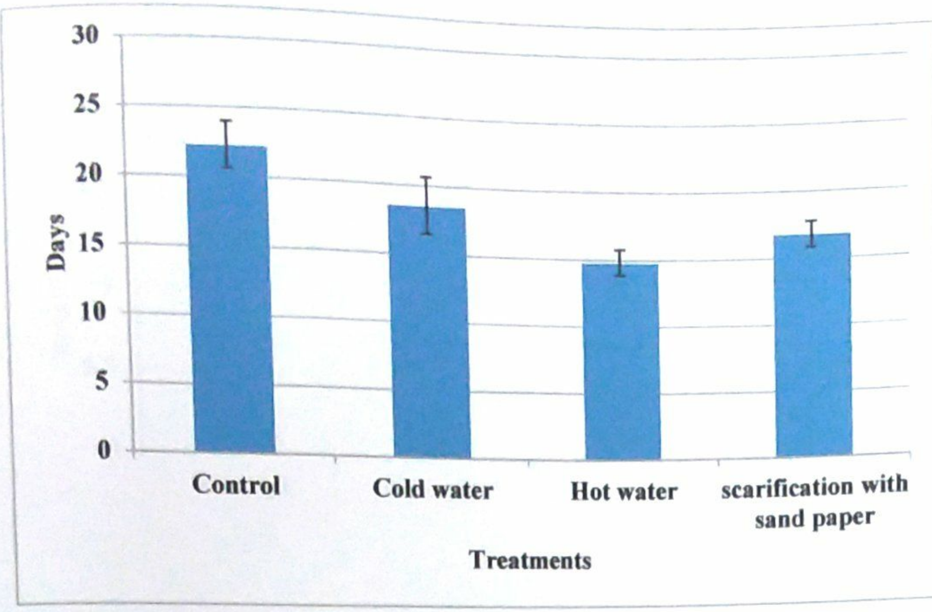

Figure 3:Figure showing germination period.

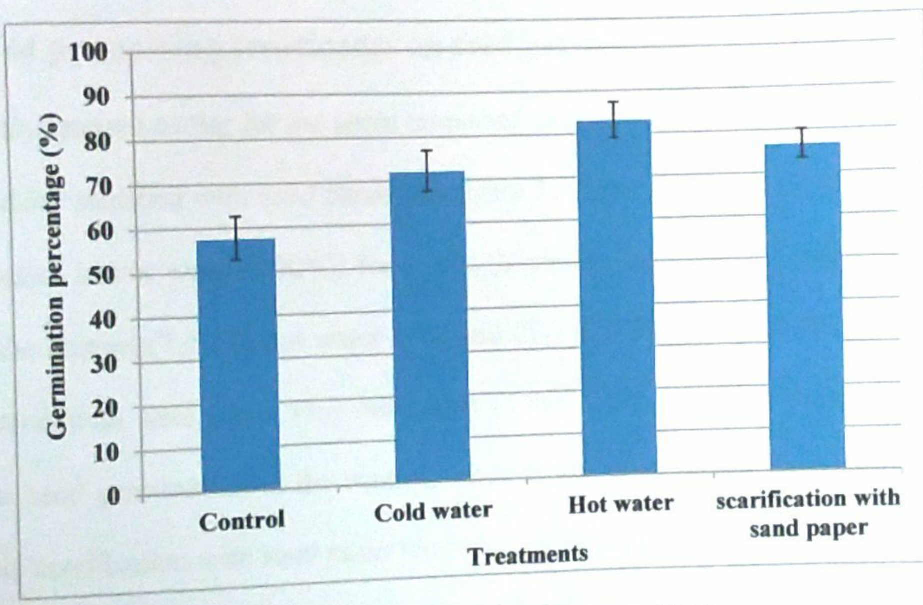

Figure 4: figure showing germination percentage.

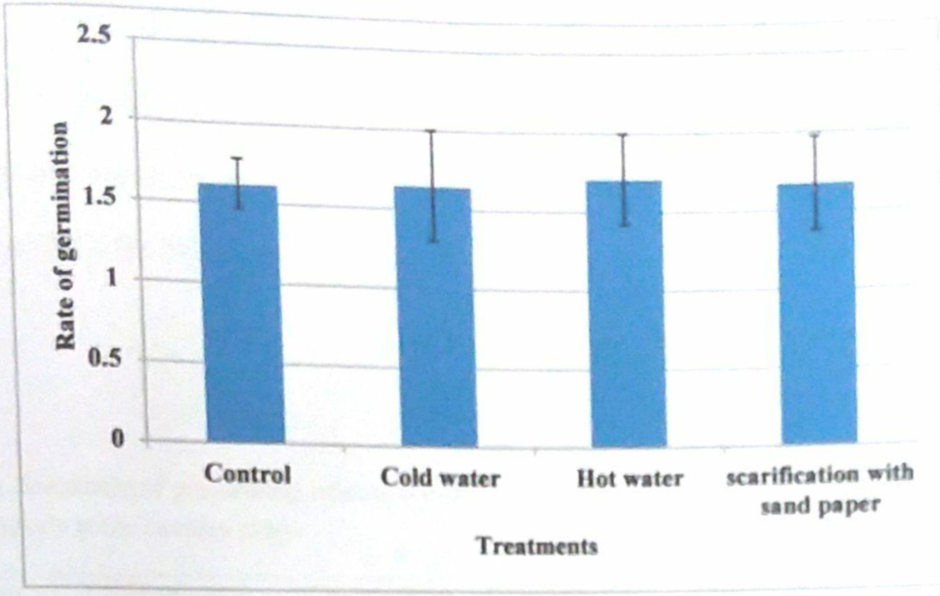

Figure 5: figure showing germination rate.

Effects of pre-sowing treatments on seed germination

Germination started earlier for the seeds immersed in hot water (100°C) for 1 min (treatment T_2) and those scarified with sand paper (treatment T_3), than in the control (treatment T_0) and seeds soaked in hot water (100°C) for 1 min (treatment T_2) (Table 1). Germination closed later in the control (T_0) and hot water treatment (T_2) ($p < 0.05$) than in cold water (T1) and scarification with sand paper (T_3) treatments (Table 1). It took an average of 39 days to complete seed germination in the control, while it was completed 7 days earlier in the hot water and scarification with sand paper treatments. Germination was completed within 33–39 days (Table 1). The highest germination success (83%) was found in the hot water treatment, which differed significantly ($p < 0.05$) from the lowest germination success (57.5%) in the control. Germination success (76.5%) scarification with sand paper (T_3) and (72%) cold water (T_1). ANOVAs showed no significant difference in rates of germination the treatments but there were significant differences in germination starting dates, germination closing dates, the germination periods and germination percentages (Table 2). DMRT showed no significant difference of seed germination between the treatments of scarification with sand paper

(76.5%) and immersion in cold water (4°C) for 24 h (72%), but these differed significantly ($p < 0.05$) from the control (57.5%) and the hot water treatment (83%) (Table 1). There was significant difference between the control and scarification with sand paper and immersion in cold water (4°C) for 24h treatment (Table 1).

Table 1: Summary of pre-sowing treatment effects on specific expressions of seed germination of *D. philippinensis* at the nursery stage

Treatment	Germination starting date (d)	Germination closing date (d)	Germination period (d)	Germination percentage (%)	Rate of germination
T_0	21.75 ± 1.70a	39 ± 1.82a	22.25 ±1.70a	57.5 ± 4.93c	1.61 ± 0.15
T_1	17.5 ± 1.29b	36 ± 0.82b	18.5 ± 2.08b	72 ± 4.69b	1.65 ± 0.34
T_2	12.75 ± 0.95b	33.5 ± 1.29c	14.75 ± 0.95c	83 ± 4.16a	1.71 ± 0.28
T_3	15.5 ± 1.29c	35 ± 1.82b	16.75 ± 0.96b	76.5 ± 3.16b	1.67 ± 0.29

Note: same letter(s) in the same column indicate(s) insignificant differences and data are shown in mean ± SD at $p < 0.05$. T_0: control, T_1: immersion in cold water (4°C) for 24 h, T_2: immersion in hot water (100°C) for 1 min, T_3: scarification with sand paper.

Table 2: Summary of p-values of treatment effects of seed germination of *D. philippinensis* at the nursery stage

Source	Particular	p-value
	Germination starting date (d)	0.0001
Treatment effects (T)	Germination closing date (d)	0.0016
	Total germination period (d)	0.0001
	Total germination (%)	0.0001
	Rate of germination	0.9743

CHAPTER SIX : DISCUSSION

Discussion

In case of *Diospyros philippinensis*, among the four treatments of seeds, hot water (100°C) immersion (1min) showed the best germination rate of 83%, followed by 72% in immersion in cold water and 76.5% in scarification with sand paper . The lowest germination (57.5%) was found in control. Ali et al. (1997) carried out an experiment on hot-water treatment (50°C) and boiling for 3minutes) on a procera and found 43% seed germination rate. The difference of germination rate may be due to the difference of temperature applied and the boiling time. It showed very clearly that the seed coat of *Diospyros philippinensis* was thinned very well at 100°C for a period of 1 minute. A previous work (Azad et al 2006a) on the seed germination of A. lebbeck indicated that the highest germination was 52% in hot-water treatment, which may be due to the variation of seed coat thickness. Anyhow, it is not difficult to measure the temperature of the boiling water for commercial purposes for the nursery owners. And, it will also be convenient for the local people if it is boiled at 100°C where they need not measure the temperature. In a previous experiment on pre-sowing treatment effect on the seed germination of *Xylia kerrii* in Bangladesh, it was found 80% success in concentrated H2SO4 treatment for 20 minutes (Azad et al.,2006a).

CHAPTER SEVEN : CONCLUSION

Diospyros philippinensis is an important tree species for its medicinal values. Thus the species is very interesting to the researchers to determine appropriate seed germination techniques. Among the four pre-sowing treatments, hot water immersion performed very well seed generation for the species. However, scarification technique is somewhat risky and troublesome. On the other hand, seed germination under hot water treatment is quite simple and inexpensive. Therefore, it is suggested to apply hot water treatment on seed germination for *Diospyros philippinensis* in rural Bangladesh.

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