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New Approach to Select Top-dying Resistant Sundari (*Heritiera fomes*) Trees from the Sundarban of Bangladesh

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Abstract

Selecting exceptionally *Heritiera fomes* seedlings from nursery is a promising and low-cost means of tree improvement, according to the study. From 2010 to 2016, 18,000 outstanding seedlings were chosen from nursery in the Sundarban and out planted. The final assessment as to efficiency of seedlings selection from selected trees of top-dying affected and non-affected (Healthy) areas awaits comparison of progeny from chosen selects with those from similarly chosen controls to see how much of the phenotypic gain is truly genetic. This investigation involved more selections and plantations than had not been tried for *H. fomes* in the past. The next step in evaluating nursery selection is to compare progeny from selects with those of controls to see how much of the phenotypic gain is truly genetic. The great promise of L₃, L₄, L₁₂, L₁₆, L₃₀, L₃₅ & L₃₆ line of *H. fomes* trees has been achieved with expected superior genetic material of unknown origin. This is reflected in the high variability observed in growth amongst individuals. Using advanced propagation techniques, these can be cloned and potentially provide the genetic base for a highly successful clonal forestry program for creation of top dying resistant *H. fomes* trees.

সারসংক্ষেপ

গবেষণালব্ধ তথ্যানুসারে প্রকাশ, নার্সারিতে বাছাইকৃত সুন্দরী চারা উন্নত জনক্রম সৃজনে সাশ্রয়ী এবং ফলদায়ক। নার্সারিতে উত্তোলিত চারার মধ্য থেকে ১৮,০০০ বিশেষ গুণ সম্পন্ন সুন্দরী চারা বাছাই করে ২০১০ সাল থেকে ২০১৬ সাল পর্যন্ত সুন্দরবনে পরীক্ষামূলক বাগান সৃজন করা হয়। সুন্দরী আগামরা এবং সুস্থ্য-সবল গাছের সংগৃহীত বীজ থেকে উত্তোলিত চারার মধ্য হতে বাহ্যিকভাবে প্রকাশিত সুস্থ্য-সবল বাছাইকৃত চারার মধ্যেই রয়েছে প্রকৃত কৌলিতাত্ত্বিক গুণাগুণ। এ ধরনের নির্বাচন পদ্ধতিতে সুন্দরী গাছের বাগান সৃজন পূর্বে করা হয়নি। পরবর্তী ধাপে জনক্রমের মধ্য থেকে প্রকৃত কৌলিতাত্ত্বিক গুণ সম্পন্ন জনক্রম নির্ধারণ করা হয়। সুন্দরী গাছের L₃, L₄, L₁₂, L₁₆, L₃₀, L₃₅ এবং L₃₆ জনক্রম সারী সমূহের মধ্যে অজানা উৎসের চাহিদামাফিক উন্নত কৌলিতাত্ত্বিক গুণাবলী রয়েছে যা বিশেষভাবে আশাব্যাঞ্জক। এর প্রভাব জনক্রমসমূহের ভিন্নতায় পরিলক্ষিত হয়। সফল ক্রোনাল ম্যানগ্রোভ বনায়ন কার্যক্রমে অত্যাধুনিক উদ্ভিদ প্রজনন কৌশল ব্যবহার করে আগামরা সহিষ্ণু ফলপ্রসু সুন্দরী গাছ সৃজন করা সম্ভব।

Keywords: Genotype-phenotype interactions, floristic composition, phenotypic gain, progeny, propagation techniques.

Introduction

The Sundarban is the largest single tract natural mangrove forest in the world. It is located at the southern part of the Ganges delta spanning over an area of 6,017 km² (5,77,285 ha) in Bangladesh. Out of this approximately 70% are lands and 30% are water bodies. The floristic composition of this forest is very rich compared to other mangrove forests of the world. Sundari (*Heritiera fomes*) and gewa (*Excoecaria agallocha*) are the major mangrove species in the Sundarban. *H. fomes* is a most commercially valuable and dominant tree species of the Sundarban mangrove ecosystem which belongs to the family- Sterculiaceae. It is used for bridge and house construction, boat building, rafter, hardboard making, fire wood, brick burning, heavy furniture, paneling, electric poles, etc. This species commands its single dominance in 52.7% and co-dominance in 14.8 % of the forest area and representing about 64% of the total standing volume (Chaffey et al. 1985, Rahman 1995).

This important species has been subjected to a serious disease syndrome called top dying. This disorder causes death from top to downwards. Top dying appears as a decline and dieback of the foliage and twigs of the upper part of the crown. Ultimately upper portion of the main stem becomes die, dries and can be broken off by strong wind. In case of older trees, one or more of the major branches may be broken off, then gradually other branches die and the crown is subsequently reduced. The symptoms of top dying was observed as early as in 1926 and recorded as such in 1960 by the Forestal during their inventory of the Sundarban (Chowdhury 1968). The history of top dying of *H. fomes* in the Sundarban is not a new thing. It has reported as an epidemic in the last few decades. Now, it has become a threat for the species and to the world's largest mangrove forest, the Sundarban. There is substantial dieback of Sundari trees in the forest. Sundari trees in the Sundarban are being destroyed following outbreak of the top-dying disease, locally known as 'agamora'. Top dying of sundari in the Sundarban was first reported by Troup (1921). He mentioned that sundari trees growing in the depressions tend to deteriorate rapidly and die off. Afterwards Curtis (1933), Ahmed (1957), Forestal (1960) recorded top dying of *H. fomes* trees in the Sundarban. Later on, it was reported by Chowdhury (1968), Sobhan (1973), Gibson (1975), Sattar (1977), Rahman et al. (1983), Rahman et al.(2003a), Rahman et al. (2003b), Chowdhury (1984), Chaffey et al. (1985), Karim (1995) and Rahman (1990, 1995, 1996, 1998, 2001 and 2004).

There are 9 blocks and 55 Compartments in the Sundarban. Top dying of *H. fomes* has been found to prevail in all 9 blocks in the forest. The incidence and distribution of top dying trees was first investigated by Chaffey et al. (1985). He reported 14 Compartments viz. 6, 14, 21, 23, 25, 26, 28, 32, 33, 38, 39, 45, 46 and 47 top dying of *H. fomes* were found to be severe. He also reported that 17% of the main forest types of the Sundarban are affected by top dying, of which 10% are moderately affected (crown less than 50% affected) and 7% are severely affected (crown 50% or more affected).

Rahman (1990) studied the extent and intensity of occurrence of top dying of *H. fomes*. He found that over 45.2 million *H. fomes* trees were affected by top dying in 22 Compartments viz. 6, 14, 17, 18, 21, 23, 25, 26, 28, 31, 32, 35, 36, 37, 38, 39, 40, 42, 45 and 47 out of 55 Compartments in the Sundarban. Out of this, 25.02 million trees covering 25,446 ha were found moderate top dying and 20.18 million trees covering 19,848 ha were severely affected.

Rahman et al. (2003a) also studied the extent of top dying by the analysis of data of top dying sundari in 1190 Temporary Sample Plots (TSP) falling in 55 compartments in the Sundarban. The data were generated through Forest Resources Management Project, Forest Inventory of the Sundarban during 1996 and 1997. They prepared a Relative Ranking Index considering the percent of top dying. Based on the value of the relative ranking index the compartment were arranged in decreasing order of occurrence of top dying of sundari trees in the Sundarban and are as follows: 37, 33, 40, 19, 36, 18, 45, 34, 22, 8, 20, 5, 31, 38, 25, 29, 32, 13, 26, 43, 28, 27, 17, 44, 10, 30, 24, 4, 9, 16, 35, 2, 3, 14, 39, 1, 11, 21, 6, 7, 12 and 15 (Rahman 2004). Top dying of *H. fomes* was seemed to be due to a single or a combination of several factors (Hossain 2015).

Disease is an abnormal unhealthy condition produced in an individual due to defective nutrition, defective heredity, unfavorable environment or infection. Disease causing organism is called pathogen. The individual in which a disease is caused by a pathogen is called host. The development of disease in a plant depends on three factors: (i) host genotype, (ii) pathogen genotype and (iii) the environment. Some host genotypes possess the ability to prevent a pathogen strain from producing disease. Such host lines are called resistant, and this ability is called resistance or disease resistance. The term strain has a similar meaning for the pathogen as line has for the host. Those lines of a host that are not resistant to the pathogen are called susceptible. A successful breeding for disease resistance depends mainly on the following two factors: (i) a good source of resistance, and (ii) a dependable disease test. In disease test, all the plants are grown under conditions in which a susceptible plant is expected to develop disease. Therefore, disease resistant plants should be produced to avoid infection.

Breeding is carried out either by conventional breeding techniques or by mutation breeding. The conventional method of breeding for disease resistance is hybridization and selection. The various sequential steps are: screening germplasm for resistance sources, hybridization of selected parents, selection and evaluation of hybrids and testing.

It is feared *H. fomes* may be soon driven to extinction due to the rapid spread of the disease that lead to slow forest growth and reduced productivity of forest sites. Although a number of works on pathological, ecological (soils), entomological and silvicultural aspects to address the problem of top dying of *H. fomes* have been conducted but no conclusive result yet achieved till to date. The purpose of this research is to discuss tree improvement methods as an evolving technology, considering the increasing levels of knowledge of the underlying mechanisms and the control of the process of generating and selecting superior plant types. Therefore, this study has been undertaken to develop a top dying resistant *H. fomes* population in the Sundarban.

Materials and Methods

The research was carried out in the Sundarban. It lies between the latitudes 21°30'N and 22°30'N, and longitudes 89°00'E and 89°55'E. The Mangrove Silviculture Division of Bangladesh Forest Research Institute has been studying selection of top-dying resistant sundari trees since 2010 by establishing healthy sundari stands. Four locations were selected from severely top dying affected (>50% trees affected), moderately affected (25-50% trees affected), less affected (<25% trees affected) and non-affected (healthy) sundari areas of the Sundarban. Ten top dying free healthy *H.*

fomes trees were selected randomly from each location and thus a total of 40 healthy trees were selected. Seeds of these trees were collected separately and seedlings were raised. From 2010 to 2016, 18,000 outstanding seedlings were selected from the mangrove nursery. Experimental plantations were raised with sundari seedlings from selected 40 trees (each tree considered as one pure line) in a completely randomized design at 1.5m x 1.5m spacing with five replications at four locations (one in top-dying free area and three in top-dying affected areas) of the Sundarbans. Plantations were initially protected by fencing against browsing up to the period it reached beyond the browsing height. The analysis of variance (ANOVA) was done to note whether there any difference existed in the treatments. All tests were at the 0.05 probability level.

Results and Discussion

The traditional method of tree improvement based on an elementary knowledge of the laws of inheritance has been the selection of plants within landraces, based on the assumption that the progenies of the best individuals are expected to be superior to the progeny of a random sample of the population. This method was formally proposed by Louis de Vilmorin in 1856, although there are mentions of the use of its principles by some farmers earlier in the 19th century (Allard, 1999). Although a more general type of cross-resistance should not be expected to be a typical feature of most resistance mechanisms (Panda and Khush, 1995; Riipi et al.,2005), it may now be important to seek to understand the degree of variation present in currently selected elite parent trees making up the seed production and breeding populations. A reduction in the number of genotypes that researchers should and can afford to work with needs to be tempered with the difficulty of accommodating more traits (Verryn, 2008), particularly if negative genetic correlations are present between traits of interest. Moreover, resistance does not always incur a physiological cost (King et al.,1997), so mechanisms of resistance that are positively correlated with growth would also be desirable ones to pursue.

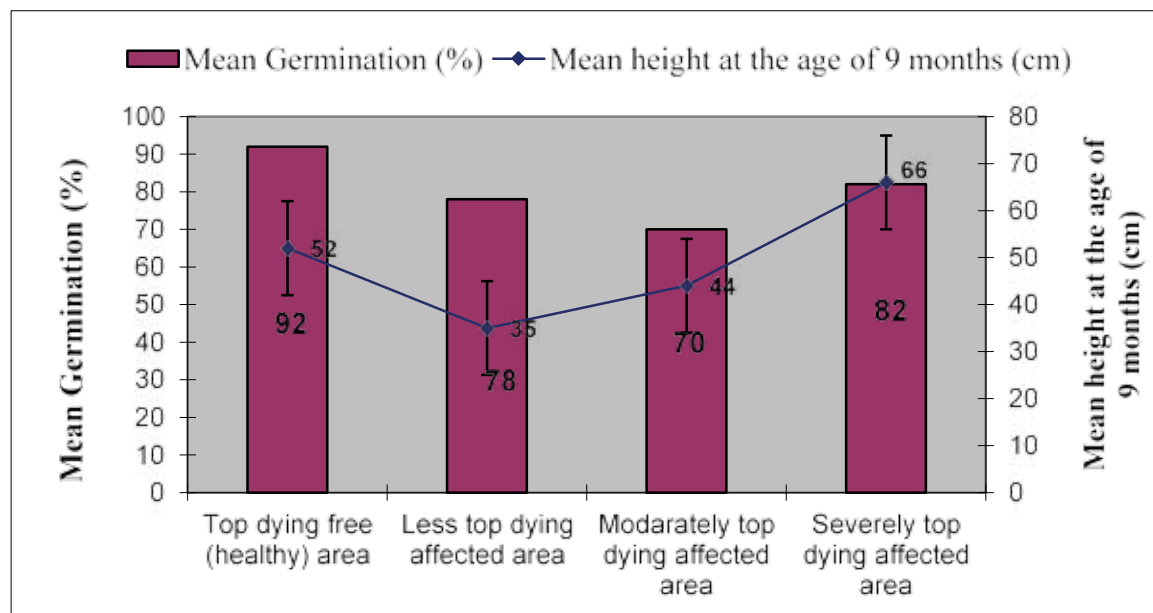


Figure 1: Germination and mean height of offspring of *Heritiera fomes* selected from top dying affected and non-affected areas of the Sundarban

The Mean height (cm) of 9 months old offspring selected from four top dying severity categories such as healthy area, less affected area, moderately affected area and severely top dying affected area were 52.0 ± 3.16 , 35.0 ± 2.12 , 44.0 ± 4.05 and 66.0 ± 5.10 as well as mean germination percentage were 92, 78, 70 and 82 respectively shown in Figure 1 & Figure 11,12,13,14.

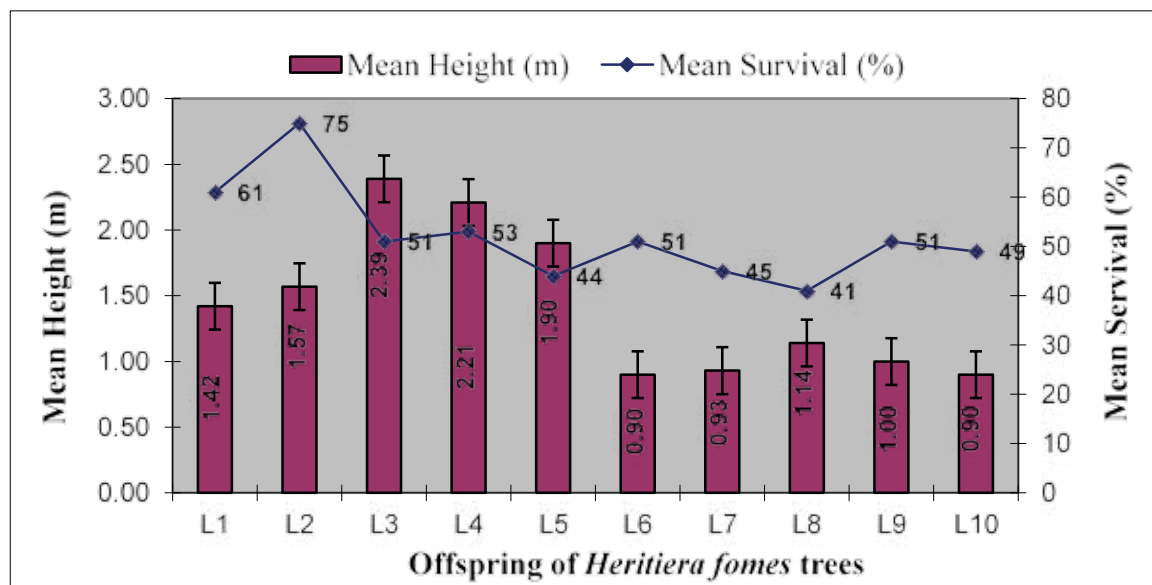


Figure 2: Field evaluation of six years old offspring of *Heritiera fomes* trees selected from natural conditions of top dying free (healthy) areas of the sundarba

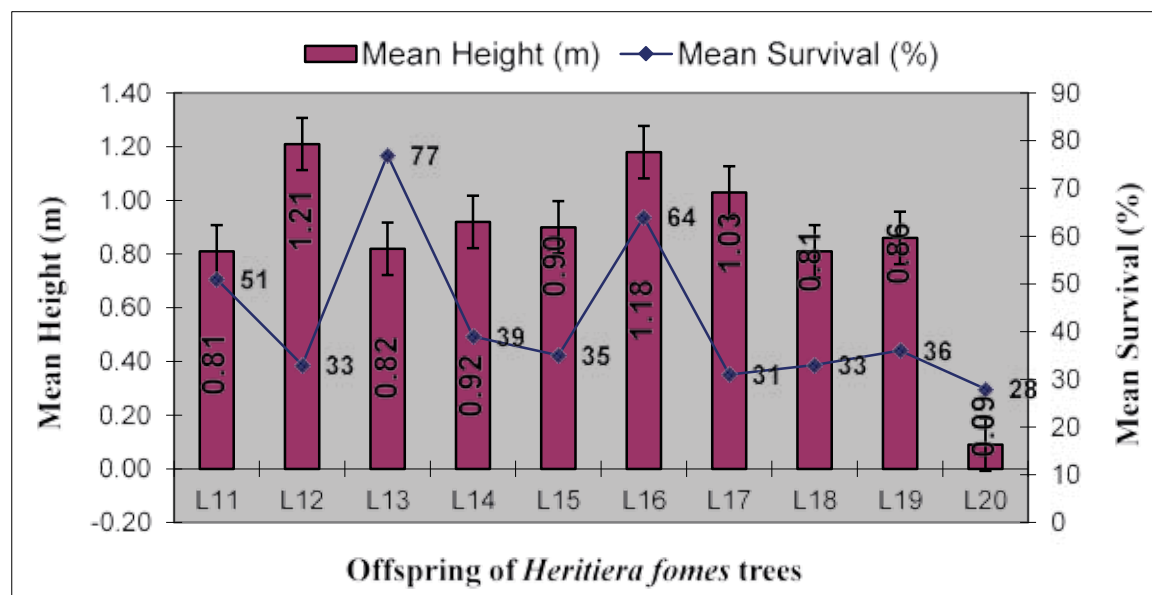


Figure 3: Field evaluation of six years old offspring of *Heritiera fomes* trees selected from natural conditions of less top dying affected areas of the sundarban

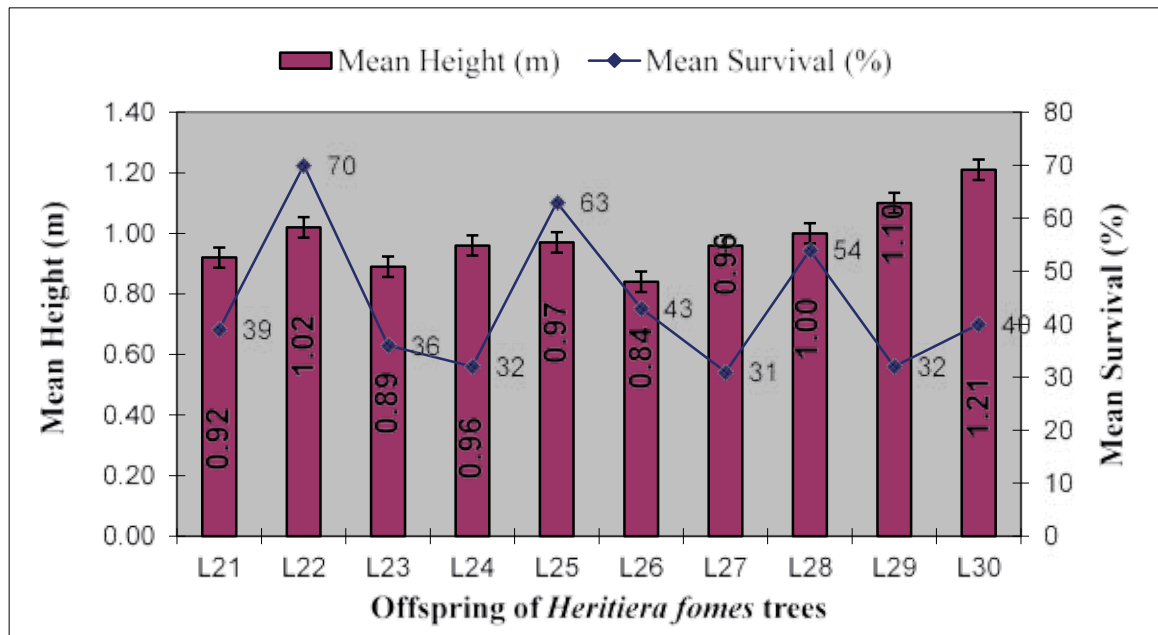


Figure 4: Field evaluation of six years old offspring of *Heritiera fomes* trees selected from natural conditions of moderately top dying affected areas of the sundarban

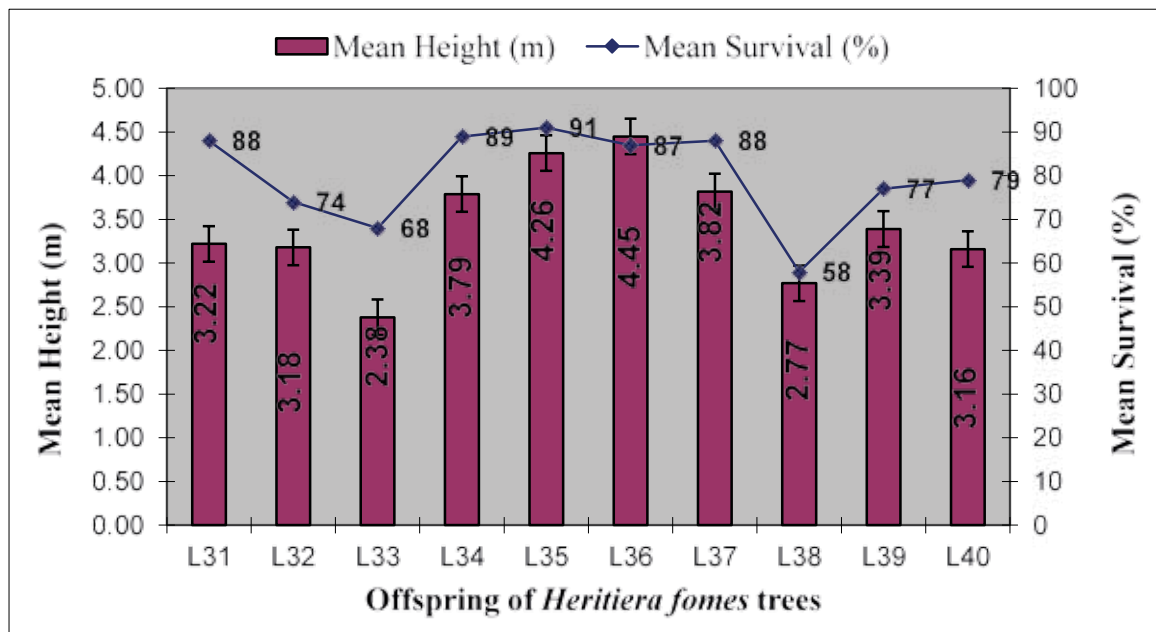


Figure 5: Field evaluation of six years old offspring of *Heritiera fomes* trees selected from natural conditions of severely top dying affected areas of the sundarban

Table 1: Growth performance of six years old offspring of *Heritiera fomes* trees selected from top dying affected and non-affected areas of the Sundarban

Indicators	Top dying free (healthy) area	Less top dying affected area	Moderately top dying affected area	Severely top dying affected area
Mean total height (m±SE)	1.44 ± 0.18	0.95 ± 0.05	0.99 ± 0.03	3.44 ± 0.20
Survival %	51	35	37	87
SD	0.56	0.15	0.11	0.64
F.05 (3)	73.16**			

The Mean total height (m) of *Heritiera fomes* at six years old offspring selected from four top dying severity categories of top dying free (healthy) area, less top dying affected area, moderately top dying affected area and severely top dying affected area were 1.44±0.18, 0.95±0.05, 0.99±0.09 and 3.44±0.20 as well as mean survival percentage were 51, 35, 37 and 87 respectively shown in Table 1. The highest mean total height (m) recorded as 3.44±0.20 of the experimental plantations, raised from selected mother trees of the severely top dying affected area of Sundarban. Here, F.05 (3) = 73.16** with 3 d.f., there is highly significant differences in the mean total height (m) of the sundari trees among five years old offspring of *H. fomes* trees selected from top dying affected and non-affected areas of the Sundarban. Experimental plantations were raised with sundari seedlings from selected 40 trees (offspring of each tree considered as a line). Field evaluation of six years old offspring of *H. fomes* trees selected from natural stress conditions of top dying affected and non-affected (healthy) areas of the Sundarban are shown in figure 2, 3, 4 & 5. Rahman M M (2003) learned that certain genotypes of *H. fomes* are more adapted to their environment than others as evidenced by their ability to survive and reproduce in that environment. He also suggested that selection and improvement of top dying resistant *H. fomes* can therefore be attempted from its natural population in the Sundarban.

Natural selection is the process of naturally ‘screening’ characteristics within individuals within a species for or against a certain outcome. Natural selection can affect morphological, physiological, biochemical and anatomical characters, however natural selection can only act on traits which can be genetically inherited and which are expressed. In summary, natural selection acts on the phenotype of an organism. The theory states that if an organism has a trait that is of benefit, then it will have a greater chance of reproducing and passing on that gene. However if an organism has a gene which is causing it disadvantage, then the organism will die and not pass on that gene.

Natural selection does not have to be gradual – in fact if there are sudden and major environmental changes then evolution (through natural selection) can occur relatively quickly. So, natural selection is a complex process in which the total environment determines which members of a species survive to reproduce and so carry on their genes to the next generation. Natural selection occurs when individuals with certain genotypes are more likely than individuals with other genotypes to survive and reproduce, and thus to pass on their alleles to the next generation. As Charles Darwin (1859) argued in *On the Origin of Species*, if the following conditions are met, natural selection must occur: i. There is variation among individuals within a population in some trait. ii. This variation is heritable (i.e., there is a genetic basis to the variation, such that offspring tend to resemble their parents in this trait). iii. Variation in this trait is associated with variation in fitness (the average net reproduction of individuals with a given genotype relative to that of individuals with other genotypes). In natural populations, the mechanisms of evolution do not act in isolation. This is crucially important to conservation geneticists, who grapple with the implications of these evolutionary processes as they design reserves and model the population dynamics of threatened species in fragmented habitats. Natural populations of trees have large variations among species, races and individuals as regards stress tolerances and this provides vast possibilities for the selection and breeding of superior types (Schreiner, 1966).



Figure 6: Germinated seeds of *Heritiera fomes* in poly-bags at mangrove nursery in the Sundarban



Figure 7: Nine months old offspring of Heritiera fomes at mangrove nursery, raised from selected mother trees of non-affected (healthy) sundari areas of the Sundarban



Figure 8: Nine months old offspring of Heritiera fomes at mangrove nursery, raised from selected mother trees of natural stress conditions of top dying less affected (<25% trees affected) areas of the Sundarban



Figure 9: Nine months old offspring of *Heritiera fomes* at mangrove nursery, raised from selected mother trees of natural stress conditions of top dying moderately affected (25-50% trees affected) areas of the Sundarban



Figure 10: Nine months old offspring of *Heritiera fomes* at mangrove nursery, raised from selected mother trees of natural stress conditions of top dying severely affected (>50% trees affected) areas of the Sundarban



Figure 11: Six years old experimental plantations of offspring of Heritiera fomes raised from selected mother trees of non-affected (healthy) sundari areas of the Sundarban



Figure 12: Six years old experimental plantations of offspring of Heritiera fomes raised from selected mother trees of natural stress conditions of top dying less affected (<25% trees affected) areas of the Sundarban



Figure 13: Six years old experimental plantations of offspring of Heritiera fomes raised from selected mother trees of natural stress conditions of top dying moderately affected (25-50% trees affected) areas of the Sundarban



Figure 14: Six years old experimental plantations of offspring of Heritiera fomes raised from selected mother trees of natural stress conditions of top dying severely affected (>50% trees affected) areas of the Sundarban

Figure 6-14 show the offspring of *Heritiera fomes* at mangrove nursery and experimental plantations, raised from selected mother trees of natural stress conditions of top dying affected and non-affected areas of the Sundarban.

The great promise of L₃, L₄, L₁₂ L₁₆ L₃₀ L₃₅ & L₃₆ line of *Heritiera fomes* trees has been achieved with expected superior genetic material of unknown origin. This is reflected in the high variability observed in growth amongst individuals. It is anticipated that some of these faults can be improved quite quickly through traditional tree improvement techniques. It is recommended that the genetic improvement of this species is a high priority for plantation development. The more promising lines are then selected for further propagation, and they are further improved by promoting as much variation as possible through advanced tree improvement techniques. Finally, selection of the plants showing greatest promise takes place. Among new generation plantation candidates, the individuals appear to have the most potential for plantation development in the Sundarban. While these results are still relatively recent (<6 years), they demonstrate great promise for the establishment of selected sundari trees. Through greater development of the genetic base of these individuals and through long-term and rigorous screening of the saplings in the plantation, it is likely that a number of exceptional individuals will be identified. Using advanced propagation techniques, these can be cloned and potentially provide the genetic base for a highly successful clonal forestry program for creation of top dying resistant *H. fomes* trees.

Conclusion

Top-dying disease of *Heritiera fomes* has already been an important factor restricting the sustainable mangrove forest development in Bangladesh. Disease decreases the quality of wood and reduces productivity of economic forest, leading to serious damages and economic loss. The common conception of evolution focuses on change due to natural selection. Natural selection is certainly an important mechanism of allele-frequency change, and it is the only mechanism that generates adaptation of organisms to their environments. Before a selection and breeding program for *H. fomes* trees can go into effect, a multidisciplinary approach is needed that involves geneticists, plant pathologists and silviculturists working closely together on a long-range research project. Research should be focused on genotypes and genotype-phenotype interactions that could form the basis of a program. Better alignment of forest genetics and forest health research program will help mitigate the predictable negative impacts of climate change on forest productivity and health. Future needs for maximum progress in genetic improvement of disease resistance in *H. fomes* trees. The research project needs to be adequately funded for 40 to 50 years or more to bring forth lasting and useful results.

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Optimization of In vitro Shoot Production and Mass Propagation of *Gynura procumbens* from Shoot Tip Culture

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Abstract

A rapid micro-propagation protocol was established for *Gynura procumbens* (Lour.) Merr., an important medicinal plant for the treatment of various ailments such as diabetes, hypertension and urinary tract infection. The shoot tips of three months old plants were used as the explants for the initiation of in vitro culture in Murashige and Skoog (MS) medium supplemented with 1mg/L BAP. Optimization of rapid proliferation of shoots was carried out by culturing the in vitro derived shoots onto MS medium supplemented with different concentrations of BAP and KIN (0.0, 0.5, 1.0, 1.5, 2.0 mg/L). Maximum shoot proliferations an average of 21.33 shoots were produced per culture from each shoot in 1.0 mg/L BAP. The effect of different strength of sugar (10, 20, 30, 40, 50 gm/L) and sub culturing on culture medium were observed for optimization of shoot producing. The micro-shoots produced normal roots within two weeks of culture on the basic ½ MS medium supplemented with 0.5 mg/L IBA. The rooted plantlets of *G. procumbens* were transferred in soil and kept under green house for hardening with a temperature of 25-29°C and 90% relative humidity for two weeks. About 99% of the plantlets survived after two weeks of transferring into polybag with soil:cowdung (3:1) in the nursery bed. The tissue culture plants showed normal growth and development in poly bag within 6-8 weeks. The regenerated shoots were macro proliferated and produced a large number of new plants in the nursery within a short period of time.

সারসংক্ষেপ

গাইনুরা উদ্ভিদের শীর্ষাঙ্গ আবাদ করে (shoot tip culture) দ্রুত চারা উৎপাদনের মাইক্রোপ্রোপাগেশন কৌশল উদ্ভাবন করা হয়েছে। Murashige and Skoog (MS) খাদ্য মাধ্যমে তিন মাস বয়সী গাছের শীর্ষাঙ্গ (shoot tip) ১.০ মিলিগ্রাম/লিটার ঘনত্বে BAP গ্রোথ হরমোন যোগ করে কালচার সূচনা করা হয়। এভাবে shoot tip থেকে উৎপাদিত প্রতিটি shoot এর সংখ্যা বৃদ্ধি করে সর্বোচ্চ সংখ্যায় উন্নিত করা হয়। Shoot উৎপাদনে MS খাদ্য মাধ্যমে বিভিন্ন ঘনত্বে গ্রোথ হরমোন BAP ও KIN (0.0, 0.5, 1.0, 1.5, 2.0 mg/L) প্রয়োগ করা হয়। নতুন shoot উৎপাদনে খাদ্য মাধ্যমে বিভিন্ন মাত্রার শর্করা (১০, ২০, ৩০, ৪০, ৫০ গ্রাম/লিটার) এবং সাবকালচার এর প্রভাব পর্যবেক্ষণ করা হয়। ফলাফল থেকে দেখা যায়, খাদ্য মিডিয়ামে ৪০ গ্রাম/লিটার সুগার এর সাথে ১.০ মিলিগ্রাম/লিটার BAP যোগ করে গড়ে সর্বোচ্চ ২১.৩৩ টি নতুন shoot পাওয়া যায়। উৎপাদিত shoot গুলি ০.৫ মিলিগ্রাম/লিটার IBA যুক্ত অর্ধমাত্রার MS মিডিয়ামে স্বাভাবিক মূল উৎপাদন করে। এভাবে উৎপাদিত অনুচারা গুলিকে পলিব্যাগে স্থানান্তর করা হয় এবং ৯৯% চারা বাইরের পরিবেশে বেড়ে উঠে। উদ্ভাবিত প্রযুক্তির মাধ্যমে কম সময়ে গাইনুরার মাতৃউদ্ভিদের গুণাগুণ সম্পন্ন প্রচুর সংখ্যক চারা উৎপাদনের মাধ্যমে ক্রমবর্ধমান চাহিদা পূরণ করা যাবে।

Keywords: Optimization, In vitro, Micro-propagation, *Gynura procumbens*, Shoot tip culture.

Introduction:

Gynura procumbens (Lour.) Merr. is an important medicinal plant. It is widely distributed in the South East Asian countries such as Malaysia, Indonesia and Thailand. This plant is commonly known as ‘sambungnyawa’, ‘kecamakar’ or ‘daundewa’ by the Malays and belongs to a family of Asteraceae. It is newly introduced in Bangladesh and known as diabetic plant. The plant height is approximately 10-40 cm. This tropical herbaceous medicinal plant is highly branched with hairy green leaves that are alternately arranged on hairy purple stem. It produces purple tubular bisexual flowers. It has several scientific synonym names such as *Gynura sarmentosa*, (DC), *Cacalia procumbens* (Lour) and *Calacia procumbens* (Lour) (Wiar 2002).

G. procumbens has been long used as ethno-herbal products to treat various ailments such as diabetes, hypertension, urinary infection and used as anti-inflammatory and anti-allergic agents (Jiratchariyakul et al. 2000). Bohari et al. (2006) reported the extracts of this plant had an enhancing effect on glucose uptake in 3T3 adiposity cell lines and they suggested that the anti-diabetic action of *G. procumbens* might be mediated through the stimulation of glucose uptake. Iskander et al. (2004) discovered that the crude ethanolic extracts of *G. procumbens* showed anti-inflammatory properties and steroid might be one class of anti-inflammatory compounds found in this plant. Zhang and Tan (2000) reported that the leaf extracts of *G. procumbens* had significantly suppressed the elevated serum glucose levels and reduced the serum cholesterol and triglyceride levels in diabetic rats. Akowuah et al. (2002) discovered that the n-butanol extracts of this plant could reduce the blood glucose levels in streptozotocin-induced type 2 diabetic rats. Two compounds 3,5-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid, identified from this plant were found to inhibit the replication of viruses (Jiratchariyakul et al. 2000). This plant traditionally been used for the treatment of fevers, rashes, kidney disease, migraine, constipation and hypertension. Due to medicinal values, there is a great potential to develop various products from this species. Propagation of this plant on a large scale will be a key step in order to maintain a sustainable raw material supply in manufacturing of *G. procumbens* products. Conventionally, this plant is propagated by cuttings. The conventional method cannot meet the increasing demand of this plant used as the raw material for the preparation of pharmaceutical, dermaceutical and aromatherapeutical products. The in vitro culture techniques can be used as the alternative for continuous supply of plantlets. In the present study, it was investigated to develop a suitable in vitro plant regeneration protocol of *G. procumbens* using shoot tip explants. The developed protocol can be used as large scale for clonal propagation of the species to produce large number of plants for future demand.

Materials and Methods

Plant materials

Plants of *Gynura procumbens* were collected from the nursery of Minor Forest Product Division of Bangladesh Forest Research Institute. Later on it was nourished in greenhouse of Silviculture Genetics Division about one month as a source of primary explants. Shoot tips were excised from

2 months old plant grown in greenhouse and brought to the tissue culture lab for in vitro culture establishment. The experiments were carried out at the tissue culture laboratory and the nursery of Silviculture Genetics Division, BFRI, Chittagong, Bangladesh.

Explants preparation and sterilization

The shoot tips of about 1.5-2.0 cm in length were washed with detergent and rinsed under running tap water for 30 minutes. After that explants carried under laminar air flow. The surface sterilization was started with one drop of tween 20 for 7-10 minutes with frequent shaking. Then washed with sterilized distilled water for 2-3 times. After washing the explants were immersed in 70% ethanol for 1 minute and then surface sterilized with 20% Clorox® for 15 minutes, and rinsed with sterilized distilled water for three times. They were again surface sterilized for 10 minutes and rinsed with sterilized distilled water. The shoot tips were cut into 1.0-1.5 cm length as the explants for inoculation onto culture medium.

Culture media preparation

The surface sterilized shoot tips were inoculated onto MS medium comprising 3% sucrose as carbon source and 2.8 gm/L gelrite as solidifying agent for initial growth. Various plant growth regulators such as; cytokinins (BAP & KIN) and auxins (IBA & NAA) were used to prepare MS medium for the regeneration of multiple shoots and roots from the base of excised new shoots. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before addition of gelrite and sterilized by autoclaving at 1.08 kg/cm² pressure and 121°C for 20 minutes.

Culture conditions

The cultures were incubated at 25±2°C under cool white and fluorescent light of 2000-2500 lux, relative humidity about 60-80% and 16/8 hours photo and dark period were maintained in growth chamber, respectively. These culture conditions were used in all the experiments mentioned below unless otherwise stated. Observations were made at regular intervals and tabulated.

Multiple shoots production and optimization

The aseptic shoot tips were cultured on MS medium supplemented with 0.0 (MS₀/control), 0.5, 1.0, 1.5 and 2.0 mg/L of BAP and KIN alone and/or in combination. Number of shoots per explants and their morphology were observed periodically. To optimize the shoot production, effect of sub culturing and the strength of sucrose level in culture medium were evaluated. Rate of multiplication of shoots and their growth were recorded up to 3-8 weeks of culture.

Development of roots at the base of the shoot, hardening and acclimatization of plantlets

In vitro elongated shoots (6-7 cm.) with at least 3-4 nodes were taken out from the culture vessel and transferred to half strength MS medium with different concentrations (0.0, 0.5, 1.0, 2.0 mg/L) of IBA for root induction.

When the plantlets developed few leaves and roots on the rooting medium, they were taken out from the culture vessels, washed thoroughly running tap water to remove the debris gelling agent with care and transferred to a pot (10 cm x 9 cm) filled with 2:1 garden soil and compost. The potted plants were then put into large poly bags (25 cm x 15 cm). To maintain high humidity, open

portion of the poly bag was made air tight and kept them in room temperature. Within 10 - 15 days the potted plants began to form new leaves and resumed new growth. After 15 days the covering bags were finally removed. Sudden removal of covering bags had adverse effect on establishment. The potted plants were brought out from the growth chamber and kept under full sunlight for 2-3 h per day. The plants were successfully acclimatized in natural conditions under sun light and they eventually became suitable for final plantation. About 90% potted plants established successfully.

Statistical analysis

All experiments were performed as Completely Randomized Design (CRD). Data were analyzed using statistical analysis system (SAS v9.3) and means were statistically compared using LSD test. The significance level was set up at $p < 0.05$. Three replications were considered for each treatment and repeated thrice.

Results and discussion

Effect of cytokinins on multiple shoot formation

The effect of plant growth regulators on multiple shoot formation and optimization of in vitro grown single shoot were tested on MS medium supplemented with different concentrations (MS0, 0.5, 1.0, 1.5, 2.0 mg/L) of BAP and KIN. The results showed that MS medium without plant growth regulators induced little number of shoots whereas the supplementation of plant growth regulators enhanced shoot formation rate. Between the two cytokinins BAP was found more potential than KIN for new shoot induction. The maximum number of shoots produced per culture in MS medium supplemented with 1.0 mg/L BAP, followed by 1.0 mg/L KIN. The mean number of shoots was found 21.33 and 9.0 per culture respectively after 8 weeks in this media combination (Figure 1).

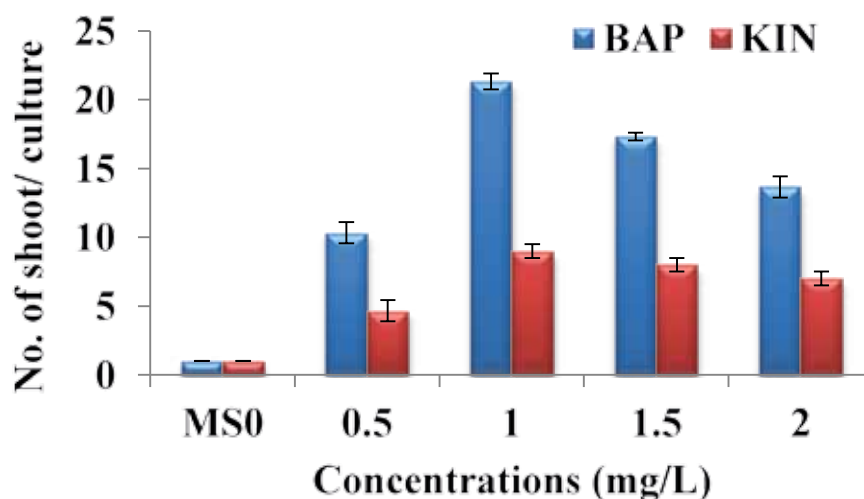


Figure 1: Effect of different concentrations of BAP and KIN supplemented with MS medium on multiple shoot production of *G. procumbens*. The vertical bar represents the standard error.

The multiple shoots production rate increased per culture for the both cytokinins BAP and KIN with between the ranges of 0.5 mg/L to 1.0 mg/L. The MS medium fortified with 0.5 mg/L and 1.0 mg/L BAP and KIN produced maximum 10.33, 21.33, 4.66 and 9.0 shoots per culture respectively. The regenerated shoots were so healthy with profuse leaves (Figure 2B & Figure 2C).

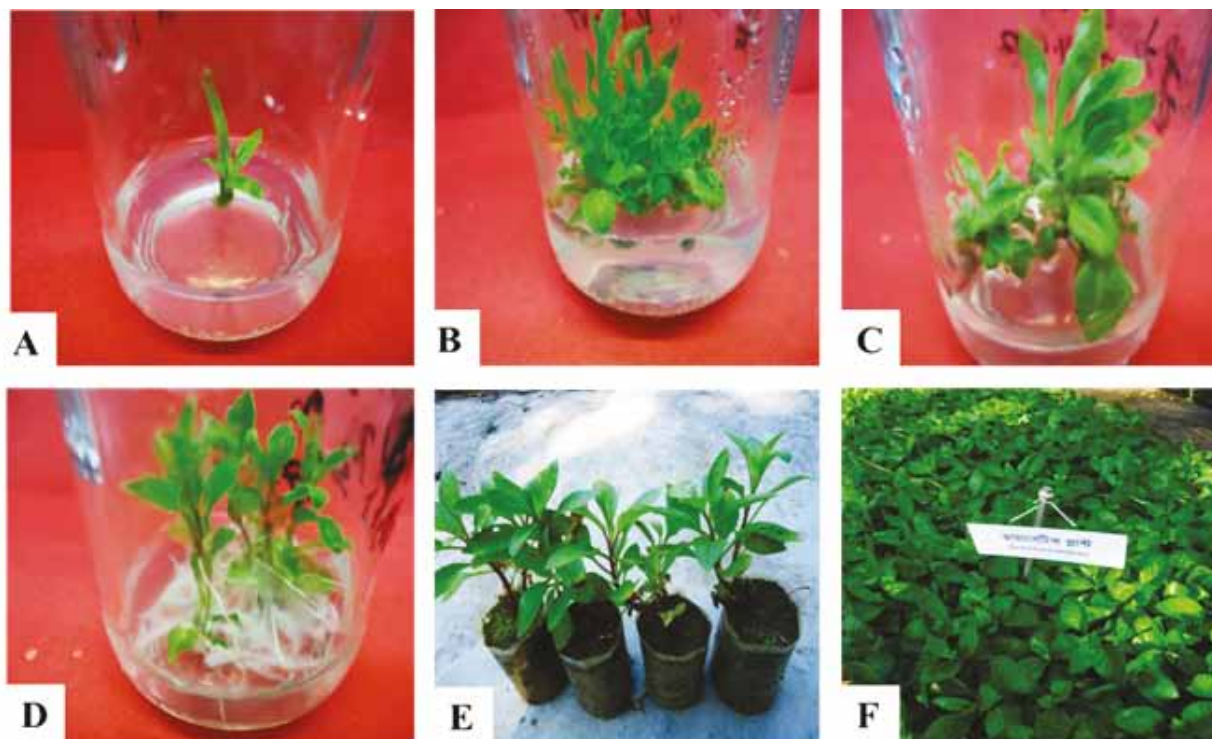


Figure 2: Effect of different concentrations of BAP and KIN in MS medium on multiple shoot production of *G. procumbens*. A. Control. B. MS + 1.0 mg/L BAP + 4% Sugar. C. MS + 1.0 mg/L KIN + 4% Sugar. D. Profuse root induced on excised in vitro grown shoots. E & F. *Gynura* tissue culture plants in polybags after hardening.

However, the number of shoot decreased with the higher concentrations of BAP and KIN. The lowest number of shoots per culture was recorded as 13.16 and 7.0 respectively in MS medium supplemented with 2.0 mg/L BAP and KIN after 8 weeks of culture.

In the present study, it was observed that the shoot tip culture of *G. procumbens* proliferated faster with the addition of cytokinins than the medium devoid of plant growth regulators. Elangomathavan and Colleagues (2003) found that in growth regulator containing media, the shoot initiation from nodal explants of *Orthosiphon spiralis* took 6-8 days whereas in hormone free media it took 10-12 days. The results showed that the concentrations of cytokinin had a crucial effect on multiple shoot formation of *G. procumbens*. The higher concentrations of BAP and KIN significantly reduced the number of shoots per culture. These findings were consonance with the findings in *Opuntia ficus-indica* (Garcia-Saucedo et al. 2005). Similarly, Thakur et al. (2006) reported that BAP increased the average number of bulblets of *Lilium* at low concentration when applied separately.

The micro shoots produced in lower levels of BAP and KIN were green, taller having bigger leaves than those produced at higher concentration of cytokinins. In MS medium containing BAP the plantlets were slightly taller than those produced in MS medium supplemented with KIN. The growth of plantlets was retarded at higher concentration of BAP. Neves et al. (2001) observed that the type of cytokinin applied had a major effect on the number of shoots induced as they promoted cell division and cell expansion in plant tissue culture. The result showed that BAP induced more shoots than KIN in regeneration of *G. procumbens*. The superiority of BAP over KIN and other cytokinins for multiple shoot formation was also demonstrated in *Salix pseudolasioogyne* (Park et al. 2008).

Effect of sub culturing on multiple shoot formation

The effect of sub culturing on multiple shoot production of *G. procumbens* was evaluated. Every 2 weeks of interval sub-cultures were maintained for multiple shoot formation. It was observed that the shoots regenerated in each sub-culture without loss of morphological responses. In the first sub-culture, the mean of shoots per culture was 6.0 and it increased up to the fourth sub-culture as 13.0 shoots/culture. However, in fifth sub-culture the shoot number decreased as 11.0 shoots/culture in the subsequent sub culture (Figure 3).

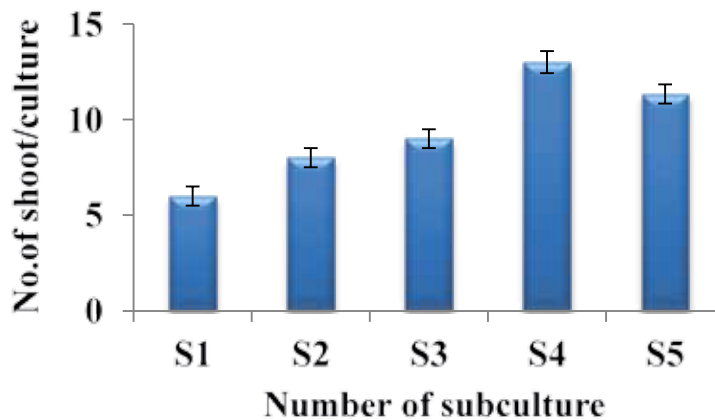


Figure 3: Effect of subculture on multiple shoot production of *G. procumbens*. The vertical bar represents the standard error.

Sub-culture exercised an important role on the multiplication of cultures (Debnath and McRae 2001). The duration of culture depended on plant species, growth rate, physical and physiological condition as well as the development stage of the plant (Moges et al. 2004).

However, sub-culturing performed at 2 weeks interval did not enhance the production of multiple shoots but produced bigger shoot which was dark green in colour. Plant tissue might have a chance to develop mutation due to repeated sub culturing, or it might produce callus, became abnormal and reduced the proliferation rate. The result revealed that *G. procumbens* did not show morphological changes after repeated sub-culturing. Likewise, it was reported that the long term culture of *Digitalis obscura* did not affect the genetic stability in vitro (Gavidia et al. 1996).

The shoot production ability varied greatly among different species. In this study, the number of shoots decreased by repeated sub-culturing. Thong (2002) reported that repeated sub-culturing caused shoots reduction in *Zingiber officinale*, *Curcuma domestica*, *Alpinia galanga*, and *Kaempferia galanga*. In contrast, repeated sub-culturing of in vitro shoot of *Spilanthes acmella* increased the multiple shoots formation by three fold (Ang and Chan 2000).

Effect of different strength of sucrose on multiple shoot formation

The sucrose level of cultures was optimized in MS medium containing 10, 20, 30, 40 and 50g/L. The number of shoots per culture increased in the media having sucrose level from 10 to 40g/L. The culture media supplemented with 40g/L sucrose produced the maximum shoots with a mean of 20.33 per culture after 8 weeks. Meanwhile 50g/L sucrose induced 16.33 shoots respectively. Among the different concentrations of sucrose 40g/L sucrose produced the highest number of shoots per culture followed by 30g/L sucrose after 8 weeks of culture (Figure 4 & Figure 2B).

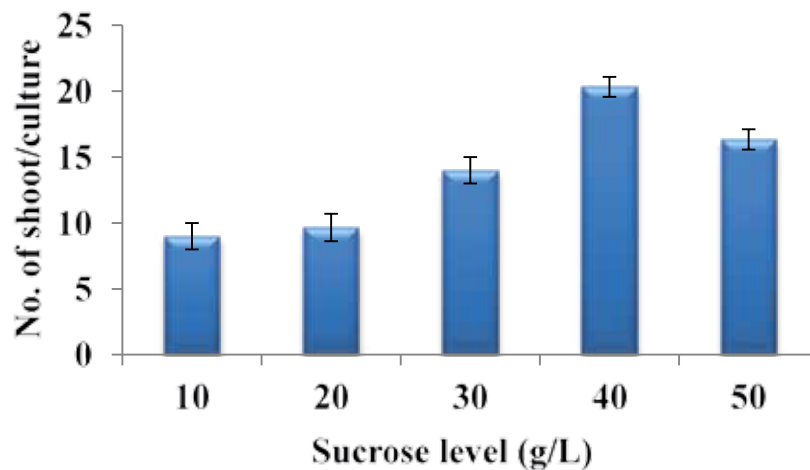


Figure 4: Effect of different sucrose concentrations supplemented with MS medium on multiple shoot production from shoot tip explants of *G. procumbens*. The vertical bar represents the standard error.

Higher plants grown in vitro were fully autotrophic (Lipavska and Vreugdenhil 1996). Therefore plant tissues culture required an exogenous carbon source and generally sucrose, is an essential ingredient of all culture media (Kozai 1991b). This is because in the culture vessels, photosynthesis was insufficient due to growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (in darkness) and the concentration of carbon dioxide (CO₂) was limited for photosynthesis. Debnath (2005) reported that specific carbohydrate may have different effects on morphogenesis in vitro, thus the carbohydrate requirements must be defined and optimized for each propagation system. The effect of carbohydrate type and concentration on shoot proliferation were genotype dependent. In this study 4% sucrose was a most optimum carbon source for in vitro multiple shoot formation in *G. procumbens* (Figure 4). Pati et al. (2006) found that sucrose concentration in culture medium had significant effect on shoot and root regeneration. High concentration of sucrose was deleterious to shoot growth and caused decrease in dry matter accumulation due to decrease in osmotic potential of the medium

(Lipavska and Vreugdenhil 1996). Increasing sucrose levels more than 7% in the medium caused osmotic stress which significantly inhibited the growth of *Parthenium argentatum* (Norton et al. 1991). In this study, no shoot proliferation was observed in the medium without carbohydrate.

Effect of different concentrations of auxin on in vitro rooting

Optimization of in vitro rooting of excised shoots were carried out in ½ MS medium supplemented with different concentrations of IBA viz. 0.0, 0.5, 1.0 and 2.0 mg/L. It was observed that no roots produced in the auxin free MS medium. The average number of root formation was significantly higher on hormone containing medium. Similarly, Chen et al. (2003a) also observed that all the shoots of *Dioscorea zingiberensis* rooted within 10 days on hormone free medium; however, medium containing 1.0 mg/L IBA induced fastest rooting and supported a higher number of roots per plantlet. Among the different concentrations of IBA, the maximum number of roots an average 9.0 per shoot was produced in media supplemented with 0.5 mg/L IBA after 4 weeks of culture. The average number of roots reduced to 7 roots/shoot at 1.0 mg/L IBA (Figure 5, Figure 2D).

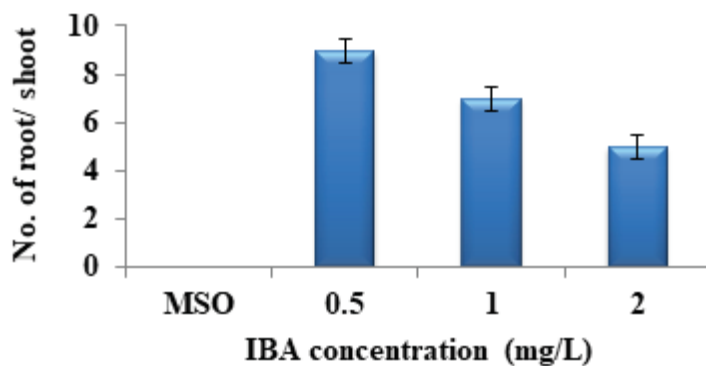


Figure 5: Effect of different concentrations of auxin, IBA supplemented with MS medium on root induction of *G. procumbens* from in vitro regenerated shoots after 4 weeks of culture. The vertical bar represents the standard error.

There was marked variation in the rooting potential of different plant species (Makunga et al. 2006) and systemic experiments were needed to define the condition for root induction. It was reported that *Stachys sieboldii* rooted on both MS and ½ MS medium with a frequency of about 95% (Li et al. 2002). However, some species required auxin for rooting such as *Aegilops longissima* (Tyankova et al. 2003). Concentrations and types of auxins used depend on species (Manickam et al. 2000). In this study, IBA 0.5 mg/L significantly supported the highest number of roots of *G. procumbens*.

Morphogenic responses of tissue culture plants under nursery condition

Variable morphogenic responses observed during the growing stage of tissue culture plants in poly bag under nursery condition. Each plant produced profuse leaves with their height. The number of leaves per plant and the plant height increased from 1 to 6 weeks of age. The maximum leaves per plant were recorded 23 with 30.16 cm plant height. It was recorded that after 6 weeks the plant height increased but the leaf number decreased gradually. At 10 weeks of plant growth average leaf numbers were recorded 9.66 with the plant height of 31.66 cm.

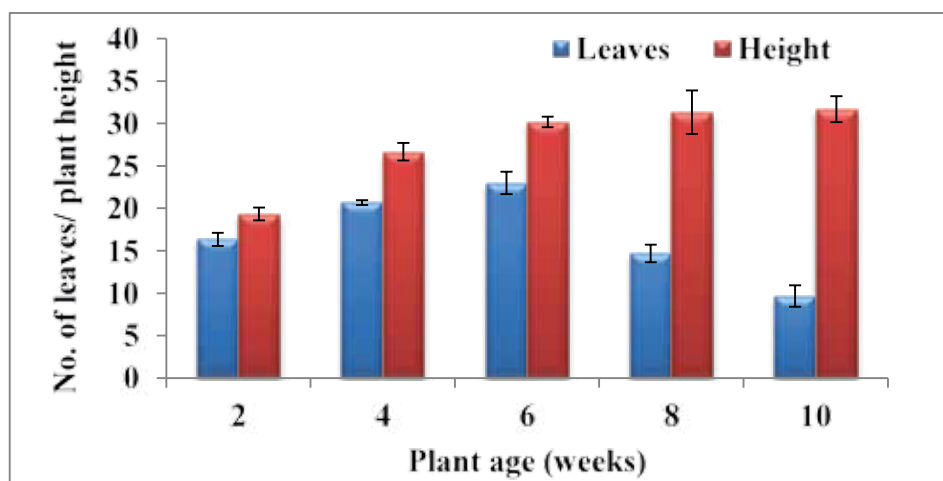


Figure: 6. Morphogenic responses of tissue culture plants at nursery. The vertical bar represents the standard error.

Each plant produced several numbers of new shoots with healthy roots in the poly bag due to rejuvenality. These new shoots were proliferated further and produced more new plants. Maximum 5 numbers of new shoots per plant were recorded after six weeks of growth in polybag (Figure 6, Figure 2E & 2F). Whereas no new shoots were produced by the seedlings grown through conventional branch cutting.

Conclusion

The plant regeneration protocol developed from shoot tip culture of *Gynura procumbens* reported through this study could be used for other medicinal plants to produce mass scale planting materials for future demand.

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Demand of Biomass Fuels for Cooking by Rural Households in Palash Upazila of Narsingdi District

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Abstract

Cooking fuels share the largest part of primary energy consumption in the rural areas of Bangladesh. Understanding the demand situation of the cooking fuels facilitates the rural energy planning in Bangladesh. This study investigates the demand of biomass fuels for cooking through rural household and market survey in the rural areas. It adopted a cluster sampling technique to select 60 households from four villages of two unions under Palash Upazila of Narsingdi district. The major cooking fuels consumed by the households were firewood, branches, leaves-and-twigs, bamboo, rice straw, rice husk and jute stick. Price elasticity of demand of firewood was 0.317 revealing that fire wood was an inelastic market commodity in that area. It is expected that this study will be helpful for rural energy development in Bangladesh.

সারসংক্ষেপ

বাংলাদেশের গ্রামীণ জনপদে রান্নার জ্বালানী মৌলিক জ্বালানী ভোগের সবচেয়ে বড় অংশ দখল করে আছে। রান্নার জ্বালানীর চাহিদা সম্পর্কে জানতে পারলে বাংলাদেশের গ্রামীণ জ্বালানী পরিকল্পনায় এটি সহযোগীতা করবে। এই গবেষণাটি গ্রামীণ গৃহস্থালী ও বাজার জরিপের মাধ্যমে রান্নার জন্য বায়োমাস জ্বালানীর চাহিদা নিরূপন করে। নরিসংদী জেলার পলাশ উপজেলায় দুটি ইউনিয়ন থেকে ৪ টি গ্রাম নিয়ে এই গবেষণাটি সম্পন্ন করা হয়। এটি ক্লাস্টার নমুনায়নের মাধ্যমে ৬০ টি গৃহস্থালী নির্ধারণ করে। গবেষণায় দেখা যায় রান্নার মূল জ্বালানীগুলো হলো জ্বালানী কাঠ, গাছের শাখা-প্রশাখা, পাতা ও কুঁড়ি, বাঁশ, ধানের খড়, ধানের তুষ এবং পাট কাঠি। জ্বালানী কাঠের চাহিদার মূল্য স্থিতিস্থাপকতা পাওয়া যায় ০.৩১৭, যার দ্বারা জ্বালানী কাঠকে বাজার চাহিদায় অস্থিতিস্থাপক বলে প্রতীয়মান হয়। এই গবেষণার ফলাফল বাংলাদেশের গ্রামীণ জনপদে জ্বালানী উন্নয়নে কাজ করবে বলে ধারণা করা যায়।

Keywords: Firewood; Price elasticity of demand; Type of cooking fuels.

Introduction

Biomass is used as the most common primary fuel for energy purposes in domestic sectors in almost all developing countries. It plays a vital role in rural energy supply in South Asia including Bangladesh sharing a major part of the total energy consumption (Barnes et al. 2011; GOB 2008). Household energy consumption in developing countries mostly covers cooking purposes (Pokharel 2004). Energy consumption pattern throughout the world varies from country to country and region to region. Due to unavailability and scarcity of modern energy supply, almost all rural people depend on biomass to meet their daily energy demand, especially for cooking. Now, there is a severe

shortage of fuel wood in many developing countries as the natural forests and village groves are being over exploited (Balat 2009). This shortage will also be severe unless the forest cover is protected and fuel wood plantation in the country is sustainably developed (Barnes et al. 2011). Many studies report that there is a gap between demand and supply of biomass energy in many countries of the world (Akther et al. 2010b; Arnold and Persson 2003; Cooke et al. 2008). A number of studies have been found on demand and supply of rural cooking fuel in different countries throughout the world. For example, Koopmans (2005) conducted study on biomass energy demand and supply for South and South-East Asia; Berndes et al. (2003) analyzed the contribution of biomass in the future global energy supply which is a review of 17 studies. Pachauri (2004), Rao and Reddy (2007) studied the effect of income and other socio-economic factors on the fuel use by the households in India.

The major sources of energy in Bangladesh are biomass fuel, natural gas, oil and coal (Ahmed et al. 2013; Huda et al. 2014). Despite many studies on rural biomass energy sources, supply and demand crossing various socio-economic factors in many parts of Bangladesh (e.g. Akther et al. 2010a; Akther et al. 2010b; Alam et al. 1999; Bala et al. 1989; Hassan et al. 2011; Jashimuddin et al. 2006; Kennes et al. 1984; Miah et al. 2003; Miah et al. 2009; Miah et al. 2010; Miah et al. 2011a; Miah et al. 2011b; Sarker and Islam 1998), demand situation of cooking fuel visualizing by elasticity measurement in Narsingdi region is unexplored. Akther et al. (2010a) studied domestic fuel use in the Meghna floodplain area of Bangladesh; Akther et al. (2010b) studied fuelwood shortage situation in the Old Brahmaputra downstream zone in Bangladesh. Although both the study sites (Akther et al. 2010a; Akther et al. 2010b) are in Narsingdi District of Bangladesh, these studies did not scaled up the demand situation in relation to price elasticity. The present study accentuates the demand situation of cooking fuels with the increase and decrease of the price on the basis of economic nature of these fuels. Likewise, it will shorten the present knowledge dearth on price-demand sensitivity of cooking fuels in Bangladesh.

Materials and Methods

The study was conducted during June 2012 through December 2012 in Palash Upazila of Narsingdi district in Bangladesh.

Description of the study area

The study was conducted at Palash Upazila of Narsingdi district. The Upazila occupies an area of 94.43 km². It is located between 23°53' and 24°03' North latitudes and between 90°34' and 90°43' East longitudes (Figure 1). It consists of 4 Union Parishads (UP), 55 Mauza and 78 villages (BBS 2012). The UP are Danga, Charsindur, Gazaria and Jinardi. The total population of the Upazila is 212,612 where male and female shares by almost 50% (BBS 2012). The total number of households in this Upazila is 46,780 with the population density 2251 km⁻¹ having an annual growth rate of 1.16. Average literacy of the Upazila is 59% including male 60% and female 57% (BBS 2012). Narsingdi district enjoys a subtropical climate having temperature ranging from 12.7°C to 36°C with average rainfall 2376 mm.



Sampling

The sampling technique conducted for the study was multistage cluster sampling. The sequence of selection was from Upazila to Union, from Union to village and then households and corresponding markets. Out of 4 Unions of Palash Upazila, 2 Unions (Gazaria and Jinardi) and 2 villages from each union (Dorichor and Gazaria from Gazaria Union, Mazerchar and Parulia from Jinardi Union) were selected randomly (Figure 1). Finally, 15 households from each village having a total of 60 households were selected randomly. From the offices of the respective UP, the list of the villages and households were obtained. Using the randomization tool of a

statistical package, the villages and corresponding households were selected for the study. After selecting the unions, villages and households, we received the help of the ward members and key persons in the village to locate the households and data collection.

Data collection and analysis

A semi-structured questionnaire was prepared for primary data collection from each of the household. Before going to collect final data, a reconnaissance survey was carried out to observe the overall situation of the study area. Through reconnaissance survey, it was observed that pipeline-gas supply was not available and most of the households were Semi-Pucca in those areas. To facilitate and enrich the study, the relevant statistical data and information about the study area were collected from the UP office. The collected data were compiled and analyzed by the statistical package SPSS statistics 17.0 and Microsoft Excel, 2007.

The study involved household and market survey. The household survey was carried out through personal visits to the households for several times during June 2012 through December 2012. The cooking fuels were identified on the basis of the determination of the price of the fuels and total quantity bought per month for each household and the responses of the households to buy these fuels at different hypothetical prices. Four hypothetical prices were assumed for this survey. They were price1, price2, price3 and price4. While price1 and price2 were derived through the reduction of present market-price at 20%. On the other hand, price3 and price4 were derived through the increase of market-price at 20%.

To determine the relationship between total income and cooking fuel use of the household, each household was brought under five income groups. The groups were selected purposively on the basis of total income per month of the household. The income groups were ≤ 9500 tk month⁻¹, 9501-13500 tk month⁻¹, 13501-20000 tk month⁻¹, 20001-45000 tk month⁻¹, ≥ 45001 tk month⁻¹. The relationship also was shown for the house-types classified as Kacha, Pucca and Semi-Pucca.

From the analysis, it was found that only fire wood (58%) was used as the market commodity as the largest scale among the other cooking fuels. So, price elasticity of demand was considered only for fire wood. From the dataset, the cases incorporating fire wood as a market commodity were abstracted only for elasticity measurement. For elasticity measurement, 5 prices and quantity demanded scenarios were used. To create these scenarios, the present market price was used as a pivotal point resulting to prices lower and two prices upper than the present price. With these changes, the corresponding quantity demanded was recorded. For calculating price elasticity of demand, the midpoint method (Mankiw 2012) was used between two point, as (Q_1, P_1) and (Q_2, P_2) .

$$\text{Price elasticity of demand} = \frac{(Q_2 - Q_1)}{\frac{(Q_2 + Q_1)}{2}} \left(\frac{(P_2 - P_1)}{(P_2 + P_1)/2} \right)$$

Where, Q_2 = Quantity demanded 2, Q_1 = Quantity demanded 1, P_1 = Price 1, P_2 = Price 2
For specifying the elasticity, the corresponding price as ‘mode’ figure was analyzed for each scenario. And then, stem-and-leaf plot was generated for showing the ‘mode’ price and the corresponding elasticity.

To find out an elasticity of firewood for the whole study area, we calculated dQ/Q and dP/P and plotted dQ/Q (Y-axis) against dP/P (X-axis). After that we calculated the slope $[(dQ/Q)/(dP/P)]$, which determined the price elasticity estimate for the firewood in the market.

The study was conducted under the academic research program designed by the authors themselves. However, after fulfilling the purpose of academic needs, the study and analysis was furthered to accomplish the full objectives of this study. The present paper is one of the series of the studies of this kind.

Results and Discussions

Socio-economic profile of the households

The average family size and number of income earners found in the selected households were 6 ± 2 and 3 ± 1 , respectively (Table 1). Among the four villages, the highest average family size was found in Parulia and the lowest in Dorichar, Gazaria and Mazerchar. Average income of the households in these villages was 29191.7 ± 3846.7 tk month-1. The highest average income (43200.0 ± 11653 tk month-1) was found in Dorichar and the lowest (18933.3 ± 3227.1 tk month-1) was in Mazerchar. The households of the study areas possessed 521.03 ± 75.85 m² homestead size and 3470.52 ± 858.89 m² agricultural lands on average.

Table 1: Socio-demographic profile of the households in Palash Upazila, Narsingdi.

Village	Homestead size (m ² homestead ⁻¹)	Homestead size (m ² homestead ⁻¹)	Agricultural land (m ² household ⁻¹)
Dorichar	43200.0±11653.0*	524.74±149.09	5943.49±2374.46
Gazaria	29166.7±6360.5	825.56±245.99	5391.77±2261.04
Mazherchar	18933.3±3227.1	277.88±42.28	631.31±267.09
Parulia	25466.7±6344.0	455.95±36.30	1915.51±405.91
Mean	29191.7±3846.7	521.03±75.85	3470.52±858.89

*Figure indicates standard error of means.

Palash Upazila, situated on the bank of Sitalakhya River, is blessed with numerous small and large scale industries. These have provided significant numbers of employment to the local people. The population census 2011 have recorded 6177 people employed in the industries including 6016 jobs in the government and non-government services in Palash Upazila (BBS 2012). In addition to this, foreign remittance was found an important source of income in many households in this Upazila (pers.comm.). Thus the present high income of the households can be explained. In contrast to this, household income revealed for Raipura and Belabo Upazila under Narsingdi district was lower than this Upazila (Akther et al. 2010a; Akther et al. 2010b). Having a total number of households 46,780, with the population density 2251 km⁻¹, Palash Upazila is experiencing a huge fragmentation of homestead and agricultural lands (BBS 2012). The tendency of rapid urbanization is also squeezing the homestead and agricultural lands in this Upazila. Thus the lower size of homestead and agricultural lands can be explained.

Cooking fuels used by the households

The main types of cooking fuels consumed by the households were firewood, branches and twigs, bamboo, rice straw, rice husk and jute stick. All of the households of Pucca and semi-Pucca types consumed firewood with the percentage of 41 and 52, respectively (Table 2). More than 90% Semi-Pucca households consumed branches and jute sticks. More than two-third of the Kacha households consumed firewood, leaves-and-twigs and jute stick, while half of that type of households consumed bamboo and rice husk. The type of biomass fuels consumed by the households in the present study site resembles to the fuel types, except cowdung, consumed by the households of Raipura and Belabo Upazila of Narsingdi district (Akther et al. 2010a; Akther et al. 2010b). In Raipura Upazila, leaves-and-twigs was found as the dominant biomass fuel for cooking (Akther et al. 2010a). The dominance of the biomass fuel use was measured by ranking of frequency of uses of each type of fuel weighted by the amount consumed (Akther et al. 2010a).

The present study did not examine the dominance as we aimed at deliberately finding the elasticity of firewood. Akther et al. (2010b) found that cowdung as a cooking fuel was consumed by the households in Belabo Upazila of Narsingdi district, as the adaptation technique at the face of biomass fuel crisis. The socio-economic profile of the present study site shows a comparatively higher income per month. It also shows a buying capacity of the households, especially living in the Pucca and Semi-Pucca houses, to buy firewood from the market. The squeezing trend of agricultural and homestead lands, and urbanization might influence the households of the present study area not to consume cowdung.

Table 2. Consumption of biomass fuels for cooking by the rural household-types in Palash Upazila, Narsingdi.

Biomass fuels	Type of house		
	<i>Kacha</i>	<i>Pucca</i>	<i>Semi-Pucca</i>
Firewood	7(88)	41(100)	52(100)
Branches	45(9)	4(50)	51(91)
Leaves-and-twigs	48(85)	4(50)	48(73)
Bamboo	46(58)	6(50)	49(53)
Rice straw	64(27)	0(0)	36(13)
Rice husk	48(50)	4(25)	48(43)
Jute stick	42(92)	7(100)	51(97)

Note: Figures without parenthesis indicate percentage of specific fuel among the fuels; figures with parenthesis indicate percentage of fuel within house types.

The highest percentage of firewood (52%) was consumed by the Semi-Pucca type household and the least (7%) by the Kacha (Table 2). More than 90% of the households of income group ≤ 9500 tk month⁻¹ consumed firewood, branches, leaves-and-twigs and jute sticks for cooking (Table 3). More than 80% of the households of income group 9501 – 13500 tk month⁻¹ consumed firewood, branches, leaves-and-twigs, and jute stick. The income group 13501-20000 tk month⁻¹ at more than 80% consumed firewood and jute stick. All of the households of income group 20001- 45000 tk month⁻¹ consumed fire wood, branches and jute stick.

Table 3. Consumption of biomass fuels for cooking by the income groups in Palash Upazila, Narsingdi.

Biomass fuels	Income group (tk month ⁻¹ household ⁻¹)				
	≤ 9500.0	9501.0 - 13500.0	13501.0 - 20000.0	20001.0 - 45000.0	45001.0+
Firewood	10(92)	23(83)	26(92)	21(100)	20(100)
Branches	25(92)	18(100)	17(69)	21(100)	19(83)
Leaves-and-twigs	41(92)	29(92)	20(69)	7(73)	3(58)
Bamboo	25(42)	22(58)	21(54)	15(45)	17(75)
Rice straw	32(25)	27(25)	19(15)	18(18)	4(8)
Rice husk	33(50)	28(50)	19(38)	15(36)	5(50)
Jute stick	29(100)	21(92)	20(85)	19(100)	11(100)

Note: Figures without parenthesis indicate percentage of specific fuel among the fuels; figures with parenthesis indicate percentage of fuel within income groups.

It is evident that the use of firewood increased with the increase of monthly income from ≤ 9500.0 to $13501.0 - 20000.0$ tk month⁻¹household⁻¹ from 10% to 26%. But it was around 20% in the groups $20001.0 - 45000.0$ and $45001.0+$. The other fuels' usages decreased with the increase of income per month of the households. Among the fuels, firewood was used as the highest quantity, 132 kg month⁻¹ household⁻¹ followed by leaves-and-twigs 119-, branches 87-, bamboo 19-, jute stick 15-, rice husk 5- and rice straw 3 kg month⁻¹ household⁻¹ (Figure 2).

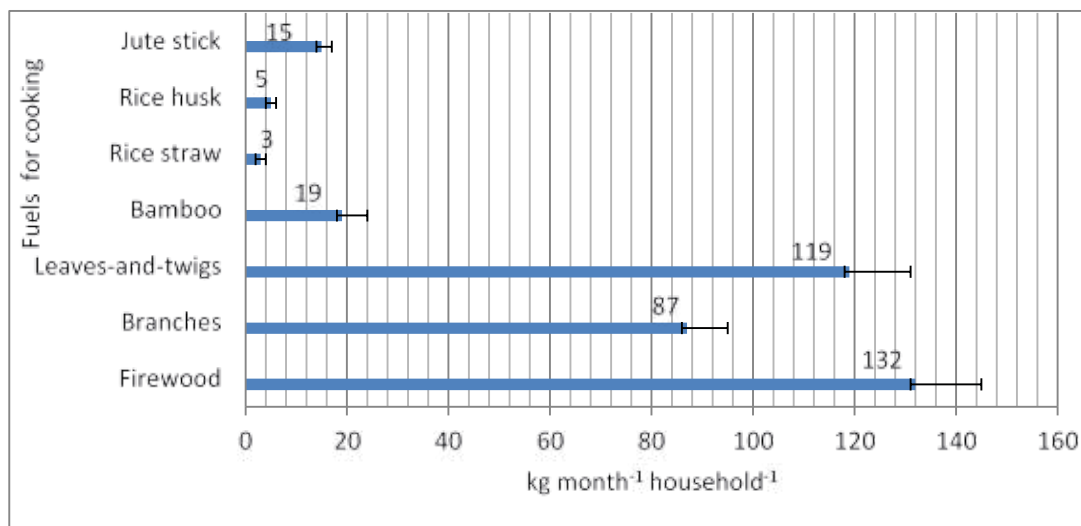


Figure 2: Consumption of cooking fuels at varying income groups and house-types in Palash

Upazila, Narsingdi.

Among the biomass fuels, only firewood was only found as the market commodity. The study found that 58% of the firewood consumed by the households was bought from the local market. The other biomass fuels were collected from the households' own and neighbors' resources. Most of the Pucca and Semi-Pucca households bought firewood from the market while a few Kacha households bought a scanty of firewoods from market. However, some of the Pucca households used LPG (Liquefied Petroleum Gas) along with firewoods and other biomass fuels. The reasons for consuming firewood by the comparatively higher-income households are simplicity of use and better burning capacity (Akther et al. 2010a; van Ruijven et al. 2008). Households' income, the major driving force, influences households' shifting of fuel use through the energy ladder in many developing countries including Bangladesh (Akther et al. 2010c; Gupta and Köhlin 2006; Heltberg 2004; Joon et al. 2009; Rao and Reddy 2007; Wijayatunga and Attalage 2002). Energy ladder follows cleanliness, convenience, efficiency and cost getting momentum with the increase of income. The concept of energy ladder incorporates three stages of fuel transitions. Dependence- on biomass fuels is the first step, on kerosene, coal is the second, and on LPG, electricity is the third one (Davis 1998; Leach 1992). Akther et al. (2010c) concluded that Bangladesh is still at the first step of the energy ladder getting place at the first half of the EKC (Environmental Kuznets Curve). However, this is clear in the present study that the households had a transition between the biomass fuels towards more cleanliness and ease.

Price elasticity of demand of firewood

The modes of 5 tk kg⁻¹ , 4 tk kg⁻¹, 3.2 tk kg⁻¹ , 6 tk kg⁻¹ elasticity was found 0 where maximum frequencies were 15, 14, 14 and 15 (Figure 3). In all the cases, it was observed that firewood was an inelastic market commodity. Increase and decrease of price could not keep effect on the consumption of firewood in the rural areas of the study area though economic value of a commodity depends on its price.

Price elasticity of demand Stem-and-Leaf Plot for present market price, 5.0 tk kg ⁻¹ Frequency Stem & Leaf 2.00 -1 . 88 3.00 -1 . 222 2.00 -0 . 69 .00 -0 . 15.00 0 . 0000000000000000 .00 0 . 1.00 1 . 0 1.00 Extremes (>=1.3) Stem width: 1.00 Each leaf: 1 case(s)	Price elasticity of demand Stem-and-Leaf Plot for market price, 4 tk kg ⁻¹ Frequency Stem & Leaf 1.00 -3 . 0 .00 -2 . .00 -2 . 5.00 -1 . 88888 2.00 -1 . 02 2.00 -0 . 68 14.00 -0 . 0000000000000000 Stem width: 1.00 Each leaf: 1 case(s)
Plot A	Plot B

Price elasticity of demand Stem-and-Leaf Plot for firewood market price, 3.2 tk kg ⁻¹		Price elasticity of demand Stem-and-Leaf Plot for firewood market price, 6 tk kg ⁻¹	
Frequency	Stem & Leaf	Frequency	Stem & Leaf
5.00	-1.00000	2.00	-3.66
5.00	-0.66666	.00	-3.00
14.00	-0.00000000012334	.00	-2.00
		3.00	-2.22
		2.00	-1.55
		1.00	-1.20
		.00	-0.00
		15.00	-0.00
			Stem width:1.00
			Each leaf:1 case(s)

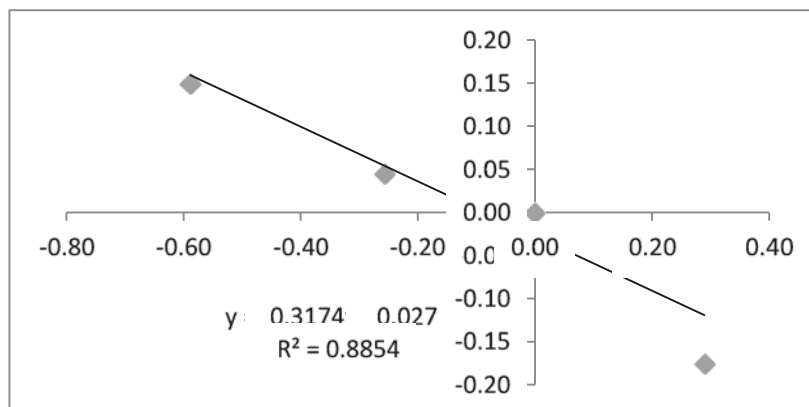
Plot C

Plot D

Figure 3: Stem-and-leaf plots including A, B, C and D mentioning price elasticity of demand of firewood for cooking by mode calculation in the rural areas of Palash Upazila, Narsingdi.

Hossain (1992) conducted a study in 1985 in Bogra and Chittagong district to find the own price elasticity of firewood. He found the price elasticity -1.51 showing a negative relationship between price and quantity demanded. But the cross elasticity, income elasticity and household size elasticity were found 0.37, 0.39 and 0.27, respectively. The study maintained the usual rule of ‘law of demand’ and gave evidence that firewood in 1985 in those districts was not ‘necessity market commodity’. However, the present study finds firewood a necessary market commodity in Palash Upazila of Narsingdi. The households in Mozambique also shows an inelastic demand of firewood evident by the coefficient -0.41 (Arthur et al. 2012).

It was observed that with the increase of price of firewood there was little change of quantity demanded. In this study, elasticity of firewood was 0.317 incorporating all the prices and households' demands (Figure 4) meaning that firewood was an inelastic market commodity in the rural areas of Palash Upazila of Narsingdi District.



A linear demand function model was constructed as presented in the figure 5 to show the relationship between price of firewood (tk kg⁻¹) and quantity demanded of firewood. The linear price demand function of firewood for cooking was found as $y = -0.104x + 19.47$ ($R^2 = 0.94$), where x denotes quantity demanded (kg month⁻¹ household⁻¹) and y denotes price (tk kg⁻¹) of firewood.

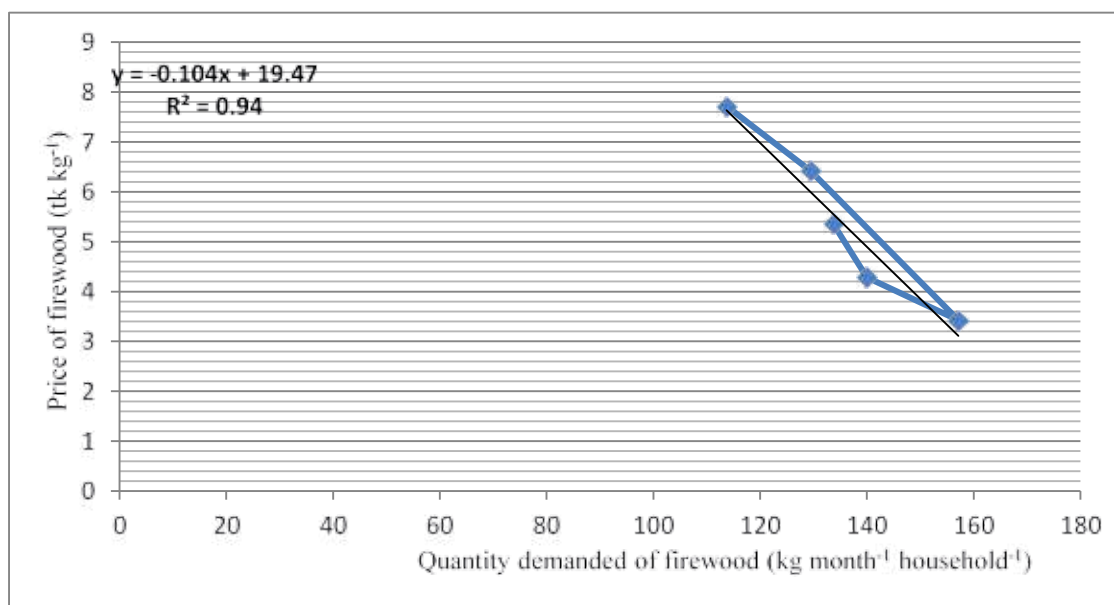


Figure 5. Linear demand functions for the firewood for cooking used in Palash Upazila, Narsingdi.

Conclusion

Consumption of biomass fuels was related with the determinants, such as house-type and total income of the households. Firewood was preferred by the Pucca, Semi-pucca and high-income households in comparison to Kacha and low income ones. Firewood was an inelastic market commodity. The study confirms that firewood was one of the essential commodities in the rural areas of Palash Upazila of Narsingdi, Bangladesh. This study will be baseline information for the energy policy makers to formulate an efficient rural energy policy.

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Field Evaluation of Trichoderma Strains as a Potential Bio-Control Agent against Fusarium Root Rot of Ashwagandha [Withania somnifera (L.) Dunal]

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Abstract

In this study, six Trichoderma strains viz. Trichoderma virens IMI-392430, T. pseudokoningii IMI-392431, T. harzianum IMI-392432, T. harzianum IMI-392433, T. harzianum IMI-392434 and T. viride FPDTV and one commercial formulation of Trichoderma (Bio derma) were evaluated alone and in combination with F. solani, to assay their efficacy in suppressing root rot disease and promoting growth and yield of W. somnifera. A pot trial experiment was conducted at the forest protection division nursery, Bangladesh Forest Research Institute (BFRI), Chattogram, Bangladesh from July 2014 to December 2014. Application of T. harzianum IMI-392433 alone (T12) or in combination with F solani (T5) significantly ($p = 0.05$) decreased the area under disease progress curve (AUDPC) (300.9 and 52.4) compared to F. solani (T1) treatment. The highest seed germination rate (90.4 %) and the highest growth and yield were also recorded in the same treatment; while F. solani treatment (T1) alone significantly decreased these values. The correlation matrix showed that root yield of W. somnifera had significant and positive correlation with plant height ($r = 0.734^{**}$), number of leaf ($r = 0.725^{**}$), number of primary branch ($r = 0.863^{**}$), number of secondary branch ($r = 0.878^{**}$), fresh shoot weight ($r = 0.749^{**}$), dry shoot weight ($r = 0.708^{**}$), number of flower at maximum flowering time ($r = 0.734^{**}$), number of pod ($r = 0.774^{**}$), number of seed ($r = 0.642^{**}$), hundred seeds weight ($r = 0.688^{**}$), seed yield/plant ($r = 0.817^{**}$), root length ($r = 0.711^{**}$), root diameter ($r = 0.970^{**}$) and fresh root weight ($r = 0.819^{**}$). The significant and negative correlation ($r = -0.619^{**}$) was observed with the root yield/plant and area under disease progress curve (AUDPC). These results revealed that T. harzianum IMI-392433 has growth promoting effects and this strain may be used as an effective biocontrol agent to control root rot disease of W. somnifera.

সারসংক্ষেপ

এই গবেষণায় অশ্বগন্ধার শিকড় পচন রোগকে নিয়ন্ত্রণ করতে এবং গাছের বৃদ্ধি ও ফলনের কার্যকারিতার মূল্যায়ন নিশ্চিত করতে Trichoderma এর ছয়টি স্ট্রেইন যেমন: Trichoderma virens IMI-392430, T. pseudokoningii IMI-392431, T. harzianum IMI-392432, T. harzianum IMI-392433, T. harzianum IMI-392434 এবং T. viride FPDTV আলাদাভাবে এবং F. solani এর সাথে সমন্বয়ে কার্যকারিতা যাচাই করা হয়েছিল। জুলাই ২০১৪ হতে

ডিসেম্বর ২০১৪ পর্যন্ত পরিচালিত গবেষণা কর্মটি বাংলাদেশ বন গবেষণা ইনস্টিটিউট, চট্টগ্রাম এর বন রক্ষণ বিভাগের নার্সারিতে মাটির টবে করা হয়েছিল। *T. harzianum* IMI-392433 (T5 Ges T12) ট্রিটমেন্টটি *F. solani* (T1) ট্রিটমেন্ট এর সাথে তুলনা করলে তাৎপর্যপূর্ণভাবে ($p = 0.005$) এরিয়া আগার ডিজিস প্রথ্রোসিভ কার্ভ (AUDPC) (৩০০.৯ এবং ৫২.৪) কমিয়েছিল। সর্বোচ্চ অঙ্কুরোদগম হার (৯০.৪%) এবং গাছের সর্বোচ্চ বৃদ্ধি এবং শিকড়ের ফলন (৬.৬৪ গ্রাম/প্লান্ট) একই ট্রিটমেন্ট এ রেকর্ড করা হয়েছিল, যদিও *F. solani* (T1) ট্রিটমেন্টটি একাই উল্লেখযোগ্যভাবে এই মানগুলি হ্রাস করেছিল। পারস্পরিক সম্পর্কের ম্যাট্রিক্স এ দেখা যায় যে, অশ্বগন্ধার শিকড়ের ফলনের সাথে তাৎপর্যপূর্ণ এবং ধনাত্মক সম্পর্ক ছিল গাছের উচ্চতা ($r = 0.734^{**}$), পাতার সংখ্যা ($r = 0.725^{**}$), প্রাইমারি শাখার সংখ্যা ($r = 0.863^{**}$), সেকেন্ডারি শাখার সংখ্যা ($r = 0.878^{**}$), ফ্রেস শটের ওজন ($r = 0.749^{**}$), শুষ্ক শটের ওজন ($r = 0.708^{**}$), ফুলের সর্বোচ্চ সময়ে ফুলের সংখ্যা ($r = 0.734^{**}$), পডের সংখ্যা ($r = 0.774^{**}$), বীজের সংখ্যা ($r = 0.642^{**}$), শত বীজের ওজন ($r = 0.688^{**}$), বীজের ফলন/প্লান্ট ($r = 0.817^{**}$), শিকড়ের দৈর্ঘ্য ($r = 0.711^{**}$), শিকড়ের ব্যাস ($r = 0.970^{**}$) এবং শিকড়ের সতেজ ওজন ($r = 0.819^{**}$)। তাৎপর্যপূর্ণ এবং ঋণাত্মক সম্পর্ক ($r = -0.619^{**}$) লক্ষ্য করা গেছে শিকড়ের ফলন/প্লান্ট এবং এরিয়া আগার ডিজিস প্রথ্রোসিভ কার্ভ (AUDPC) এর মধ্যে। ফলাফলগুলি প্রমাণ করে যে, *T.harzianum* IMI-392433 স্ট্রাইনটির গাছের বৃদ্ধির ক্ষেত্রে প্রভাব আছে এবং এই স্ট্রাইনটি অশ্বগন্ধার শিকড় পচন রোগ নিয়ন্ত্রণে একটি কার্যকর জৈব নিয়ন্ত্রক এজেন্ট হিসেবে ব্যবহার করা যেতে পারে।

Keywords:

Biological control, *Withania somnifera*, Root rot disease, *Trichoderma*, *Fusarium solani*.

Introduction

Ashwagandha [*Withania somnifera* (L). Dunal.] is an important medicinal plant belonging to the family Solanaceae. It grows well in dry and sub-tropical regions of India, Sri Lanka and Bangladesh (Agarwal et al. 2004). The plant is most important due to presence of alkaloids, withanoloids, steroids, lactones which have antimicrobial properties. So that the plant is used to preparing the medicines (Baraiya et al. 2004). The roots of this medicinal plants are also prescribable for curing general sexual weakness in human. Root rot of Ashwagandha caused by *Fusarium solani* is a major concern in many medicinal plant growing areas in Bangladesh leading to enormous plant losses. This pathogen attack nursery seedlings and young plant and showed symptoms of yellowing, drooping and leading to 30-50% mortality (Gupta et al. 2004). Diseased areas enlarge with age and gradually turn brown and invades the vascular bundles, causes severe wilting and death of the above ground parts of plants by blocking the xylem transport system. It is extremely difficult to control this soil-borne fungus using conventional method such as the use of synthetic fungicides. Since their spores are able to survive for many years in the soil. Biological control strategies for this pathogen can be carefully selected and handled in an eco-friendly way instead of using chemical fungicides. *Trichoderma* spp. are considered as potential bio-control and plant growth promoting agents for many crop plants (Verma et al. 2007; Savazzini et al. 2009). The genus *Trichoderma* is known to be secreting to the environment various secondary metabolites. Among the metabolites, the production of harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthy- α -pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others have been described (Vey et al. 2001), which provide to protect plant from disease (Chet et al. 1997). *Trichoderma* populations can be established relatively easily in different types of soil and can continue to persist at detectable levels for months. In the above context, the present study was undertaken to evaluate the bio-control potential of *Trichoderma* strains to control the root rot

disease of Ashwagandha under field conditions. Also, the effect of Trichoderma strains on growth parameters and root yield attributes were evaluated under field conditions.

Materials and Methods

To evaluate the efficacy of Trichoderma strains for controlling Fusarium root rot of Ashwagandha, a pot trial experiment was conducted at the forest protection division nursery, Bangladesh Forest Research Institute (BFRI), Chattogram, Bangladesh from July 2014 to December 2014.

Isolation, identification and pathogenicity test of the causal organism

Samples of Ashwagandha plants showing root rot symptoms were collected from forest protection division nursery at BFRI, Chattogram, Bangladesh. The infected roots were thoroughly washed with running tap water, cut into small fragments, superficially sterilized with sodium hypochlorite (5%) for 2 min, washed several times with sterile distilled water and dried between sterilized filter paper. The sterilized pieces were transferred into potato dextrose agar (PDA) medium and incubated at 25°C for observing fungal growth. The fungal colonies were purified using single spore or hyphal tip techniques suggested by Booth (Booth 1985) and Dhingra and Sinclair (1985) and then identified according to their morphological and microscopical characters as described by Booth (1985) and Barnett and Hunter (1986). Pathogenicity tests were conducted on potted plants and re-isolation of Fusarium solani.

Sources of Trichoderma strains

Five Trichoderma strains, including *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were collected from the Biotechnology and Microbiology Laboratory, Department of Botany, Rajshahi University, Bangladesh. These strains were previously verified by CABI Bioscience, Surrey, UK. The culture of *Trichoderma viride* FPDTV were procured from Forest pathology laboratory, Forest Protection Division (FPD), BFRI, Bangladesh. Commercially available Bio derma product (Ispahani Agro Limited, Bangladesh) was collected from local market of Gazipur, Dhaka, Bangladesh.

Preparation of inocula of Fusarium solani and Trichoderma strains

In order to prepare *F. solani*, Erlenmeyer flasks containing 100 g of wheat grains and 100 ml of water were autoclaved at 121 °C for 1 h on three successive days. After cooling, about 5-7 small plugs of seven- day- old culture of *F. solani* were dropped into each flask under sterilized condition. The flasks were kept at 25 °C for 4 weeks. Colonized wheat grains were then transferred into paper pockets, dried and ground. Ten gm (105 CFU/gm) of prepared powder was used to infest 1 Kg of soil (Frommel et al. 1991). For preparation of Trichoderma inocula moistened wheat bran was poured into Erlenmeyer flasks which were autoclaved at 121 °C for 1 h on three successive days. The substrate mixture was then inoculated with a homogenized suspension of spore and mycelia of seven days old culture of Trichoderma strains under aseptic conditions. Erlenmeyer flasks were incubated at 27 °C for 14 days. Ten gm of this inoculum (108 CFU/gm) was used to add in 1 Kg of pot soil (Ommati and Zaker, 2012).

Sterilization and application of soil

Soil was collected from the research field of FPD Nursery of BFRI, Bangladesh and sterilized with formaldehyde (formalin: water; 1:5 v/v) and covered with polythene (Begum et al., 2010). After 30 days of sterilization, sterilized soil, sand and decomposed manure were put in the earthen pot (30 × 20 cm) each of 5 kg capacity. Fifty gm of prepared each Trichoderma inoculum powder or 50 gm Bio derma singly and in combination with *F. solani* [Ten gm (10⁸ CFU/gm)/Kg soil] was mixed well in each pot soil. Only soil filled in pot used as control.

Collection and sowing of Seeds

Seeds of Ashwagandha were obtained from FPD, BFRI, Chattogram, Bangladesh. Seeds were first disinfected superficially in 0.05% sodium hypochlorite solution for 3 min then washed three times in sterilized distilled water. These moistened seeds were spread over the polythene sheet for two hours and these seeds were sown 10 seeds/pot. After 7 to 10 days of sowing 5 seedlings were randomly selected from each pot for determination of seedling vigour index. Individual shoot and root length were measured for each seedling. The vigour of the seedlings was determined by the following formula of Abdul-Baki and Anderson (1973).

Vigor index = [mean of root length (cm) + mean of shoot length (cm)] × percentage of seed germinations.

Treatments

The treatments were as following combinations:

T₀: Control (without pathogen); T₁: Control [with *F. solani* (10⁵cfu/g)]; T₂: *T. virens* IMI 392430 (10⁸cfu/g) + *F. solani* (10⁵ cfu/g); T₃: *T. pseudokoningii* IMI-392431 (10⁸cfu/g) + *F. solani* (10⁵cfu/g); T₄: *T. harzianum* IMI-392432 (10⁸cfu/g) + *F. solani* (10⁵cfu/g); T₅ : *T. harzianum* IMI-392433 (10⁸cfu/g) + *F. solani* (10⁵cfu/g); T₆: *T. harzianum* IMI-392434 (10⁸cfu/g) + *F. solani* (10⁵cfu/g); T₇: *T. viride* FPDTV (10⁸cfu/g) + *F. solani* (10⁵cfu/g); T₈: Bio derma + *F. solani* (10⁵cfu/g), T₉: *T. virens* IMI 392430 (10⁸cfu/g); T₁₀: *T. pseudokoningii* IMI-392431 (10⁸cfu/g); T₁₁: *T. harzianum* IMI-392432 (10⁸cfu/g); T₁₂ : *T. harzianum* IMI-392433 (10⁸cfu/g); T₁₃ : *T. harzianum* IMI-392434 (10⁸cfu/g); T₁₄: *T. viride* FPDTV (10⁸cfu/g); T₁₅: Bio derma.

Data recording

Seed germination (%), growth, yield and yield contributing parameters were collected at different stages of plant growth after sowing. Observations were recorded for plant height, number of leaf, number of primary branch, number of secondary branch, fresh shoot weight, dry shoot weight, number of flower at maximum flowering time, number of pod, number of seed, hundred seed weight, root length, root diameter, fresh root weight, seed yield/plant and root yield/plant.

Disease severity was estimated at 10 days interval until 120 days after sowing of seeds according to Abdou et al. (2001) using a rating scale of (0 – 5) based on leaf yellowing grading, viz., 0 = healthy, 1= one leaf yellowing 2= more than one leaf yellowing, 3= one wilted leaf, 4= more than one leaf wilted, and 5= completely dead plants.

Disease severity index (DSI) described by Liu et al. (1995) was adapted and calculated as follows:

$$DSI = \frac{\sum d}{d_{\max} \times n} \times 100.$$

Where: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of tested plants/samples examined in each replicate.

The mean of area under disease progress curve (AUDPC) for each replicate was calculated as suggested by Pandey et al. (1989).

$$AUDPC = D \left[\frac{1}{2} (Y_1 + Y_k) + (Y_2 + Y_3 + \dots + Y_{k-1}) \right]$$

Where D= Time interval; Y₁= First disease severity; Y_k= Last disease severity; Y₂, Y₃,.....Y_{k-1}= Intermediate disease severity.

Experimental design and statistical analysis

The experiment was carried out following Randomized Block Design with three replications and ten plants were used for each replicate. Data on percentage of seed germination, vigour index, growth, yield and yield contributing characteristics were recorded and statistically analyzed by DMRT and correlation matrix with the help of the computer package program SPSS (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Isolation and identification of pathogens

Colonies grown in PDA became whitish to brown after 7 days of inoculation and produced macro and micro conidia (Figure 1a). Macroconidia sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell. Microconidia were 1 to 2 celled, hyaline, puriform, fusiform to ovoid, straight or curved. The chlamydospores located in middle of hyphae (intercalary), on tip of the hyphae (terminal) and some chlamydospores were seen in middle of macro conidia. The chlamydospores were thick walled, rough, globose to oval shaped and measured 8.95-12.65 × 6.10-9.55 μm (Figure 1b).

Pathogenicity Test

The first sign of wilting on Ashwagandha appeared at 90 days after inoculation and gradually intensified. Lower leaves developed the wilting first, then extended to the upper leaves. Vascular discoloration was evident from the early stages of infection, extending, upward throughout the plant (Figure 1d). Healthy Ashwagandha plants were also grown adjacently (Figure 1c).

Effect of Trichoderma strains on AUDPC, seed germination %, growth and yield components of Ashwagandha

A) *Effect of Trichoderma strains on area under disease progress curve (AUDPC)*

Values in the bar Figure 2 indicate that all Trichoderma strains exhibit significant protection against root rot disease compared with control (T1). The highest AUDPC was recorded of T1 (alone with F. solani) treatment and the lowest was recorded of T. harzianum IMI-392433 (T12) followed by T. harzianum IMI-392433+ F. solani (T5). In control (T0), a remarkable AUDPC was

also observed. In this case infection may be occurred from the seeds or environment. In a similar study, Rahman et al. (2012) were used culture filtrates of five *Trichoderma* strains to control anthracnose fruit rot disease of chili under field condition. Their result showed that seed treatment with culture filtrates of *T. harzianum* IMI-392433 significantly ($p=0.05$) suppressed the disease percentages (94.97 %) compared to *Colletotrichum capsici* treatment.

B) *Effect on germination percentages and vigour index*

Seed germination and the vigour index were significantly ($p\leq 0.05$) affected by the treatments. The highest percentage of seed germination (90.43 %) and vigour index (838.29) were recorded for the treatment T12 (*T. harzianum* IMI-392433) and the lowest (42.41 % and 128.28) was recorded for *F. solani* (T1) treatment alone. Seed germination was drastically reduced for T1 (*F. solani*) and control (T0) (Table 1). These results revealed that *T. harzianum* IMI-392433 might promote *Ashwagandha* seed germination. In a similar study, Islam et al. (2011) were evaluated five *Trichoderma* strains for their potentiality on seed germination and seedling parameters in chili both laboratory and field condition. Among the five *Trichoderma* strains, *T. harzianum* IMI-3924332 gave the highest germination percentage both in laboratory and field conditions, while control decrease these value.

Seedling vigour was found higher when *T. harzianum* IMI-392433 (T12) was applied, whereas *F. solani* (T1) showed the worst seedling vigour followed by control (T0) (Table 1). Consistent with the results, Mukhtar (2008) observed the highest vigour index when okra seeds were treated with spore suspension of *T. harzianum*. In a similar study, Begum et al. (2010) were evaluated five *Trichoderma* strains to assay their efficacy in suppressing *Alternaria* fruit rot disease of chili and promoting chili plant growth and yield and observed that application of spore suspension of *T. harzianum* IMI-392432 significantly suppressed the disease and improved highest seed germination percentage and vigour index.

C) *Effect on growth, yield and yield contributing parameters*

Plant height, number of leaf, number of primary branch, number of secondary branch, fresh shoot weight, dry shoot weight, number of flower at maximum flowering time, number of pod, number of seed, hundred seed weight, root length, root diameter, fresh root weight, seed yield/plant and root yield/plant were highest for T12 (*T. harzianum* IMI-392433) and lowest for T1 (*F. solani*) treatment, with a significant difference ($p<0.05$) (Table 2 & 3) (Figure 3). These results indicated that *T. harzianum* has growth-promoting effects on *Ashwagandha*. Earlier work on cabbage supports this result, that is, the regular application of *T. harzianum* increased cabbage growth, leaf area, shoot and root dry weight (Rabeendran et al. 2000). *T. harzianum* IMI392433 increased yield and yield contributing characters by 81.07 % for number of pod, 83.15% for number of seed, 75.94% for hundred seeds weight, 79.62 % for root length, 61.94% for root diameter, 91.26% for fresh root weight, 77.27% for seed yield and 91.21% for root yield compared to *F. solani* (T1) treatment (Table 3). The results revealed that the yield and yield contributing characteristics were significantly influenced by the application of *T. harzianum* IMI-392433. With *T. harzianum* treatment of the seeds, many workers found much higher yields compared to control. Bal and Altintas (2006) observed that *T. harzianum* has positive effect in the early yield of tomato plant (*Lycopersicon esculentum*), which produced 527g/plant in comparison to the control with 374g/plant.

D) *Correlation matrix*

The correlation matrix among different plant parameters are presented in Table 4. The correlation matrix showed that root yield per plant of Ashwagandha had significant and positive correlation with plant height ($r = 0.734^{**}$), number of leaf ($r = 0.725^{**}$), number of primary branch ($r = 0.863^{**}$), number of secondary branch ($r = 0.878^{**}$), fresh shoot weight ($r = 0.749^{**}$), dry shoot weight ($r = 0.708^{**}$), number of flower at maximum flowering time ($r = 0.734^{**}$), number of pod ($r = 0.774^{**}$), number of seed/pod ($r = 0.642^{**}$), hundred seed weight ($r = 0.688^{**}$), root length ($r = 0.711^{**}$), root diameter ($r = 0.970^{**}$), fresh root weight ($r = 0.819^{**}$) and seed yield ($r = 0.817^{**}$) per plant. On the other hand, AUDPC showed negative correlation with all the growth and yield contributing characters (Table 4). The significant and negative correlation ($r = -0.619^{**}$ and $r = -0.564^{**}$) was observed with the root yield/plant, seed yield/plant and AUDPC. These results indicated that root yield/plant of Ashwagandha were dependence on growth characteristics of the plant. The higher seed yield which depends on the higher root yield related to plant growth parameters were negatively influenced by the AUDPC. All Trichoderma strains singly control the disease significantly. Among those T12 control the disease effectively even in presence of *F solani*.

Conclusion

From the above findings it may be concluded that *T. harzianum* IMI-392433 could be more effective for controlling *F. solani* which is causal organism of root rot disease of Ashwagandha under field condition, and this strain also showed promising results on seed germination, growth and yield characteristics of Ashwagandha. The results suggest that this strain may be used as an effective bio control agent to control root rot disease of Ashwagandha.

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Table 1. Seed germination % and vigour index of Ashwagandha under different treatments at 10 DAS

Treatments	Seed germination (%)	Shoot length (cm)	Root length (cm)	Vogour index
T ₀	66.58 j	2.68 ab	3.19 ab	390.83 o
T ₁	42.41 k	1.16 b	1.86 b	128.08 p
T ₂	78.59 f	2.89 ab	3.38 ab	491.19 k
T ₃	70.32 h	2.87 ab	3.27 ab	431.77 n
T ₄	76.38 g	3.29 a	3.48 ab	517.09 j
T ₅	83.38 d	3.86 a	3.96 a	652.03 e
T ₆	81.46 e	3.54 a	3.67 ab	587.32 h
T ₇	80.95 e	3.64 a	3.38 ab	568.29 i
T ₈	68.43 i	2.75 ab	3.59 ab	433.85 m
T ₉	84.51 d	3.79 a	3.89 a	649.04 f
T ₁₀	81.58 e	3.98 a	3.84 a	637.96 g
T ₁₁	88.98 ab	3.86 a	4.16 a	713.62 d
T ₁₂	90.43 a	4.29 a	4.98 a	838.29 a
T ₁₃	88.21 b	4.18 a	4.58 a	772.72 b
T ₁₄	86.32 c	3.86 a	4.67 a	736.31 c
T ₁₅	71.64 h	2.98 ab	3.62 ab	472.83 l

T₀: Control (without pathogen); T₁: Control (Only *F. solani*); T₂: *T. virens* IMI 392430 + *F. solani*; T₃: *T. pseudokoningii* IMI-392431 + *F. solani*; T₄: *T. harzianum* IMI-392432 + *F. solani*; T₅: *T. harzianum* IMI-392433 + *F. solani*; T₆: *T. harzianum* IMI-392434 + *F. solani*; T₇: *T. viride* FPDTV + *F. solani*; T₈: Bio derma + *F. solani*; T₉: *T. virens* IMI 392430; T₁₀: *T. pseudokoningii* IMI-392431; T₁₁: *T. harzianum* IMI-392432; T₁₂: *T. harzianum* IMI-392433; T₁₃: *T. harzianum* IMI-392434; T₁₄: *T. viride* FPDTV, T₁₅: Bio derma.

Table 2. Effect of formulated Trichoderma strains on growth characteristics of Ashwagandha under field conditions

Treatments	Plant height at 90 DAS (cm)	Number of leaf at 90 DAS	Number of primary branch at 90 DAS	Number of secondary branch at 90 DAS	Fresh shoot weight at 90 DAS (gm)	Dry shoot weight at 90 DAS (gm)	Number of flower at the maximum flowering stage
T ₀	28.32 i	20.37 kl	2.17 bc	3.68 de	132.94 n	53.92 i	16.95 l
T ₁	23.98 j	18.98 l	1.21 c	2.12 e	98.53 o	42.38 l	5.82 m
T ₂	32.84 g	26.48 i	2.42 bc	3.87 de	154.32 k	51.42 j	21.95 j
T ₃	30.86 h	24.92 ij	2.37 bc	3.68 de	149.94 l	42.46 l	16.73 l
T ₄	34.72 f	21.38 k	2.28 bc	4.76 cd	163.73 i	58.53 h	25.78 h
T ₅	40.37 c	32.84 f	2.78 bc	5.17 bc	169.62 h	62.19 ef	32.52 f
T ₆	38.26 d	30.96 g	2.62 bc	4.96 cd	162.39 i	60.53 g	28.74 g
T ₇	36.52 e	28.92 h	2.59 bc	4.81 cd	159.48 j	54.82 i	23.84 i
T ₈	29.98 hi	23.74 j	1.98 bc	3.79 de	142.53 m	46.83 k	18.95 k
T ₉	39.68 cd	37.46 cd	2.91 bc	6.18 bc	182.28 e	63.36 e	38.75 e
T ₁₀	35.18 ef	35.68 e	2.85 bc	5.94 bc	179.18 f	61.42 fg	32.63 f
T ₁₁	45.37 b	36.47 de	2.98 bc	6.28 bc	188.16 c	76.42 b	49.62 b
T ₁₂	49.68 a	43.28 a	4.86 a	8.97 a	192.28 a	79.39 a	58.84 a
T ₁₃	46.36 b	40.19 b	3.28 ab	8.27 a	190.16 b	73.94 c	51.28 b
T ₁₄	44.86 b	38.42 c	3.17 abc	7.64 ab	186.24 d	66.58 d	46.83 c
T ₁₅	32.53 g	33.97 f	2.74 bc	5.29 cd	177.18 g	62.83 ef	42.84 d

T₀: Control (without pathogen); T₁: Control (Only *F. solani*); T₂: *T. virens* IMI 392430 + *F. solani*; T₃: *T. pseudokoningii* IMI-392431 + *F. solani*; T₄: *T. harzianum* IMI-392432 + *F. solani*; T₅: *T. harzianum* IMI-392433 + *F. solani*; T₆: *T. harzianum* IMI-392434 + *F. solani*; T₇: *T. viride* FPDTV + *F. solani*; T₈: Bio derma + *F. solani*; T₉: *T. virens* IMI 392430; T₁₀: *T. pseudokoningii* IMI-392431; T₁₁: *T. harzianum* IMI-392432; T₁₂: *T. harzianum* IMI-392433; T₁₃: *T. harzianum* IMI-392434; T₁₄: *T. viride* FPDTV, T₁₅: Bio derma.

Table 3. Effect of formulated Trichoderma strains on yield and yield contributing characteristics of Ashwagandha under field conditions

Treatments	Number of pod	Total Number of seed (count 10 pod/plant)	100 seed weight (gm)	Seed yield /plant (gm)	Root length at 120 DAS (cm)	Root Diameter at 120 DAS (cm)	Fresh root weight at 120 DAS	Root yield/ plant (gm)
T ₀	22.12 j	38.27 j	0.62 cd	3.83 d	11.27 i	3.27 def	39.37 j	3.93 cd
T ₁	8.43 k	36.28 k	0.51 d	3.63 d	6.37 j	2.39 f	23.18 k	2.32 d
T ₂	26.75 h	39.97 ij	0.84 bcd	4.1 d	14.29 h	3.97 cdef	42.37 i	4.23 bc
T ₃	24.96 i	39.32 ij	0.81 bcd	3.93 d	12.43 i	3.16 ef	40.34 j	4.03 bcd
T ₄	28.53 fg	38.28 j	0.89 bcd	3.83 d	18.12 ef	4.98 abcde	49.83 g	4.98 abc
T ₅	36.28 c	44.38 g	1.14 abcd	4.43 cd	18.37 fg	5.27 abc	54.38 de	5.44 abc
T ₆	32.29 d	42.14 h	0.97 bcd	4.21 cd	17.98 ef	5.12 abcd	51.94 f	5.19 abc
T ₇	30.95 de	40.97 hi	0.92 bcd	4.09 d	15.62 gh	4.61 abcde	46.37 h	4.64 bc
T ₈	28.74 fg	40.17 i	1.12 abcd	4.01 d	17.68 ef	4.25 bcde	44.92 h	4.49 bc
T ₉	32.28 d	52.83 d	0.98 bcd	5.28 bcd	18.62 e	4.98 abcde	54.18 de	5.42 abc
T ₁₀	29.73 ef	50.34 e	1.69 abc	5.03 bcd	27.95 b	4.86 abcde	52.91 ef	5.29 abc
T ₁₁	37.68 bc	62.86 b	1.74 abc	6.29 ab	24.17 c	5.97 ab	57.61 c	5.76 abc
T ₁₂	44.53 a	67.29 a	2.12 a	6.73 a	31.27 a	6.28 a	66.38 a	6.64 a
T ₁₃	39.12 b	64.12 b	1.86 ab	6.41 ab	29.38 b	5.86 abc	52.96 ef	5.31 abc
T ₁₄	36.19 c	58.67 c	1.81 ab	5.87 abc	22.26 d	5.74 abc	59.67 b	5.97 ab
T ₁₅	27.73 gh	48.37 f	1.24abcd	4.84 bcd	16.37 fg	5.29 abc	55.82 d	5.58 abc

T₀: Control (without pathogen); T₁: Control (Only *F. solani*); T₂: *T. virens* IMI 392430 + *F. solani*; T₃: *T. pseudokoningii* IMI-392431 + *F. solani*; T₄: *T. harzianum* IMI-392432 + *F. solani*; T₅: *T. harzianum* IMI-392433 + *F. solani*; T₆: *T. harzianum* IMI-392434 + *F. solani*; T₇: *T. viride* FPDTV + *F. solani*; T₈: Bio derma + *F. solani*; T₉: *T. virens* IMI 392430; T₁₀: *T. pseudokoningii* IMI-392431; T₁₁: *T. harzianum* IMI-392432; T₁₂: *T. harzianum* IMI-392433; T₁₃: *T. harzianum* IMI-392434; T₁₄: *T. viride* FPDTV, T₁₅: Bio derma.

Table 4. Correlation matrix among different parameters of Ashwagandha as influenced by treatments

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1															
2	0.889**	1														
3	0.660**	0.652**	1													
4	0.892**	0.882**	0.830**	1												
5	0.873**	0.892**	0.580**	0.825**	1											
6	0.909**	0.864**	0.613**	0.858**	0.859**	1										
7	0.912**	0.933**	0.630**	0.887**	0.918**	0.956**	1									
8	0.936**	0.836**	0.653**	0.839**	0.906**	0.840**	0.867**	1								
9	0.887**	0.916**	0.614**	0.877**	0.810**	0.908**	0.945**	0.782**	1							
10	0.673**	0.692**	0.697**	0.771**	0.624**	0.676**	0.687**	0.600**	0.705**	1						
11	0.781**	0.798**	0.789**	0.908**	0.678**	0.772**	0.793**	0.690**	0.847**	0.798**	1					
12	0.849**	0.849**	0.638**	0.873**	0.851**	0.851**	0.857**	0.858**	0.859**	0.694**	0.753**	1				
13	0.787**	0.742**	0.826**	0.896**	0.755**	0.771**	0.771**	0.789**	0.689**	0.705**	0.844	0.749	1			
14	0.864**	0.856**	0.635**	0.825**	0.946**	0.862**	0.908**	0.927**	0.770**	0.610**	0.664**	0.828**	0.802**	1		
15	-0.681	-0.747**	-0.473**	-0.698**	-0.836**	-0.807**	-0.806**	-0.725**	-0.713**	-0.527**	-0.564**	-0.696**	-0.581**	-0.817**	1	
16	0.734**	0.725**	0.863**	0.878**	0.749**	0.708**	0.734**	0.774**	0.642**	0.688**	0.817**	0.711**	0.970**	0.819**	-0.619**	1

1= Plant height at 90 DAS, 2=Number of leaf at 90 DAS, 3=Number of primary branch at 90 DAS, 4= Number of secondary branch, 5= Fresh shoot weight at 90 DAS, 6= Dry shoot weight at 90 DAS, 7= Number of flower at maximum flowering time, 8 = Number of pod, 9 = Number of seed, 10 = Hundred seed weight, 11 = Seed yield/plant, 12 = Root length, 13 = Root diameter, 14 = Fresh root weight, 15 = Area under disease progress curve, 16 = root yield/plant.

** . Correlation is significant at the 0.01 level.



Figure 1. Photograph shows colony, conidia and pathogenicity test of *F. solani* and mass production of *Trichoderma* and *Fusarium solani*; a: colonies of *F. solani* on PDA after 7 days. b: conidia of *F. solani*. c: healthy plant (without inoculum, Control) after 90 DAS of sowing; d: typical wilting symptoms were visible in Ashwagandha plant after 90 days of sowing. e: *Trichoderma* mass production in wheat grain medium after 7 days of incubation. f: *F. solani* mass production in wheat grain medium after 7 days of incubation.

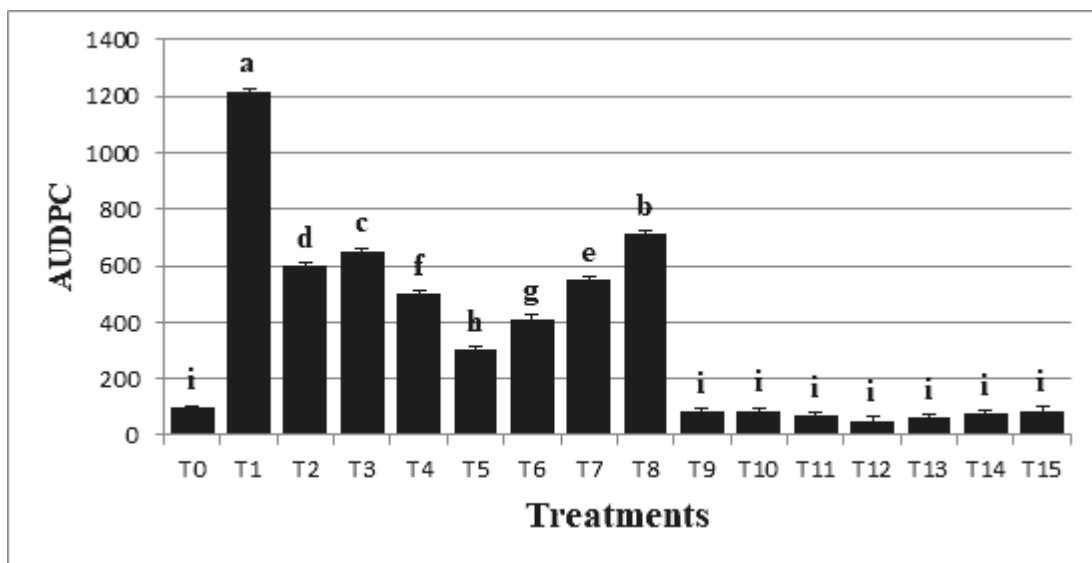


Figure 2. Effect of formulated Trichoderma strains on area under disease progress curve (AUDPC) of Ashwandha under pot experiments

Values in the bar followed by different letters indicate significant differences and same letters are not significantly different among treatments according to DMRT test ($P \leq 0.05$).

T0: control (without pathogen); T1: control (Only *F. solani*); T2: *T. virens* IMI 392430 + *F. solani*; T3: *T. pseudokoningii* IMI-392431 + *F. solani*; T4: *T. harzianum* IMI-392432 + *F. solani*; T5: *T. harzianum* IMI-392433 + *F. solani*; T6: *T. harzianum* IMI-392434 + *F. solani*; T7: *T. viride* FPDTV + *F. solani*; T8: Bio derma + *F. solani*; T9: *T. virens* IMI 392430; T10: *T. pseudokoningii* IMI-392431; T11: *T. harzianum* IMI-392432; T12: *T. harzianum* IMI-392433; T13: *T. harzianum* IMI-392434; T14: *T. viride* FPDTV, T15: Bio derma.





Figure 3. Effect of Trichoderma on growth character of Ashwagandha at 90 DAS

T0: Control (without pathogen); T1: Control (Only *F. solani*); T2: *T. virens* IMI 392430 + *F. solani*; T3: *T. pseudokoningii* IMI-392431 + *F. solani*; T4: *T. harzianum* IMI-392432 + *F. solani*; T5: *T. harzianum* IMI-392433 + *F. solani*; T6: *T. harzianum* IMI-392434 + *F. solani*; T7: *T. viride* FPDTV + *F. solani*; T8: Bio derma + *F. solani*, T9: *T. virens* IMI 392430; T10: *T. pseudokoningii* IMI-392431; T11: *T. harzianum* IMI-392432; T12: *T. harzianum* IMI-392433; T13: *T. harzianum* IMI-392434; T14: *T. viride* FPDTV, T15: Bio derma.

Machining and Handtool Properties of Mahogany (*Swietenia macrophylla*) Wood grown in Bangladesh

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Abstract

Wood is widely used in all over the world because of its excellent physical, mechanical and finishing properties. However, the machining and handtool properties of mahogany (*Swietenia macrophylla*) wood were ascertained for the characterization of working properties in this study. The effects of machining properties, such as- planing, shaping, boring, mortising and turning were tested on this wood species along with handtool test. The evaluation of each test was based on frequency of occurrence of defect free sample. The applications of two types of polishing materials, namely: shellac and carpa were used for the purpose of finishing property evaluation. Each sample was visually observed and classified based on five quality grades. This wood showed an excellent working performance in all properties except handtool property in planing test.

সারসংক্ষেপ

ভৌত, যান্ত্রিক ও পলিশিং গুণাগুণ থাকায় কাঠ সমগ্র বিশ্বে ব্যাপকভাবে ব্যবহৃত হয়। যাহোক, অত্র গবেষণায় ওয়ার্কিং গুণাগুণ বৈশিষ্ট্যায়িত করার জন্য মেহগনি কাঠের মেশিনিং ও হ্যান্ডটুল গুণাগুণ নিরূপণ করা হয়েছে। উক্ত কাঠের হ্যান্ডটুল ও মেশিনিং গুণাগুণ, যেমন: প্লানিং, শেপিং, বোরিং, মরটাইজিং, টার্নিং পরীক্ষাগুলির প্রভাব পর্যবেক্ষণ করা হয়েছে। প্রতিটি পরীক্ষণে ত্রুটিমুক্ত নমুনার উপর ভিত্তি করে মূল্যায়ন ও গ্রেডিং করা হয়েছে। পলিশিং গুণাগুণ নির্ণয়ে দুই ধরনের পলিশ, যথা: শেলাক ও কারপা ব্যবহার হয়েছে। নমুনাগুলোকে খালি চোখে নিরীক্ষণ করার মাধ্যমে পাঁচটি গুণাগুণের গ্রেডে ভাগ করা হয়েছে। এই কাঠ প্লানিং পরীক্ষণে হ্যান্ডটুল গুণাগুণ ছাড়া অন্যান্য কাজের গুণাগুণ উন্নতমানের প্রদর্শন করেছে।

Keywords: Working properties, Planing, Boring, Mortising, Shaping, Turning.

Introduction

Mahogany (*Swietenia macrophylla*) tree species is native to tropical America and it is one of the most important timber species in world trade (Gillies et al, 1999). The plant mahogany commonly known as “sky fruit”, because its fruits seen to point upwards to the sky is a beautiful, lofty, evergreen large tree usually 30-40 m in height and 3-4 m in girth (Goh and Kadir 2011). The average tree is 3 to 6 feet in diameter and has a long trunk that is generally free of branches from 60 to 80 feet above the heavy buttress (Kukachka 1959). This tree has been planted in homestead and road side as timber species, and it has become popular to the people for the growing nature of it under a little bit shadow in Bangladesh.

Wood has traditionally been the basic raw material for furniture and joinery industries. One of the most important advantages of wood is its easy machine ability in contrast to metal and plastic products. However, its non-uniform characteristics within and between species plays a significant role on its efficient and effective machining. Any surface defects due to an improper machining process will also reduce the quality of the final product, resulting in an increase in the cost of the manufactured unit. Therefore, it is important to evaluate machining parameters and relate them to raw material characteristics (Sofuoglu and Kurtoglu 2014). Wood is considered as the prime material for the survival of mankind and also as the fundamental one for the enhancement of civilization (Sattar et al. 1981).

Commercial wood has excellent physical, mechanical and appearance properties and is highly used in markets all over the world (Tu et al. 2014). Variation of machining properties of different wood is influenced by their density, fiber structure, chemical and mineral contents and many other characteristics. As machining is involved in all common wood working operations, knowledge of the machine ability of different wood is helpful in selection of a particular species for a specific use. The importance of this information lies in marketing of new and inexpensive species and in their conversion for many important wood products (Qasem et al. 1981).

The quality of commercial timber varies according to local conditions, and the variations encountered are no greater over its entire range than those which might be found within the confined geographic area. These variations chiefly effect the weight, hardness, and color of the wood (Kukachka, 1959). Knowledge of machining properties of wood is required specially for fabrication of furniture and cabinet work (Hossain et al. 1978). Now-a-days, mahogany wood is being widely used for the purpose of making furniture, cabinet, interior and construction works in Bangladesh. But it is unknown to the users of its working properties. The study was thus carried out to ascertain the machining and handtool properties along with finishing properties which wood help to regulate the proper use of the species.

Materials and methods

Mahogany (*Swietenia macrophylla*) wood aged about 27 years was procured in the log form from Chattogram. This log was converted into different sizes by plane sawing. The sawing quality of this wood was determined by manual feeding of logs to the saw blade. The sawn timber was seasoned to less than 15 percent moisture content (Table-1). The seasoned timber was dressed and 20 samples of 20 mm × 126 mm × 1224 mm in size were prepared according to ASTM standard. All the test samples were sound and free from all defects including knots, stain, incipient decay, end splits and surface checks. For different experimental conditions, each sample was cut into three parts which are 20 mm × 106 mm × 915 mm, 20 mm × 20 mm × 153 mm and 20 mm × 78 mm × 307 mm in size. First sized were used for the tests of planing and finishing properties, and second were used for turning property tests. The third sized samples were used for the tests of boring, mortising and shaping properties.

Table 1: Moisture content, age and specific gravity of mahogany wood species.

Parameter	Quantity	Unit
Moisture content of wood	14	Percentage
Age of the tree	27	Year
*Specific gravity	0.58	No unit

* Sattar, et al 1999.

Ten samples were tested with machines and ten samples with hand tool but twenty samples were tested for the purpose of planing and finishing tests. After the completion of machining tests, the samples were visually examined for sorting out the defect free ones immediately. The occurrence of defects, namely- fuzzy grain, torn grain, raised grain, chipped grain, broken corner, tear out and roughness was recorded. The percentages of defect-free samples based on total samples were determined and these percentages were considered to be the measure of their machining qualities. Then, each sample was visually examined and classified based on five quality grades which are shown in Table 2.

Table 2: Quality grades of different property tests

Quality grade	Performance	Defects
1	Excellent	No defect
2	Good	Few slight defects
3	Fair	Lots of slight defects
4	Poor	Serious defects
5	Very poor	Very serious defects

The performance criteria and suitable pieces for different test samples used for the tests are presented in Table 3. The machining tests were carried out according to American Society for Testing and Materials standard test method - "Conducting Machining Tests of Wood and Wood-Based Products (ASTM D 1666-64 Standard International, 2004)". Similar tests were carried out using carpenter's handtool.

Table 3: Qualified grade and performance criteria based on different test along with dimension of different test samples

Tests	Dimension (mm)	Qualified grade	Criteria of performance
Planing	20 x 106 x 915	1 and 2	Excellent and good
Shaping	20 x 78 x 307	1 and 2	Excellent and good
Boring	20 x 78 x 307	1 and 2	Excellent and good
Mortising	20 x 78 x 307	1,2 and 3	Excellent, good and fair
Turning	20 x 20 x 153	1 and 2	Excellent and good
Finishing	20 x 106 x 915	1	Excellent

Planing

The planing test was carried out in a single surface planer with a cutter head speed of 3000 rotation per minute. The depth of cut for all the runs was 1.59 mm. The machine was equipped with a variable feed rate of 154 mm to 2540 mm per minute and the feed rate was adjusted to 636 mm per minute so that the target numbers of knife mark were 40 per 2.54 cm. The run was made with a cutting angle of 25 degrees and sharpness angle of 30 degrees. The test samples were fed into the machine one by one to complete a full rotation of test. Each of the samples was carefully examined visually for planing defects after each run. The test samples of the species were tested in a group separately. The same number of samples were tested with the carpenter's hand planner and similar procedure of testing was applied.

Boring

The boring test was carried out in a 508 mm single spindle hand feed drill press. Two thorough holes were bored on each sample. A one-inch single twist solid center bred point type of wood boring bit was used for the test. The drill was adjusted to maintain a spindle speed of 2850 rpm. In hand tool test, boring was done by a carpenter's hand drill. A one-inch single twist solid center screwed point type wood boring auger bit was used for the test. Solid hardboard was used as backing underneath in order to avoid the tearing and splintering of samples at the bottom during boring both for machining and handtool test.

Mortising

The samples used for boring test by machine and hand tool were also used for carrying out the mortising test by machine and handtool respectively. Two thorough mortises were cut on each sample extending through into a hard board backing. Each mortise was cut with two sides parallel and two sides perpendicular to the grain. The tests in machining were carried out in a foot feed vertical square hollow chisel mortiser. The spindle speed of 3600 rpm and the chisel of 12.7 mm were used for mortising test.

Shaping

The test samples used for boring and mortising were also used for shaping test. In machine, the test was carried out in a special jig to shape the sample to a curved pattern. A hand feed single spindle shaper with two high speed steel knives having a spindle speed of 6500 rpm was used. The cutter used to obtain a quarter round pattern had a radius of curvature of 12.70 mm and the cutting angle was set 25°. In hand tool test, ripping of the sample was done by carpenter handsaw to obtain the quarter round pattern. The shaping was carried out by carpenter's chisel of half-round type.

Turning

The turning test was carried out in a variable speed wood lathe at 2400 rpm. A single high speed steel cutter was used to give head and cove for having different turning features as well as the ability to cut at different angles with the grain.

Finishing

The planing test samples were used for the finishing test after completing all planing tests. In finishing test, two types of polish, viz.: shellac and carpa were applied and performance was recorded on the basis of the surface finish and physical appearances. Gum copal finish is locally known as 'carpa' or 'chandra' polish. The polish is prepared by dissolving gum copal in denatured alcohol. The standard mixture is 70% denatured alcohol and 30% gum copal. The method of applications is the same as that of shellac polish (Qasem 1987).

Results and Discussion

Mahogany wood was moderately hard and heavy according to the variation of its density, grain orientation and the load applied to the saw blade, and it indicated medium sawing quality.

The results of planning, shaping, boring, mortising, turning and finishing properties for mahogany wood species are presented in Table 4.

Table 4: Property wise grade percentage of different properties for mahogany wood

Properties		Grade of different properties (%)					
Name	Type	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Qualified grade
Planing	Machining	70	25	5	0	0	95
	Handtools	85	15	0	0	0	100
Shaping	Machining	100	0	0	0	0	100
	Handtools	100	0	0	0	0	100
Boring	Machining	100	0	0	0	0	100
	Handtools	100	0	0	0	0	100
Mortising	Machining	90	10	0	0	0	100
	Handtools	90	10	0	0	0	100
Turning	Machining	100	0	0	0	0	100
Finishing		100	0	0	0	0	100

The qualified grades of planing, shaping, boring, mortising and turning properties were assumed the summation of grade 1 and grade 2. Among these operations, shaping, boring and turning showed only grade 1 that referred 100% qualified grade. On the other hand, machining and handtool properties of planing tests resulted three grade (grade 1, 2 & 3) and two grade (grade 1 & 2) respectively. In terms of planing test, machining property indicated 70 % grade 1, and it rated only 95 % qualified grade where as hand tool property indicated 100 % qualified grade. In case of this test, handtool property showed 85 % grade 1 which is higher than that of machining.

In terms of mortising tests for both machining and hand tool properties, the qualified grades were considered the sum of grade 1, 2 and 3. In this case, both machining and handtool properties showed 90% grade 1 and 10 % grade 2, and these properties indicated 100 % qualified grade. In the finishing operation, qualified grade was assumed only grade 1, and it scored 100 % qualified grade.

The defect free samples for mahogany wood in different tests are shown in Fig.1. In case of machining properties, this wood showed excellent shaping, boring, mortising and turning results, but planing operation indicated good quality. In terms of hand tool properties, all showed excellent qualities.

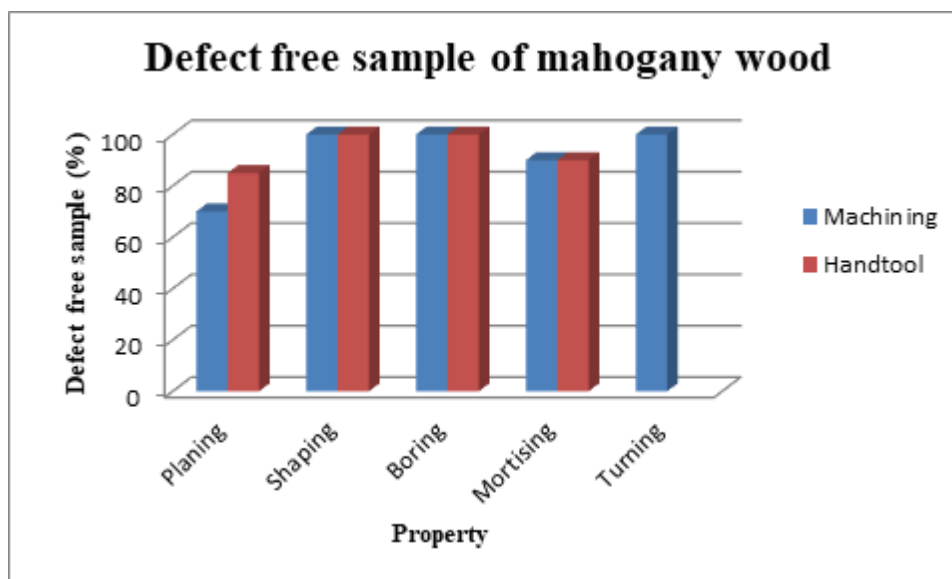


Figure 1: Comparison of the defect free samples among different tests

Defects of planing and mortising operations along with average percentage of defects are presented in Table 5. Defects typically observed for mahogany wood species were fuzzy grain for both machining and handtool properties in planing operations. A few tear out and crushing out were present on the transverse side of the hole for machining and handtool properties respectively in mortising tests.

Table 5: Defects of planing and mortising tests for machining and hand tool properties

Test name	Property	No. of defected sample	Defects	Average %
Planing	Machining	06	Fuzzy grain	21.67
	Handtool	03	Fuzzy grain	20
Mortising	Machining	01	Tear out	20
	Handtool	01	Crushing out	20

For good machining quality, the cutters used in the machine should be maintained properly. Generally, deep cuts should not be made. The number of blade traces in unit distance should be high. High feed speed can cause a poor surface. The defects may be caused by feed speed. The feed speed of planing test should be slow, but capacity should also be considered.

The evaluation of machining defects was based on visual inspection. But it was not possible to quantify them properly. In the defective samples, particularly in planing and turning test, the degree and frequency of incidence of defects were negligible and the defects could easily be removed by adequate sanding.

Because of the limitation of available equipments, extensive investigation of the machining tests could not be carried out. For optimum results, further research work is still needed.

Conclusion

This experiment was carried out to ascertain the behaviour of mahogany wood species for the characterization of different important machining, handtool and finishing properties generating some essential information on the suitability of various utilizations. These evaluated properties indicated that this wood species should have potential suitability in different purposes. Mahogany wood could be suitable for quality furniture, cabinet, fixture, music instrument, door, paneling and turnery works along with interior designs and construction purposes. By using this wood commercially, it may reduce the pressure on the traditional timber species and people of our country could fulfill their quality furniture demand. However, the result of this study is an indicative value and may be used when and where a particular property or a group of properties are to require in the selection of this species for specific use.

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Effect of Storage Condition and Duration on Germination Of Agar (*Aquilaria malaccensis* Lamk.) Seed

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Abstract

A nursery trial was conducted at National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute, Chittagong to evaluate the effect of storage condition and duration on germination of Agar seed. Agar seed were stored at five different storage condition viz. open air (control), sand, chalk powder, normal refrigerator (0~40C) and saw dust for different storage durations viz. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 and 36 days. Storage condition, duration and their interaction were found significant on germination of Agar seed. Refrigerator (0~40C) showed the highest germination (82%) at 3 days duration. It also prolonged the seed viability (12%) up to 33 days. Such technique of maintaining viability of Agar seed may be useful for raising seedlings and plantations at large scale.

সারসংক্ষেপ

বাংলাদেশ বন গবেষণা ইনস্টিটিউটের অধীনস্থ বীজ বাগান বিভাগের ন্যাশনাল ফরেস্ট সীড সেন্টারে আগর বীজের অংকুরোদগমের উপর বিভিন্ন সংরক্ষণ পদ্ধতি ও উহার সময়কালের প্রভাব মূল্যায়নের জন্য বর্তমান পরীক্ষণটি পরিচালনা করা হয়েছে। ৫টি সংরক্ষণ মাধ্যম যথা : খোলা অবস্থায় (কন্ট্রোল), বালি, চক পাউডার, রেফ্রিজারেটর (০-৪° সে.) ও কাঠের গুড়া এবং বিভিন্ন সময়কাল যথা: ৩, ৬, ৯, ১২, ১৫, ১৮, ২১, ২৪, ২৭, ৩০, ৩৩ ও ৩৬ দিন সংরক্ষণ করে আগর বীজের অংকুরোদগমের হার এবং জীবনীশক্তি নির্ণয় করা হয়েছে। এদের মধ্যে রেফ্রিজারেটরে (০-৪° সে.) ৩ দিন পর্যন্ত সংরক্ষণ করা বীজে সর্বোচ্চ ৮২% অংকুরোদগম এবং ৩৩দিন পর্যন্ত ১২% জীবনীশক্তি পাওয়া গিয়েছে। এই পরীক্ষার ফলাফল বৃহৎ পরিসরে চারা উত্তোলন ও বাগান সৃজনের ক্ষেত্রে সহায়ক ভূমিকা রাখবে।

Keywords: *Aquilaria malaccensis*, germination, seed, storage condition.

Introduction

Agar(*Aquilaria malaccensis* Lamk.) occurs predominantly in the Indo-Burma hotspot of biodiversity (Whitmore 1973). It is also found in Nepal, Bhutan, North-Eastern India (Assam, Meghalay, Nagaland, Manipur and Tripura), Myanmar, Thailand, Laos, Vietnam, Cambodia, Indonesia, Malaysia, South-Eastern China, Brunei Darussalam, The Philippines, Papua New Guinea and islands of East India (Baksha et al. 2009, Burkhil 1966). In Bangladesh, it is found naturally in the forests of Sylhet, Chittagong and Chittagong Hill Tracts of Bangladesh (Rahman and Basak 1980). Population of Agar has markedly decreased in natural forests of Bangladesh due to unsustainable harvesting of natural trees for agar wood trade. Hence, it is enlisted in 'The World

List of Threatened Trees' since late 2000s (Chakrabarty et al., 1994). The high value of the wood has resulted in indiscriminate felling of natural populations in some cases. Thus, the knowledge of its regeneration ecology is extensively desired for developing protocols for raising large-scale plantations of Agar (Donovan et al. 2004).

Generally seedling is regenerated after storing of seed over variable period. Efficient storage of seeds is necessary to ensure continuous and cost effective supply of seedlings, which is a prerequisite for the success of any afforestation programme. Seed storage is also important for conserving the genetic resources which are ravaged by deforestation as well as by catastrophes such as forest fire, draught and floods. However, storage potential of tree seeds is highly species-specific and large variation has been encountered across the tree species (Berjek and Pammenter, 2002). Based on the inherent storage potential, seeds are grouped into two main categories viz. recalcitrant and orthodox (Berjek and Pammenter, 2002). Recalcitrant (desiccation-sensitive) seeds are metabolically active when shed from the mother plant and possess relatively high moisture content. Even under ambient temperature and low relative humidity their post-harvest life is very short which also depends on the species. Since sensitive to desiccation, these seeds lose viability when their moisture content falls below 20 to 30% (Farrant et al. 1988, Pritchard 2004). As Agar seed is recalcitrant, it is imperative to find out a suitable storage method which can prolong its viability to raise seedlings in large scale at nursery. However, there is a scanty literature regarding the storage method of Agar seed. Therefore, the present study was undertaken to evaluate the effect of storage condition and duration on germination and viability of Agar seed.

Materials and Methods

The experiment was conducted at National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute, Chittagong during July to August 2014. Mature fruits of Agar were collected from the plus trees in the month of July. Seeds were extracted from depulping the fruits manually.

Fresh seed germination and viability test was conducted with four replications of 100 seeds each. Seeds were sown into moist sand bed and germination was calculated following standard method (ISTA 2006). To test the influence of storage conditions and durations a total of 3,250 seeds were taken and divided equally into five seed lots. Each lot has subjected to a specific storage condition as follows: open air/control, sand, chalk powder, refrigerator and saw dust. Seeds in all conditions were stored for different durations viz. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36 and 39 days. From stored seed, ten seeds with five replications were taken out after every three days and germination was tested by sowing in moist sand bed. Seeds were sown 0.5 cm depth and 4.0 cm distance between seeds in sand beds and then pressed lightly into the sand. Proper shade was provided until germination starts. Routine watering and weeding activities were carried out. Seeds were considered germinated when the cotyledons protruded from the sand surface. Germinated seeds were remarked with small sticks to differentiate them from newly germinated seeds. Germination was observed on alternate days until completion. The factorial experiment was followed as complete randomized block design with two factors - storage condition and duration. A variation (ANOVA) in germination potential under different conditions was analyzed using statistical package MSTAT.

Results and Discussion

Analysis of variance(ANOVA) for germination potential of Agar seed under different conditions and durations was done and found highly significant within storage conditions , durations and their interaction(Table 1). This indicated that viability of Agar seed is highly dependent on both storage conditions and storage durations which was in accordance with Manjkhola et al(2005) and Panwar et al. (2015).

Germination of Agar was epigeous. Both fresh and stored seeds started germination within 6 to 12 days. Similar results were observed for *A. crassna* where seeds germinated within 9 to 15 days (Soehartono and Newton 2001, Shankar 2012) and for *Gyrinops walla* Garten. within 7 to 14 days(Alwis et al. 2016). Gemination was completed within 26 days which is similar to Beniwal (1989) and Adelina et al. (2004).

Figure 1. A) Fruits, B) Seeds and C) Seedlings of Agar



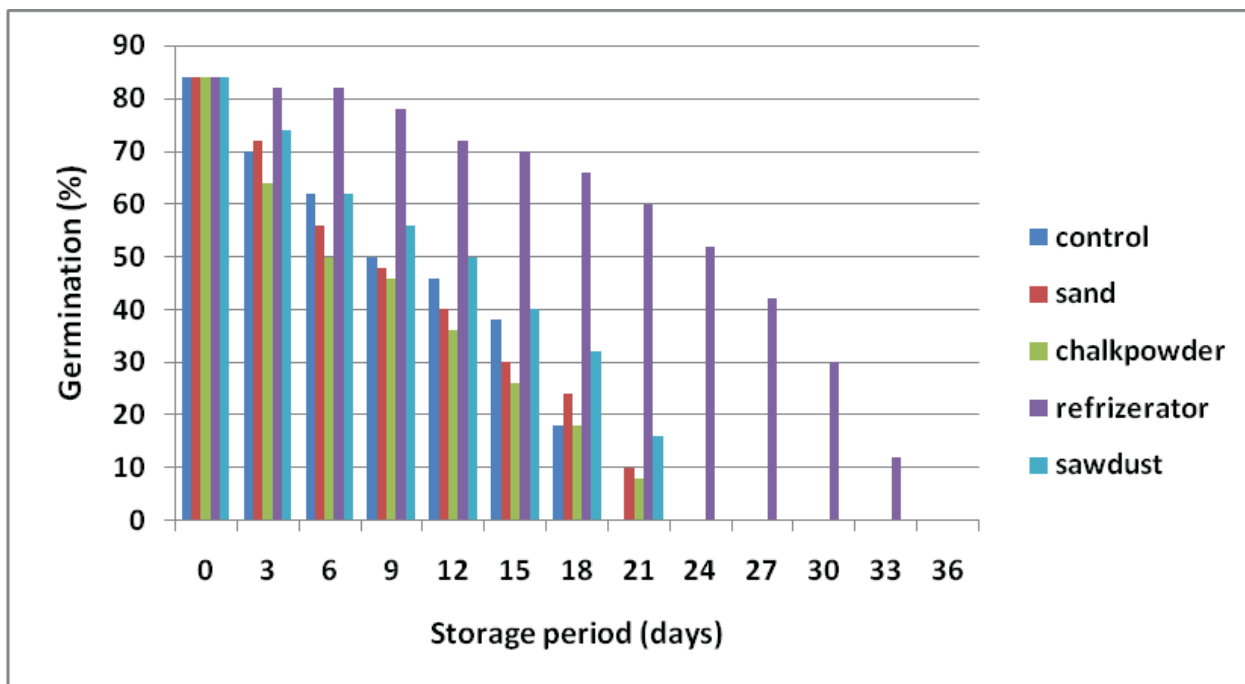
The highest germination (82%) was recorded with both fresh seeds sown immediately after harvest and stored in refrigerator (0~4⁰C) for 3 days. Tabin and Srivastava (2014) recorded 92% and Adelin et al. (2004) recorded 70-80% germination when direct sowing.

Table 1. Two-way ANOVA for seed germination at different storage conditions in relation with storage periods

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Storage condition(C)	4	458.240	114.560	110.7346**	0.0000
Storage duration (D)	10	1559.542	155.954	150.7466**	0.0000
C X D	40	108.240	2.706	2.6156**	0.0000
Error	220	227.600	1.035		
Total	274	2353.622			

**Values are significant at p > 0.01 level

Figure 2. Effect of storage conditions and durations on germination of Agar seeds



A reduction trend on seed germination percent over the period was evident and significant differences in the germination among the storage conditions and durations used in the experiment. The seeds stored for 3 days in refrigerator showed the highest (82%) germination (Fig. 2) followed by the seeds stored in saw-dust (74%), sand (72%), control (70) and chalk powder (64%). After a period of 21 days storage, the seeds of refrigerator found 60 % germination followed by the seeds of saw-dust, sand and chalk powder 16%, 10% and 8% respectively, although the seeds of control had no germination. After the storage of 24, 27 and 30 days, the seeds of refrigerator showed 52%, 42% 30% and 12% of germinability respectively, while others storage conditions showed no germination at all. Present study revealed that storing seeds in cool conditions such as in a refrigerator can prolong viability 12% up to 33 days. No more seeds were found with germinability after storage of 33 days.

Analysis of variance (ANOVA) for viability potential under different media and durations was done and F value was found highly significant (Table 1) within the interaction of media and storage durations. LSD values (1.267 at 5% and 1.672 at 1% level) were for grading the combination more precisely. Germination level in earlier interval period of followed all storage media were found all most same and maximum. But, emphasizing the prolonging period of viability in desired level, seeds stored in refrigerator, germination after 15, 18 and 21 days were found statistically same and optimum at 5% & 1% level of significance. Seed viability of Agar other than those conditions in various interval periods were found poor & below desired level (Table 1).

Therefore, viability period of Agar in desired level could be selected for the period prolonging 21 days in refrigerator condition. In the same condition Agar seed prolonged only 12% germinability up to 33days.

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Suitability of Acacia hybrid for making hardboard

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Keywords: *Acacia hybrid*, hardboard, steamed chips, chemically treated.

The *Acacia hybrid*, a cross between *Acacia mangium* and *Acacia auriculiformis*, grows in Indonesia, Malaysia, Thailand, Vietnam, and China (Kha 2000; Kijkar 1992; Rufelds 1987, 1988). At present forest department and local people of Bangladesh have been planting thousands of hectares of these species. *Acacia hybrid* is a fast growing medium sized leguminous tree. The species is more productive than either of the parent species. The wood density is slightly higher than *A. mangium*, and moreover the shape of the log is almost completely round, which renders *Acacia hybrid* as a valuable and excellent source of timber (Jusoh et.al 2014). In Bangladesh it has very limited use. Due to this Bangladesh Forest Research Institute has been conducting research to determine its end use for efficient utilization. Scientists found that the species is fine grained and may be used for making furniture, small hand tools, cabinet door frame, window frame and pulp and paper (Rokeya et.al 2010). It peels easily and produces the best quality veneer which can be used for decorative purpose, plywood for general use and particleboard (Rahaman et.al 2012). To this end, hardboard making study is undertaken for knowing the suitability of the species.

Acacia hybrid logs were procured from Banshkhali, Chattogram. The freshly cut *Acacia hybrid* logs were debarked and sawn to 10 cm X 10 cm X 100 cm size. Then these were chipped in the laboratory model Murray chipper machine and screened to remove oversized and pin chips. In addition, the knots, barks and decayed wood chips were removed. The defect free chips were then air dried. The *Acacia hybrid* chips were cooked by steaming and different chemical pretreatment process. By the steaming process, chips were cooked in laboratory model stainless steel rotary digesters where the digester pressure was 7.03 and 10.55 kg/cm². The digesting time was 30, 60 and 90 minutes for each pressure. In the chemical pretreatment process, chips were soaked in 1% NaOH, 2% NaOH, 3% NaOH, 3% Na₂SO₃ and mixture of 3% (NaOH and Na₂SO₃) solution under atmospheric pressure for 24 hours soaking time. The chips (steamed and chemically treated) were then refined in a single rotating disk attrition mill at different plate clearances. Three pulps of different freeness were made from each cook. For the preparation of hardboard, at first 10 litre volume of slurry was made from 128 g oven dry pulp in water. Pulp freeness was recorded in the freeness tester each time. Mat was formed after dewatering of water from the freeness tester. The mat was then pressed in cold press to reduce the thickness and remove excess water. At last the cold- pressed mat was compressed between the cauls of a hydraulic hot press at about 190°C. The pressing time was six minutes where first two minutes pressure at 35 kg/cm² then one minute breathing at 7 kg/cm² and last three minutes again pressed at 35 kg/cm². Thus S-1-S (smooth in

one side) hardboards were made. At least five boards of each pulp were prepared for sampling in size 12.7 cm x 5.08 cm. Three samples were obtained from each board. Test samples were conditioned at 50 ± 2 % relative humidity and $23 \pm 1^\circ\text{C}$ temperature in a humidity control room. Strength properties (MOR) of the boards were determined by static bending process and water absorptions were measured according to ASTM standards (Anon 1954). Five boards of each pulp were tested. The average values against pulp freeness are shown in Table 1 and 2.

Table 1. Strength and water resistant properties of steamed hardboard made from *Acacia* hybrid wood chips.

Species	Cooking condition		Freeness in seconds	Modulus of rupture(MOR) kg/cm^2	Water absorption (%)			
	Digester pressure (kg/cm^2)	Steaming time (minute)			Change in weight	Change in thickness		
<i>Acacia</i> hybrid	7.03	30	19	38	38	87		
			21	45	47	46		
			25	45	81	59		
		60	21	21	58	39	29	
				22	59	43	30	
				23	59	77	50	
			90	29	29	80	17	11
					29	87	16	11
					30	112	13	9
	10.55	30	18	31	123	78		
			21	35	109	74		
			35	80	12	13		
		60	23	23	53	60	43	
				24	48	77	57	
				25	61	58	43	
			90	20	20	54	38	28
					20	46	31	27
					21	64	56	39
	¹ Sundri		60	35	175	60	16	

¹Khan and Shafi 1988

It may be mentioned that pulp freeness is an important consideration in the manufacturing process, and a freeness value exceeding 40 seconds (defibrator freeness) is ordinarily unacceptable for industrial purpose (Lyll 1969). From Table 1 it is seen that the boards were not strong. But the boards made from the pulps under 7.03 kg/cm² digester pressure and 90 minute steaming time was moderately strong. Pre-treatments with NaOH and that with mixtures of NaOH and Na₂SO₃ produced boards with better strength property shown in Table 2. It is also seen that strength properties (MOR) were increased with increasing the chemical concentration. But the boards obtained from pre-treatment with Na₂SO₃ alone were very weak.

Water resistance is another important property expressed in terms of the amount of water absorbed by the samples and their thickness swelling. These values were very poor of pre- treatment boards compared to those made by steam- softening of the chips. The hardboard made from the pulps under 7.03 kg/cm² digester pressure and 90 minute steaming time was fairly water resistant.

Table 2. Strength and water resistant properties of hardboard made from chemically treated Acacia hybrid wood chips.

Species	Chemicals	Freeness in seconds	Modulus of rupture(MOR) kg/cm ²	Water absorption (%)	
				Change in weight	Change in thickness
<i>Acacia</i> hybrid	1% NaOH	31	92	126	93
		34	153	117	86
		40	183	107	79
	2% NaOH	30	137	128	95
		30	170	120	90
		32	180	127	94
	3% NaOH	28	129	136	105
		30	179	130	96
		31	185	122	74
	3% Na ₂ SO ₃	23	35	131	94
		32	42	121	91
		42	54	100	81
	3% Mixture NaOH+Na ₂ SO ₃	39	198	117	85
		40	199	125	90
		41	206	125	93
¹ Sundri	3% NaOH	35	395	143	112

¹Khan and Shafi 1988

As compared to sundri , which was used in Khulna Hardboard Mills, hardboards made with Acacia hybrid are found to be inferior. The strength MOR is comparatively poor at the same freeness level of good stock. Hence it can be inferred that boards made from Acacia hybrid is less suitable compared to sundri. However, there is scope to improve properties by using additives, sizing materials and heat treatment etc.

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