EVALUATION OF SEED SOURCE VARIATION IN *Strychnos nux-vomica* L. (KANJIRAM) FROM DIFFERENT FOREST TYPES OF KERALA

by

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Evaluation of seed source variation in *Strychnos nux-vomica* L. (Kanjiram) from different forest types of Kerala"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of other University or Society.

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Finally I blow my head before the ALMIGHTY.

Shine

SHINE, G.

Dedicated to my family, my teachers and friends

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Introduction

INTRODUCTION

Strychnos nux-vomica L. (Loganiaceae) is a medium sized deciduous tree known as Karaskara in Sanskrit, Kajra in Hindi and Kanjiram in Malayalam. It is a valuable medicinal plant native to South and South East Asia especially in India and Myanmar (American Cancer Society, 2000). The plant is distributed throughout India in deciduous forests up to 1200 m elevation. Its natural distribution also extends to Sri Lanka, Thailand, China and Malaysia (Joy et al., 1998). In Kerala, the tree is found in the natural forests, waste lands, river banks and homesteads.

Flowering occurs during March- April and fruiting during May- December. Ripe fruits are available, 8-9 months after flowering in January to May (Joy et al., 1998). The tree is a shade bearer. The fruit is a berry, 5-6 cm diameter, globose indehiscent, thick shelled, orange red when ripe with fleshy pulp enclosing the seeds. The fruits contain many discoid seeds, and are light grayish in color with a satiny or glistening appearance, extremely compact and horny (Dey, 1980).

S. nux-vomica is a source of drugs in Ayurvedic and Homoeopathic Systems of Medicine (Rathi et al., 2008). The seeds and leaves are highly toxic to man and animals due to the highly toxic alkaloids and producing stiffness of muscles and convulsions, ultimately leading to death. Hence, the tree is known as snake wood. The seeds of *S. nux-vomica* are used in the treatment of arthritis. Being extremely toxic, the raw seeds must be processed before clinical use. The cotyledons of the seeds are rich in alkaloids like strychnine and brucine. It serves in small doses as efficacious cure for different forms of paralysis and other nervous disorders. The seeds are used as a remedy in intermittent fever, dyspepsia, chronic dysentery, paralytic and neuralgic affections, worms, epilepsy, chronic rheumatism, insomnia and colic. It is also useful in impotence, neuralgia of face, heart disease, spermatorrhoea, skin diseases, toxins, wounds, emaciation, cough and cholera. Leaves are applied as poultice in the treatment of chronic wounds and ulcers and the leaf decoction is useful in paralytic complaints (Joy et al., 1998). Root and root bark is used in fever and dysentery (Kirtikar et al., 1933). The plant has been used as folk medicine for alleviating inflammation, joint pains and allergic symptoms. The suppressive activity of *S. nux-vomica* on allergen-specific IgE antibody response is confirmed and its possible application in allergic conditions is suggested (Duddukuri et al. 2008). The tree stem, having good tensile strength, is used for making agricultural implements, tool handles such as ploughs, shares, axe-handles, hammer shafts, clod breakers, cart wheels, cots, fancy cabinet works and for ornamental panels (CSIR, 1976).

The plant belongs to high volume trade or consumption category i.e., 100 tonnes/year (Ved *et al.*, 2008) among medicinal plants in India. The seeds are having more demand than the other parts of the tree. It is estimated that 300 kg/year is the demand of *S. nux-vomica* seeds by herbal industries and Ayurvedic drug producers of Maharashtra (Khadiwale, 2000). According to Indian Institute of Foreign Trade, the annual collection of seeds in India is 2000 tonnes and total potential yield in Orissa is 360 tonnes per annum. Three factories are present, one each in Kolkata, Hyderabad and Amritsar with a total installed capacity of 36 tonnes per year for the processing of *S. nux-vomica* seeds. The seeds are exported mainly to UK, USA, Netherlands, Morocco, Israel and West Germany (Wealth of India, 1976)

To meet the future needs of raw materials for pharmaceutical industries and local medicinal consumption, urgent attention need to be paid to propagation and cultivation of *S. nux-vomica.* As propagation by seed is the most common and cheapest method, knowledge of seed handling is a prerequisite for growing this species successfully. Despite its enormous industrial potential and the declining status of its natural population, little or no attention has been given to conservation and establishment of large scale plantations of *S. nux-vomica*. It is one of the species suggested for reforestation of open, fallow, degraded lands of low rainfall zones in the eastern part of the Sharavathi upper catchment in Karnataka (Ramachandra and Kumar, 2003).

By the domestication and cultivation of the tree, pressure on natural stands can be reduced. The natural regeneration of this species in its habitat is heavily constrained by massive collection of fruits for industries or local medicinal preparations. This reduces the availability of the diaspore in the forest floor for the establishment of new seedlings. Identification of seed source variation of the species will be a valuable tool for its use in tree improvement programmes.

The study aims to evaluate the variation in terms of seed quality, germinability and seedling growth in selected natural populations of *S. nux-vomica* in three forest types (dry deciduous, moist deciduous and riparian) of Kerala for identifying the best possible source for future use in tree improvement programmes, domestication and cultivation and thereby control its overexploitation from natural habitats. The specific objectives of the study are to:

1. find out the variations in fruit and seed characteristics among the seed sources
2. identify the best seed source in terms of the germination aspects
3. identify the best seed source based upon the seedling growth
4. identify the best seed source through alkaloid estimation
5. identify the variations among the seed sources through isozyme analysis

Review of literature

REVIEW OF LITERATURE

2.1 Seed source variation

Seed source is defined as the area of plant population of a particular species which is set aside for seed collection and maintained either naturally or artificially. Use of proper seed source helps to attain maximum gains in most of the tree improvement programmes (Zobel and Talbert, 1984). The plants belonging to same species may show variations in morphological, anatomical and physiological characteristics and growth patterns according to the seed sources.

Seed source variation can be exploited as the first step in any tree improvement programmes. Forest tree improvement programme starts with the study of available variations in the entire range of species (Suri, 1984). Knowledge of variability within a species is a prerequisite for developing effective tree improvement or breeding strategies (Vakshaya et al., 1992). The significance of the genetic variation studies and provenance testing in forest tree improvement is very well realized by different authors. Literature citing the variation existing between different seed sources of *Strychnos nux-vomica* is lacking.

The variations in fruit traits are very common in the plants among different seed sources. In a study conducted in *Adansonia digitata* from five land use types of South Africa, it was found that both individual fruit size and total volume production were significantly different among the fruit sources (Venter and Witkowski, 2010). Study conducted on the phenotypic variations of the fruits of *A. digitata* from 10 different provenances over the rainfall gradient at Mali have also shown that fruit weight has significant difference among the provenances (De Smedt et al., 2011). Sivaprasad and

Channabasappa (2011) have observed greater fruit weight in *Terminalia alata* growing in moist deciduous forests compared to semi-evergreen and dry deciduous forest types.

In a study on *Acacia nilotica*, 20 provenances covering five countries (India, Senegal, Sudan, Yemen and Pakistan) were compared for variation in certain seed and seedling characteristics under nursery and field conditions of Jabalpur, India (Ginwal et al., 1996). Mandal et al. (1997) found that seed germination, seedling growth and nodule numbers of *A. nilotica* were greatest from large sized seeds and decreased as seed size decreased. In a study to investigate the effect of seed source on physical and physiological qualities of *A. nilotica* seeds, Vanangamudi et al. (1999) also showed significant differences between seed sources for all seed characteristics (length, width, thickness, 100 seed weight). A significant relationship was found between seed weight and growth of seedlings in nurseries and plantations, indicating it to be an important trait for the early selection of provenances. Ten quantitative characters (plant, collar-bole length, collar diameter, number of branches, first, second and third inter branch distances and angles of first, second and third branches with reference to the main stem) were recorded in 8-month old seedlings. Studies were undertaken at the Forest Research Institute, Dehra Dun (Uttar Pradesh) to assess the magnitude of seed source variations among 27 seed sources of *A. nilotica* (Bagchi, 1999). Ginwal and Gera (2000) studied seed germination behavior and growth characteristics of 12 different *A. nilotica* populations in a provenance trial at Jabalpur in Madhya Pradesh. The study showed significant differences between the provenances with respect to seed germination and growth (height, diameter, survival percentage).

Uniyal et al. (2003) have found that the species *Grewia oppositifolia* has morphological and physiological variation between provenances for percent of sound seed, seed weight, speed of germination, time to complete germination and percent germination in the nursery.

Many studies have been carried out on the seed source variation of *Santalam album* in South India. Effendi and Singa (1994) sorted sandal seeds into eight size classes ranging from 0.100-0.109 to 0.170-0.179 g. The 0.160-0.169 g seed size class (the seventh largest) gave better germination, survival, seedling diameter and height growth, but differences between the classes were only significant in the case of seedling height. In a study on sandal seeds from various seed sources exhibited significant variation in morphology and physiology (Sindhuveerendra et al., 1999). They also found that seed parameters (seed length, seed width and seed weight) of nine provenances from Karnataka and Kerala (India) had significant amount of variation. Manonmani and Vanangamudi (2002) conducted an experiment to delineate the effect of seed size on the seed germination capacity and seedling vigour of *S. album*. The seeds collected from the four sources recorded three different size grades in varying frequencies. Within the same size grade, the seed dimensions, namely, seed length, seed width and 100 seed weight were highest for Coimbatore and lowest in Metupalayam. It was suggested that it is best to collect only big sized sandal seeds from Coimbatore in order to obtain maximum germination percentage and increased seedling vigour.

Assis et al. (1992) could obtain positive correlation between seed weight and height of seedlings in case of *Terminalia catappa* while there was no such correlation in *Terminalia bellerica* seedlings. The variation in seed size and its effects on early growth in *Terminalia arjuna* was reported by Srivastava et al. (2001). The seed size was having a positive influence in initial stage of seedling growth. The relationship between seed weight and seedling size reduced with age of seedling. The larger seeds showed better growth in initial stage of seedling growth as compared to middle and smaller sized seeds due to higher nutrient concentration.

Studies on the seedlings of *Leucaena leucocephala* from large medium and smaller seeds showed the respective superiority of large seeds for raising nursery (Gupta et al., 1983). Moideen et al. (1990) noted that in the case of *Casuarina equisetifolia,* cone weight is a major determinant in seed germination and seedling vigour. The estimation of three weight grades of cones into light, medium and heavy revealed that seeds from heavy cones have a high germination percentage, vigour index and maximum weight.

Ponnammal et al. (1993), grouped *Hardwickia binata* seeds into small, medium (normal) and large categories based on seed size and weight and reported that seed germination, seedling growth and biomass of seedlings increased with increase in seed size and seed weight. Moreover, initial growth rate was found to be faster in larger seeds compared to that of medium and smaller ones. Arjunan et al. (1994) graded seeds of *Pongamia pinnata* into large, medium and small sizes and their germination percentage was recorded. Large sized seeds germinated better (98%) than medium sized (80%) and small sized (70%). Biomass production was higher in seedlings produced from larger seeds.

It was also found that small seeds of some species give higher germination than larger ones. Very small seeds of *Glycine max* were slower to germinate than small, medium and larger ones when incubated on a wet paper; however very small seeds had the fastest germination when seeds of whole sizes were incubated on wet sand (Hooper et al., 1979). The germination was faster in small seeds than in large seeds of *Medicago sativa* (Cooper et al., 1979), *Oryza sativa* (Krishnasamy and Seshu, 1989), *Calkie edentate* (Zhang, 1993) and *Leymus arenarius* (Greipsson and Devi, 1995). In the case of *Erobium brachicarpum*, small seeds became permeable faster to water than large ones; therefore these are likely to germinate in the year in which they are produced; the germination of large seeds being delayed until the subsequent year (Stamp, 1990).

In contrast to these observations, Krishnaswamy et al. (1982) noted that in the case of *Eucalyptus tereticornis* neither germination percentage, nor seedling attributes like root length, shoot length etc. were affected by capsule weight. But the seedling dry weight varied significantly due to capsule weight. Sujith et al. (1994) found that seed size did not have any influence on germination of *Ceiba pentandra*. Quiraishi et al. (1996) found that the quality of seedlings of *Cleistanthus collinus* was not correlated with seed size. But Dileep et al. (1994) found that large sized seeds (> 0.055 g) in combination with good rooting media yielded better seedlings in *C. pentandra*. Nazeem et al. (1980) in their investigations on correlation between seed and seedling characters in Jack (*Artocarpus heterophyllus* L.) observed that the weight of the seeds does not influence the seedling characters. The germination was found to be independent of seed size in *Medicago sativa* (Beveridge and Wilsie, 1959), *Zea mays* (Eagles and Hardcare, 1979), *Rumus obtusifolius* (Cideciyan and Malloch, 1982), *Dactylis glomerata* (Bretagnolle et al. 1995) and *Diplotaxis virgata* (Perez-garcia et al., 1995).

In an investigation on genetic and germination aspects carried out by Manga and Sen (1996), genetic parameters of six seed traits (weight, length, width, thickness, volume and density) and percentage germination were studied in 51 accessions of *Prosopis cineraria*, mostly from the state of Rajasthan, but with four each from Gujarat and Haryana. Seed weight and volume exhibited high genetic variability, heritability and genetic gain. The correlation study showed that seed weight, seed volume and seed thickness had significant and positive associations with germination percentage. Therefore, these traits should be given priority for improving germination in *Prosopis cineraria*. Variation in germination traits were investigated (Siril et al., 1998) in seeds of the multipurpose tree *Sapium sebiferum* obtained from three populations in Uttar Pradesh, India. The influence on seed weight and treatment on germination was also studied. Within each population, seed germination was much higher (84.3-89 %) for larger seeds than medium (24.6- 44.3 %) and small (9.6-11.6 %).

Variation in seed germination and juvenile growth of 12 provenances of *Albizia lebbeck* in arid India was studied by Kumar and Toky (1996). Significant differences among most of the provenances in seed germination, with variations of 5-94 percent in the incubator and 8-50 percent in the nursery was observed. The variations did not show a significant relation with the latitude or longitude of the seed source but are likely to be important in the selection of vigorous provenances based on seed germination and juvenile characters. In another study, differences in seed morphological traits (length, width, thickness, volume and 100 seed weight) and germination were compared for seven provenances of *Grewia obtiva* from different parts of the Tehri-Garhwal district of Uttar Pradesh and the Solan district of Himachal Pradesh (Tyagi et al., 1999). The analysis demonstrated genetic variation between the provenances and highly significant correlations between different pairs of characters. Multiple regression analysis showed that seed length and 100 seed weight might be used as the predictors of germination in *G. optiva*.

Variation in plant height, collar diameter and survival rate of six neem provenances were examined at three test sites in Bangladesh and India after a growing period of about seven months in the field (Kundu et al., 1998). Three out of the six provenances showed significant genotype x environmental interactions. Positive correlations between collar diameter and survival rate at two sites were detected among provenances. Clinal variations were observed for collar diameter, survival rate and production percentage. In case of studying provenance variation of *Azadirachta indica* in Bangladesh and India (Jodhpur, Rajasthan) 20 sites were taken. Significant differences were observed between provenances in height and collar diameter. Eco-climatic attributes played an important role in the differentiation of neem populations and thereby affected their growth and survival during the early stages of establishment (Kundu, 2000).

A study was conducted by Jalil et al. (2000) to investigate the performance of 10 provenance of *Azadirachta indica* at Seoni in Madhya Pradesh, India. Significant variation was observed among the ten provenances with respect to germination percentage, height, collar diameter and survival. The provenance Betul showed best results with respect to height and survival percentage after four years of growth. Amarkantak provenance was found superior in terms of collar diameter while germination percentage was recorded highest in Indore provenance. Based on the results obtained after four years, provenances of Betul and Bilaspur were ranked as overall best. Study furnished that, except provenance of Ujjain (the lowest growth performer), all the provenances are suitable for the plantation with fastest growth rate.

A provenance trial in teak (*Tectona grandis*) involving seven provenances from Kerala (India) was conducted (Jayasankar et al., 1999). Germination characteristics did not vary significantly among the provenances in the nursery. But profound variation in seedling growth rates among the provenances was observed. Performances of the provenances in the field followed nursery growth patterns confirming that growth was under strong genetic control. Grading of seedling based on their height and collar diameter at an early stage was recommended for selection of best performing seedlings in the field. A study was conducted by Jijeesh and Sudhakara (2007) to determine the natural variability of teak (*Tectona grandis* Linn.f.) fruits belonging to different plantations in Nilambur Division, Kerala and the influence of fruit size on the number of seeds and significant variations were found. Sudhakara and Jijeesh (2008) reported that *Tectona grandis* drupes belonging to largest size grade gave higher germination percentage but the size variation did not affected the germination value. Jijeesh and Sudhakara (2011) also reported that the drupes belonging to largest size grade have given the seedlings with higher chlorophyll content which greatly influence the total performance of the seedlings. Plantations did not have any influence on the volume and weight of fruits.

2.2 Isozyme characterization in plants

Seed sources differ significantly among species with different geographic ranges, life forms, and taxonomic affinities. Various methods using different molecular markers are employed for drawing the genetic variations in plants. Analysis of structurally different primary products of allelic genes, i.e., isozyme analysis, has opened the gate to population genetics and is one of the method for knowing the variations in plants at the genetic level (Lundkvist and Rudin, 1977). Variation in isoenzymes might be arising from the allelic segregations at a single locus, representing more suitable changes in the enzyme molecules. The inheritance of several polymorphic isozyme systems in forest trees have been elucidated in the last few years: alcohol dehydrogenases (Conkle, 1971), esterases (Bartel, 1971), peroxidases (Feret and Stairs, 1971), acid phosphatases (Bergmann, 1974), leucine aminopeptidase (Lundkvist, 1974) and glutamate-oxalate-transaminase (Rudin, 1975). The isozymes are isolated by various methods like Polyacrylamide Gel Electrophoresis in which the isozymes are detected by the differences in the net electrical charges. A number of biological researches on evolution of population, the transformation, the regulation of gene expression and metabolic regulation in differentiated tissues are done with the isozyme analysis. Isozymes are active in different tissues and also had different molecular properties.

In a study conducted by Lundkvist and Rudin (1977), eleven populations of *Picea abies* in Sweden were analysed for isozyme variation in four polymorphic loci and it was found that the major part of genic differences occurred in the esterase-A and acid phosphatase-A loci. When populations originating from foreign seeds were compared with indigenous populations, higher values of genetic differentiation were found.

Tao et al. (1999) commented that isozymes are the widely used protein markers in plant breeding. Zeidler (2000) reported that isozymes are powerful tools for creating genetic variability within and between populations of plants and animals. He also concluded that isozymes are able to solve other questions of population biology, conservation biology and ecology as well. Ishikava et al. (2000) found that isozymes serve as useful gene markers in genetic studies at the plant and cellular level and are useful for uniting conventional linkage and restriction fragment length polymorphism linkage maps. Suma and Balasundaran (2003) have found low degree of genetic variability between five provenances of *Santalum album* in India through the isolation and evaluation of five isozymes viz., peroxidase (PRX), shikimate dehydrogenase (SKDH), glucophosphate isomerase (GPI), malate dehydrogenase (MDH) and esterase (EST).

It is now recognized that peroxidase isozymes are present throughout the plant kingdom. While all peroxidase isozymes appear to catalyze the same reaction, the individual isozymes may differ markedly in physicochemical and kinetic properties (Shannon, 1968). Genetic evidence suggests that gene flow among local populations is high, but geographically separated populations show moderate levels of genetic differentiation. Ecological and historical processes provide the mechanisms influencing the genetic architecture of plant species. Data on isozyme variation in tropical trees are related to breeding systems, seed dispersal mechanisms, demography, and patterns of environmental heterogeneity (Loveless, 1992).

2.3 Seed germination in *Strychnos nux-vomica*

The fruit of *Strychnos nux-vomica* is a berry with one or more discoid seeds. The seeds are light grayish in color with a satiny or glistening appearance, extremely compact and horny (Dey, 1980). The storage and germination aspects of *Strychnos nux-vomica* were studied by Krishnamurthy (1993). The results showed that freshly harvested seeds germinate poorly after 70–120 days. Seeds that fail to germinate under favourable environmental conditions are considered to be dormant. The various types of dormancy in general, and their underlying causes and methods to break them, are described in Bewley and Black (1994) and Baskin and Baskin (1998). Seeds of *S. nux-vomica* have slow and erratic germination; thus different presowing treatments were applied to enhance the germination of its seeds. Germination of *S. nux-vomica* seed is epigeal (Sivakumar et al., 2006). It was found that germination of *S. nux-vomica* seeds differed between different storage periods and moisture contents of the seed. The highest germination (92%) was achieved when seeds with 10% moisture content were stored at ambient temperature for 30 weeks. It was found that *S. nux-vomica* seeds possess physiological dormancy that can be broken effectively by after-ripening. The seeds of *S. nux-vomica* do not possess physical dormancy. However, GA_3 treatment for 24 h and alternate wetting and drying for 14 days improved germination to 32 % and 37 % respectively compared to 10 % germination of control. This could be because of the presence of chemical and physiological dormancy.

2.4 Biochemical characteristics and medicinal properties of *Strychnos nux-vomica*

The seeds of *Strychnos nux-vomica* are greatly important in various herbal medicinal industries due to the valuable alkaloids present in the seed cotyledon. Out of several alkaloids, seven alkaloids viz., strychnine, brucine, beta colubrine, strychnine N-oxide, pseudo strychnine and icajine have been isolated from the processed seeds of *S. nux-vomica* by Ma Lian et al. (2009). Other alkaloids present in the plant are vomicine, alpha colubrine, novacine and N-methyl sec- pseudobrucine. Pseudo strychnine is non-toxic. The plant contained a significant quantity of non-enzymatic antioxidants like ascorbic acid, alpha tocopherol, reduced glutathione and enzymatic antioxidants like super oxide dismutase, ascorbate peroxidase, catalase peroxidase and polyphenol oxidase in the seeds and leaves. Apart from this, glycoside loganin is also present in the seeds (Vijayakumar et al., 2009).

The medicinal properties of *S. nux-vomica* seeds are due to the various alkaloids present in the seeds and these alkaloids are extracted for use in various medicinal preparations in Ayurveda, Homoeopathy, Unani etc. Strychnine and brucine are the major alkaloids present in the seeds. Strychnine is violently poisonous crystalline alkaloid, slightly soluble in cold water and highly soluble in chloroform and alcohol. It affects the cerebro-spinal system, but it kills without producing marked anatomical change and the fatal dose of Strychnine is as low as 4 grain. Brucine is very bitter, freely dissolves in cold alcohol. It resembles the physiological qualities of strychnine, but in a markedly less degree and is decidedly less dangerous than strychnine. The antidotes and treatment for poisoning by brucine are the same as for strychnine (Howes, 1904).

Nux-vomica is prepared using the ethanolic extract from the seeds, used in homeopathy against ethanol induced illness (Sukul et al., 2001). The alkaloids brucine and brucine N-oxide extracted from the seeds of *S. nux-vomica* are having analgesic and anti-inflamatory properties (Wu Yin, 2003). Antiplasmodical activity was observed in the root extract of *Strychnos melladora* and *Strychnos variabilis* (Philippe et al., 2004). Ma Lian et al. (2009) observed anti-tumour activity of seven alkaloids extracted from the seeds. The morphological assessment of seed extract treated cells showed significant features associated with apotopsis and so useful against tumour (Rao et al., 2009). Anti-ulcer properties have been discovered from *Strychnos pseuduquina* (Bonamin et al., 2011). Singh et al. (2010) observed the aphrodisiac potential of the *Strchnos nux-vomica* plant extract in men.

The dried seeds are used as a remedy in intermittent fever, dyspepsia, chronic dysentery, paralytic and neuralgic affections, worms, epilepsy, chronic rheumatism, insomnia and colic. It is also useful in impotence, neuralgia of face, heart diseases, spermatorrhoea, skin diseases, toxins, wound, emaciation, cough and cholera. Leaves are applied as poultice in the treatment of chronic wounds and ulcers and the leaf decoction is useful in the paralytic complaints (Joy et al., 1998). Leaves when fed to cows, impart a bitter taste to milk which is credited with digestive and tonic properties (Chopra, 1958)

Nux-vomica is a powerful poison in large doses producing titanic convulsions and eventually death and lesser dose may result in mental disorders. In Konkan, small doses of the seeds are given with aromatics in colic and in Cambodia, the seeds are used as emetic. Seeds are also used in the preparations of medicated products for the hair scalp (Chopra et al., 1956). Nux-vomica is an effective animal poison. It is also used as an insecticide against the vermins in the field. In South-Eastern Asian countries, tribals used the same in preparation of arrow and dart poisons. Powdered Nux-vomica often adulterated with powdered olive stones and seeds of *Strychnos potatorum*, *Strychnos nux-blanda* etc. (Chopra, 1958). Estimation of alkaloids is usually done by High Performance Liquid Chromatography (HPLC) method in *Strychnos nux-vomica* seeds (Han, Quan-Bin et al., 2008).

Materials and methods

MATERIALS AND METHODS

The present study on evaluation of variation in seed sources of *Strychnos nux-vomica* L. (Kanjiram) collected from three forest types of Kerala was carried out during the period 2009-2011 at the College of Forestry, Vellanikkara, Kerala, India. The details of the materials and methods used for the study are described in this chapter.

3.1 Identification of the seed sources for the research work

Strychnos nux-vomica is one of the important tree species in the dry deciduous, moist deciduous and riparian forest types of Kerala. Dry deciduous forests at Chinnar Wildlife Sanctuary (SS 1), moist deciduous forests at Pattikkad Range of Thrissur Forest Division (SS 2) and riparian forests (at the flood plains) at Peechi-Vazhani Wildlife Sanctuary (SS 3) were identified as the three seed sources for seed collection.

Chinnar Wildlife Sanctuary is located in the eastern part of the High Ranges of southern Western Ghats of Kerala state. The terrain is highly undulating with altitudes ranging from 500 m to 2300 m. The area is drained by two perennial rivers namely Pambar and Chinnar. The maximum temperature is 35.7^{o} C and minimum temperature is 12.7^{o} C in the sanctuary. The sanctuary is situated in the rain shadow region of the Western Ghats, getting rains mostly during the north-east monsoons (October-December). The average annual rainfall is 500 mm. The plains are generally hot but the higher altitudes are cool. *S. nux-vomica* is seen in the mixed dry deciduous forests of Chinnar wildlife sanctuary.

The trees growing in the mixed moist deciduous forests of Pattikkad Range of Trichur Forest Division in Kerala were selected for seed collection as the second seed source. Pattikkad forest range is blessed with a great extent of mixed moist deciduous forests, semi-evergreen forests and a small patch of evergreen forests. The altitude ranges from 100 m – 500 m. The average annual rainfall is 3000 mm. The mean maximum temperature and minimum temperature are 39°C and 19°C respectively.

Peechi-Vazhani Wildlife Sanctuary receives both Northeast and Southwest monsoons. Pre-monsoon showers are often received in the month of April. Southwest monsoon starts from June and extends up to September. Northeast monsoon brings reasonable rains during October-November. Average annual rainfall is 3000 mm. The Sanctuary is blessed with salubrious weather with cooler months during November to January and hotter days during February to May. Mean maximum temperature recorded is 39.4° C with a minimum temperature of 18.9° C. There are two reservoirs in the sanctuary, Peechi and Vazhani formed by the construction of two dams across Manalipuzha and Vadakkancherry puzha. The water spread of the two reservoirs is 14.793 sq. km. The trees which are growing in the flood plains around the Peechi dam (Constructed during 1947- 1957) were selected for seed collection and it comes under the Peechi Range of the sanctuary.

The details of the seed sources are shown in Table 1. From each seed source, five or six distant located trees were selected for fruit collection depending on the availability. The geographical locations of each tree using GPS were noted along with the dimensions of the trees. Figure 1 shows the locations of seed collection.

3.2 Fruit collection

Fruits were collected from the seed sources Chinnar and Pattikkad using a long bamboo pole laden with a basket. Fruits were harvested from the riparian Peechi forests and moist deciduous Pattikkad forests during December 2010

Table 1. Geographical descriptions of the seed sources and growth parameters of *Strychnos nux-vomica* trees selected for fruit collection

Sl. No.	Seed sources	Location	Latitude (N)	Longitude (E)	Altitude (m)	Tree height (m)	GBH (cm)
1	Dry deciduous forests (SS 1)	Chinnar Wildlife Sanctuary	10^{0}18’16”	77^{0}11’28”	656	8.5	66
			10^{0}18’15”	77^{0}11’29”	635	10	73
			10^{0}18’15”	77^{0}11’29”	642	7	71
			10^{0}18’16”	77^{0}11’29”	647	8	84
			10^{0}18’15”	77^{0}11’29”	648	9	79
			10^{0}18’15”	77^{0}11’28”	655	7.5	87
2	Moist deciduous forests (SS 2)	Pattikkad Range, Thrissur Forest Division	10^{0}34’02”	76^{0}18’47”	363	8	69
			10^{0}34’00”	76^{0}18’32”	320	9.5	93
			10^{0}34’07”	76^{0}18’38”	338	10.5	89
			10^{0}34’56”	76^{0}18’15”	406	10	104
			10^{0}34’56”	76^{0}18’13”	420	9	90
			10^{0}34’51”	76^{0}18’13”	400	7.5	76
3	Riparian forests (SS 3)	Peechi-Vazhani Wildlife Sanctuary	10^{0}81’47”	76^{0}22’12”	90	9	58
			10^{0}81’34”	76^{0}22’29”	90	7.5	61
			10^{0}81’12”	76^{0}22’01”	83	7	65
			10^{0}81’15”	76^{0}22’28”	95	8.5	73
			10^{0}81’25”	76^{0}22’02”	99	10	76

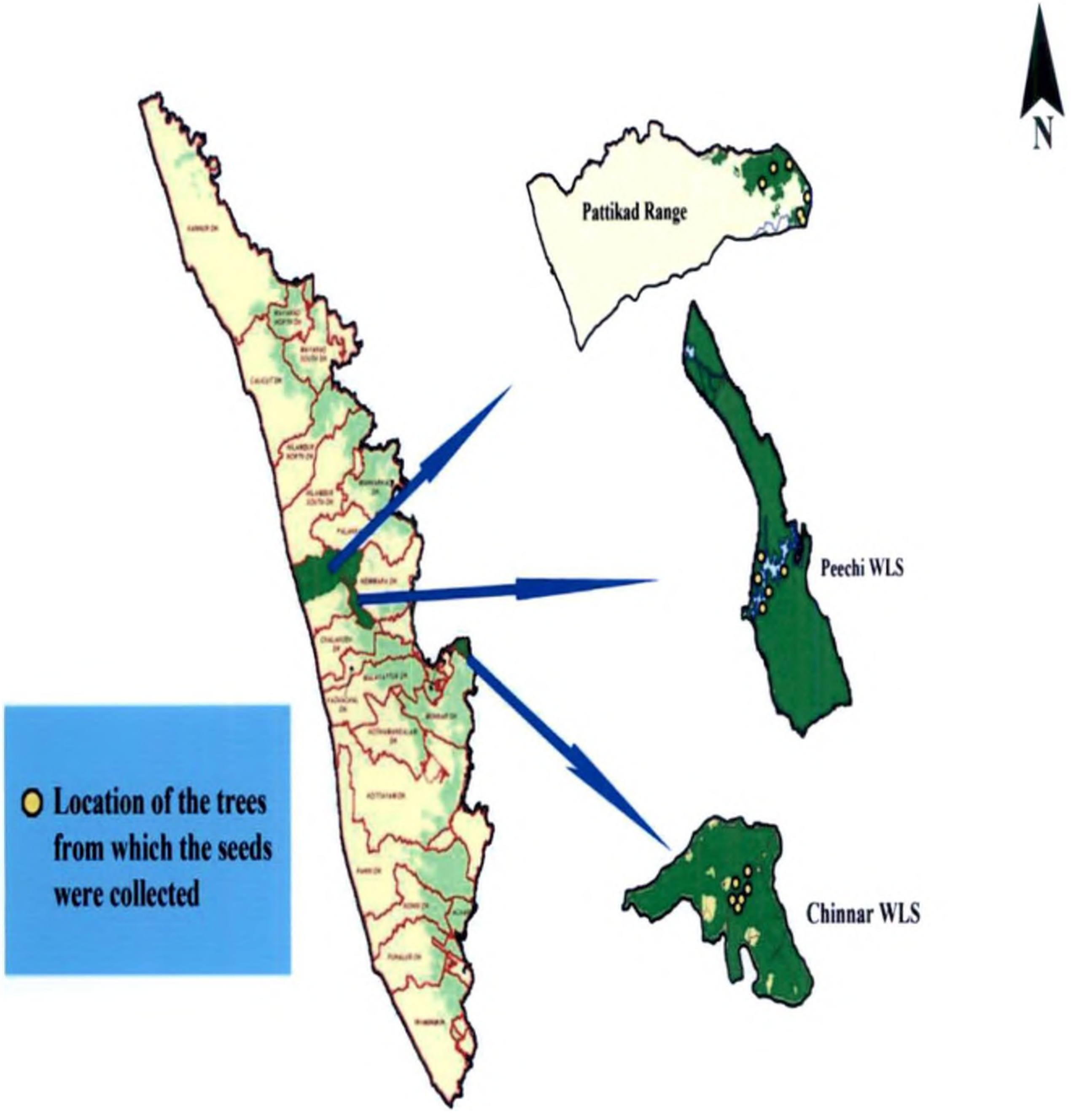


Fig. 1: Seed sources of *Strychnos nux-vomica*

and from dry deciduous Chinnar forests during January 2011. In the riparian seed source SS 3, as the trees were in waist-deep waterlogged condition the trees were climbed and the fruits were dropped directly to the water body. About 200-300 ripe and near-ripe fruits (Plate 1) were collected from each of the three seed sources.

3.3 Measurement of the fruit parameters

All the collected fruits were used for measuring the three fruit parameters viz., fruit weight, fruit volume and number of seeds per fruit. Fruit weight was determined by using an electronic weighing balance of accuracy 0.0001g and expressed in grams. Fruit volume was measured by the water displacement method. A 250 mL beaker filled to the brim with water was kept inside a one litre beaker and the individual fruit was carefully dropped into the 250 mL beaker using a ball point pin. The over flown water in the one litre beaker was measured using measuring cylinders of 25 mL, 50 mL and 100 mL capacity. Volume was expressed in cubic centimeters. Fruit wall was broken manually and the number of seeds in each fruit was counted. The under-developed and malformed seeds were also counted and included in the total number. The height and width of all the collected fruits were measured using a thread and measuring scale.

3.4 Seed extraction

Seed extraction was done manually after noting down the morphological parameters of the fruits described in 3.3. The seeds along with the pulp (Plate 2b) were extracted after splitting the fruit wall (Plate 2a) and soaked in water overnight. Afterwards, the seeds were extracted by rubbing off on a wire mesh with continuous flow of water to remove the pulp.

Plate 1: Ripe fruit of *Strychnos nux-vomica*

Plate 2A: Manual breaking of fruit wall of *Strychnos nux-vomica*

Plate 2B: *Strychnos nux-vomica* seeds with surrounding fruit pulp

3.5 Size grades of seeds and other seed parameters

Seeds were graded based on the seed size. Seed size was calculated as the average of the longest diameter and its orthogonal diameter. The measurements were made using a high precision vernier caliper of least count = 0.02. It was found that the average size of the seeds ranged from the lowest of 18 mm to the highest of 30 mm. A class interval of 3 mm was fixed and the seeds were graded into four size classes viz., 18 - 21 mm (SG 4), 21 - 24 mm (SG 3), 24 – 27 mm (SG 2) and 27 - 30 mm (SG 1). Further experiments were done based on these seed grades from the three seed sources.

For the morphometric measurements of seeds, random samples of 25 seeds from each size grade from each source were taken. The characters studied and techniques adopted to record the observations are as follows: Seed size was measured as described above. Seed thickness was measured at the middle portion of the seed using a digital vernier caliper of least count = 0.02 and expressed in mm. Seed weight was taken for each seed by using an electronic weighing balance of accuracy = 0.0001 g and expressed in grams. Seed density was taken by using an instrument known as specific gravity module (Schimadzu AUY 220) (Plate 3). Seed submerged in water using combination pans, receives buoyancy that is equal to the weight of the water equal to the seed volume. The balance computes the specific gravity and displays the result based on the Archimedes' Principle with an accuracy of 0.0001.

The position of the embryo in the seeds of *S. nux-vomica* was determined near the micropyle and that portion was split open using dissection blade. The radicle and plumule of the embryo were identified using an image analyzer (Digizoom of Labomed) and is shown in the Plate 4. The diameter containing the embryo axis (generally the longest diameter), was taken as the seed length

Plate 3. Specific gravity module (Schimadzu AUY 220)

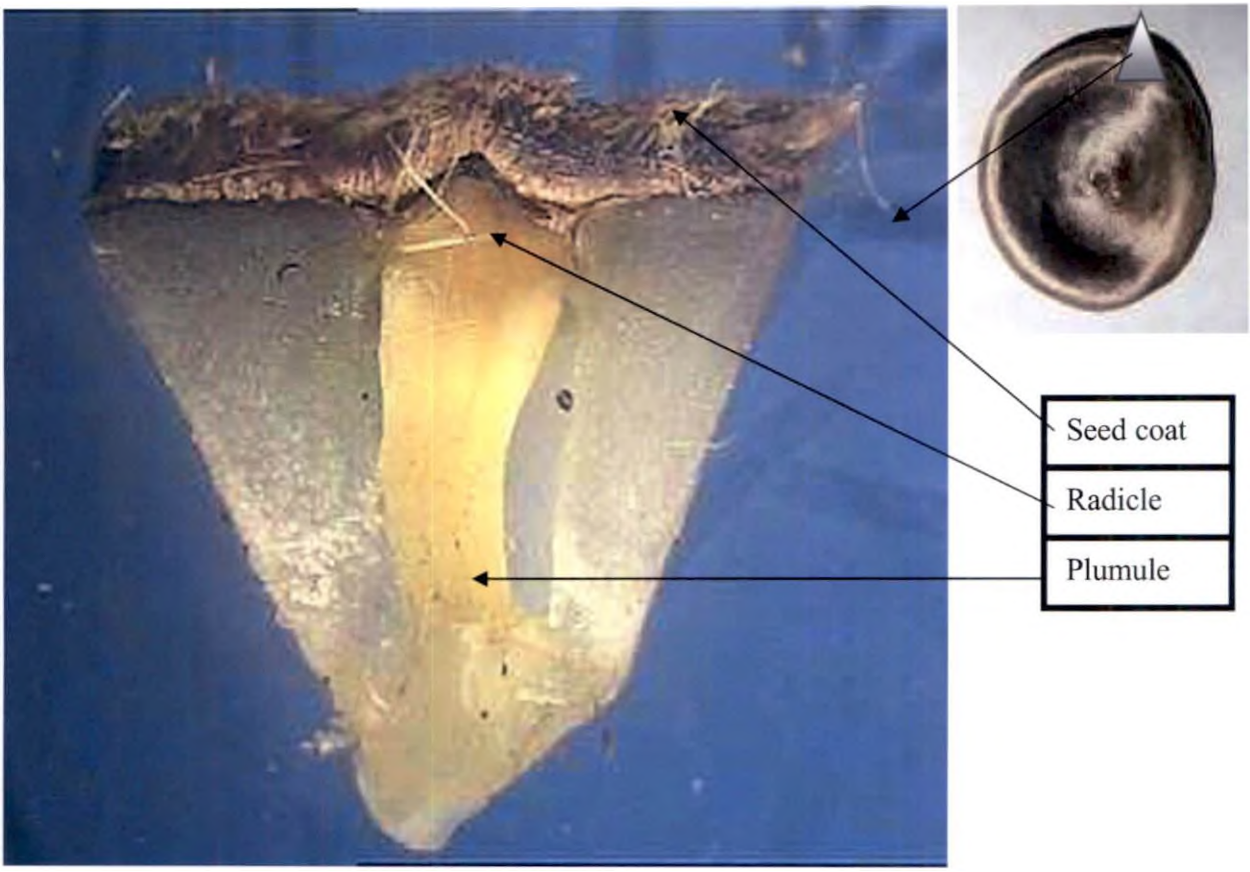


Plate 4: Seed and microscopical image (2x) of the seed embryo of *Strychnos nux-vomica*

and its orthogonal diameter was taken as the seed breadth. The length measurements were done, using a high precision digital vernier caliper having least count = 0.02. Both seed length and seed breadth were measured in millimeters.

3.6 Nursery trials

In order to sow the seeds from all the three sources on a single date, temporary storage of seeds was done for almost two months. The seeds were sown in the root trainers of 24 cells (Specifications: Length of the tray= 35 cm, Breadth= 22 cm, with cells of 5x2x10 cm^3) after filling with the potting mixture having a composition of soil, dried cattle dung and sand in the ratio of 3:2:1. The seeds were soaked in water for 48 hours before sowing. The design followed was Simple Factorial Completely Randomized Block Design having three seed sources with four seed grades. The three seed sources were taken as factors and four size grades were taken as levels with six replications. The root trainers were kept in the green house and each seed source was represented by 576 seeds in the nursery. Each source x size was represented by 144 seeds. Timely weeding was done in the root trainers. Irrigation was done daily using rose can.

3.7 Seed germination characteristics

First germination was observed 33 days after sowing and the observation was continued daily for further 45 days i.e., till the last day of germination. The germination parameters viz., germination percentage, germination energy, speed of germination, mean daily germination, peak value of germination and germination value were calculated as follows.

3.7.1 Germination percentage

Germination percentage was calculated by using the formula,

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

3.7.2 Germination energy

Germination energy is calculated as the per cent, by number of seeds in a given sample which germinate up to the time of peak germination (Willan, 1985).

$$\text{Germination energy} = \frac{\text{Number of seeds germinated upto the peak germination}}{\text{Total number of seeds germinated in the sample}} \times 100$$

3.7.3 Speed of germination

Speed of germination is computed daily by dividing cumulative germination by the number of days (Czabator, 1962).

$$\text{Speed of germination} = \sum \frac{\text{Daily germination}}{\text{Number of days after sowing}}$$

3.7.4 Mean daily germination (MDG)

Mean daily germination was assessed by using the following formula given by (Czabator, 1962).

$$\text{Mean daily germination} = \sum \frac{\text{Cumulative germination percentage}}{\text{number of days after sowing}}$$

3.7.5 Peak value of germination (PV)

Peak value is the maximum mean daily germination reached at any time during the period of germination test (Czabator, 1962).

3.7.6 Germination value (GV)

Germination value was estimated according to the method prescribed by Czabator (1962).

Germination value (GV)

$$= \text{Peak value of germination (PV)} \times \text{Mean daily germination (MDG)}$$

3.8 Seedling attributes

Seedling attributes were recorded for all the seedlings. Following parameters of the seedlings were recorded at monthly interval (30 days) after the completion of germination and continued for six months (180 days). The seedling height was measured from the collar region in the root trainer cells to the topest nodal portion of the stem by using a meter scale up to centimeter accuracy. Collar diameter of the seedlings was measured with a high precision digital vernier caliper of least count 0.02 and it was expressed in millimeters (mm). Number of fully opened leaves was counted at the time of each observation. Occurrence of pest and disease was observed throughout the study period.

3.9 Biomass of the seedlings

At the end of the study period (180 days), three seedlings from each replication were uprooted from the root trainer randomly for the measurements. The seedlings were uprooted carefully from the soil after watering the soil. The soil was completely removed from the roots by washing. The dry weights of the stem, leaves and roots were determined separately for each uprooted seedling. The plant samples were put in to the paper cover separately and were oven dried for 48 hours at 70^0C and the dry weight was measured (average weight of three seedlings representing one replication) by using an electronic weighing balance of accuracy 0.0001g and expressed in grams.

3.10 Seedling vigour index

Seedling vigour index was calculated as the product of the germination percentage of the seed lot (Seed source or size grade) and the seedling dry weight (Yari et al., 2010). Seedling vigour index was calculated for the uprooted seedlings.

Seedling Vigour Index

$$= \text{Germination percentage} \times \text{Seedling dry weight (g)}$$

3.11 Biochemical characteristics

Estimation of the alkaloid contents was done for the seed sample. The isozyme analysis was done for both the seed and seedling leaf samples of *Strychnos nux-vomica.*

3.11.1 Alkaloid estimation in *Strychnos nux-vomica*

Estimation of the quantity of alkaloids was done at the Aromatic and Medicinal Plants Research Station, Kerala Agricultural University, Odakkali, Kerala. The quantity of the major alkaloids, strychnine and brucine were estimated in the seed samples taken from the three seed sources. The seed samples were replicated six times. The extraction and clean up of Nux-vomica samples was done based on the method suggested by Manske (1950). Modifications were done for the estimation of the alkaloids from *Strychnos nux-vomica* by High Performance Liquid Chromatography (HPLC).

3.11.1.1 Preparation of the seed sample

Seeds of *Strychnos nux-vomica* were found to contain about 2.5% fixed oil (Joy et al., 1998). Seeds were de-oiled before extraction of alkaloids. For this, the powdered seed material (100 g) was repeatedly shaken with 100 ml (for one time) of petroleum ether (75 %) till all the fat was removed. 0.5 g of the finely powdered de-oiled seed material was taken in a 250 ml round bottom flask and refluxed with 50 ml redistilled methanol (Boiling point = 65°C) for 48 hours in Soxhlet apparatus (Plate 5).

The methanol extract was decanted and filtered through Whatman No. 1 filter paper. The solvent was evaporated and the resultant residue was dissolved in water. Two ml of 10% lead acetate solution was added and the contents warmed on a water bath for 30 minutes at 80° C for coagulating the impurities. Two ml of 10% oxalic acid was added to remove the excess lead acetate and the solution was filtered and the coagulated impurities were removed. The pH of the filtrate was adjusted to 8.0 by adding 5% Na_2CO_3 solution and allowed to stand for 30 minutes. Alkaloids in the extractives were then separated four times into 30 ml portions of chloroform. The combined chloroform extract was washed twice with 25 ml each of 5% NaCl solution and dried over anhydrous sodium sulphate. Chloroform was evaporated and the residue was analysed for alkaloids by HPLC.

3.11.1.2 Analysis of the seed sample

The alkaloids in *S. nux-vomica* seed extract were analyzed by HPLC. The instrumental parameters are given in the Table 2.

Table 2: The instrumental parameters for the HPLC analysis of *Strychnos nux-vomica* seed sample

Column	250 x 4 mm SS column packed with 5µ Lichrosorb DIOL material
Mobile phase	Hexane (50) + Dichloromethane (50) + Methanol (1) + Triethylamine (1) at a flow rate of 1 ml min^{-1}
Detector	Photometric (256 nm)
Injection volume	100 µl

Plate 6a shows the photograph of High Performance Liquid Chromatograph at AMPRS, Odakkali and Plate 6b shows the injection of the prepared seed sample for the HPLC analysis.

3.11.2 Isozyme analysis

Isozymes are generally made up of a number of subunits and it is the varying combination of the subunits which give rise to isozymes. Isozyme (Esterase) analysis was done in the seed sample and also in the leaf sample of the seedlings of the three seed sources and the method followed are hereunder.

Peroxidase and Esterase separation using native page

Polyacrylamide Gel Electrophoresis (PAGE) was carried out using "Holfer mighty small" vertical slab gel electrophoresis unit. Acrylamide monomer ($CH=CHCONH_2$) was polymerized with bisacrylamide [CH2(NHCONH=NH2) bis] to obtain the gel. Freshly prepared ammonium per sulphate (APS) was used as chain initiator and N,N,N',N', tetra methylene diamine (TEMED) as catalyst. Polyacrylamide gel was preferred because of its chemical inertness, high resolution, ease in handling and preparation.

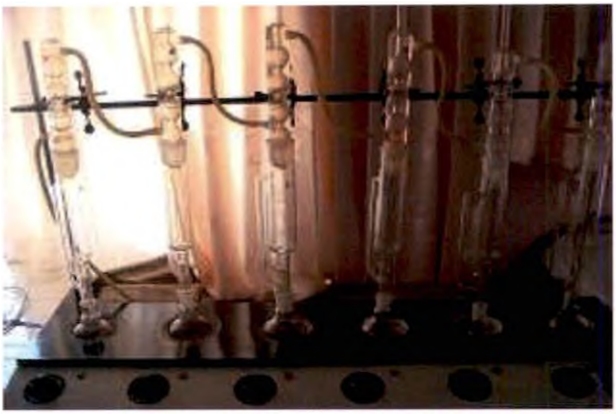


Plate 5. Soxhlet apparatus for extracting the seed extractives of *Strychnos nux-vomica*

Plate 6a. High Performance Liquid Chromatograph (Shimadzu LC-10AT) at AMPRS, Odakkali

Plate 6b. Injection of the prepared seed extract sample of *Strychnos nux-vomica* into the High Performance Liquid Chromatograph

Preparation of the reagents for the gel

The separation of isozyme was carried out in the anionic system. The following stock solutions were used.

Acrylamide – bis acrylamide stock solutions:

Acrylamide - 29.2 g

Bisacrylamide - 0.8 g

Volume was made up to 100 ml with distilled water and stored in amber coloured bottles away from light.

Resolving gel buffer (1.5M Tris-HCl, pH 8.8):

Tris base - 18.5 g

18.5 g of Tris base was dissolved in 70 ml distilled water and the pH was adjusted to 8.8 with 6N HCl and the volume made up to 100ml with distilled water. This solution was stored at 4^0C.

Stacking gel buffer (0.5 M Tris HCl, pH 6.8):

Tris base 6 g

6 g of Tris-base was dissolved in 70 ml distilled water and the pH was adjusted to 6.8 with 6 N HCl and volume was made up to 100 ml and stored at 4° C.

Ammonium per sulphate (APS):

Ammonium per sulphate solution was freshly prepared each time by dissolving 0.1 g APS in 1 ml distilled water.

For esterase isozyme separation, Gels having 5% acrylamide concentraton for staking gel and 10% acrylamide concentration for resolving gel were prepared.

Working solutions:

The reagents and their quantity required for preparing the stacking gel and resolving gel are given in the Table 3.

Table 3: The reagents used for the preparation of stacking and resolving gel solution

Reagents	Stacking gel solution (5%)	Resolving gel solution (10%)
Distilled water (ml)	5.7	4.1
Monomer solution (ml)	1.7	3.3
Gel buffer (ml)	2.5	2.5
10% APS(μl)	100	100
TEMED (μl)	15	20

Pouring polyacrylamide gels:

1. The glass plates were assembled in a vertical electrophoresis apparatus
2. Resolving gel solution was prepared as above. Polymerization will begin as soon as TEMED is added.
3. Acrylamide solution was poured into the gap between the glass plates (leave sufficient space for staking gel). Using a Pasteur pipette, isopropanol was overlaid (10% acrylamide gels) to prevent oxygen diffusing into gel and inhibit polymerization and placed the gel at room temperature in vertical position.
4. After polymerization, overlaid isopropanol was poured off and washed several times with distilled water.
5. The stacking gel solution was poured directly on the surface of the polymerized resolving gel. Immediately inserted a cleaned Teflon comb into the stacking gel solution (avoided trapping of air bubbles).

Preparation of the sample

Dried seeds and pulverized seedling leaf were used for the isozyme assay. For extraction of peroxidase, 500 mg of the sample was taken and homogenized in a pre-cooled mortar, along with 30 µl of ascorbic acid (50 Mm), 30 µl of soluble Poly Vinyl Pyrrolidone (PVP) (50 mg/50 ml water) and 0.5 ml of Tris-HCl extraction buffer (pH 7.0). The samples were ground at 4^0C by keeping the mortar and pestle in an ice tray. For extraction of esterase, 500 mg of the sample was ground in 0.5 ml of Tris-HCl buffer (pH 7.0).

For the extraction of esterase 500 mg of sample was ground in 1-2 ml of 0.2 M Tris HCl pH 8.0. The homogenized samples were centrifuged at 5°C. After centrifugation, the supernatant was collected in eppedorf tubes. The reagents required for the sample buffer preparation are given in the Table 4.

Table 4. Rreagents used in sample buffer preparation

Reagents	Volume
Deionised water (ml)	3.55
0.5M Tris HCl PH 8.0 (ml)	1.25
Glycerol (ml)	2.5
Bromophenol blue 0.5% (w/v) (ml)	0.2
Total volume(ml)	7.5

20 µl of the sample were mixed with 20 µl sample buffer and loaded into the wells.

Electrode buffer (0.025M Tris, 0.192 M Glycine, pH 8.3):

Tris base - 1.5125 g

Glycine - 7.2 g

Tris base and glycine were dissolved in 350 ml of distilled water, pH adjusted to 8.3 and the volume was made up to 500 ml with distilled water.

The Electrophoresis apparatus was attached to an electric power supply and a voltage of 50 V was applied to the gel. After the dye front has moved to the resolving gel, the voltage was raised to 150 V and the gel was run until the bromphenol blue reaches the bottom of the resolving gel (~4 hours). Then the power supply was turned off. A constant current of 20 mA per plate was maintained throughout the run.

Staining solution for peroxidase (modified from Shaw and Koen, 1968):

Staining solution (100 ml) contained the following reagents and their quantity required are given in the Table 5.

Table 5. Reagents used for the preparation of the staining solution for Peroxidase

Reagents	Quantity
0.2 M acetate buffer, pH 5.6	100 ml
Coomassie Brilliant Blue	0.1 g
H_2O_2 (3%)	0.4 ml

Fresh stain was prepared each time. Acetate buffer and Coomassie brilliant blue dye were mixed, boiled, cooled, filtered and then H_2O_2 was added to the mixture just before immersing the gel in staining solution. The gels were immersed in the staining solution for about 15 min. in dark with continuous shaking for the full development of the bands.

Staining solution for Esterase (Sadasivam and Manickam, 1992):

Staining solution (200 ml) contained the following reagents as given in the Table 6

Table 6. Reagents used for the preparation of staining solution

Reagents	Quantity
Sodium dihydrogen phosphate (g)	2.8
Disodium hydrophosphate (g)	1.1
Fast blue RR salt (g)	0.2
α Napthyl acetate (g)	0.03
Distilled water (ml)	200

After the run was over, the gels were taken out and incubated in staining solution for 30 min at 37^0 C in dark. The gels were destained with 7 per cent acetic acid. Gels were photographed.

3.12 Statistical analysis

The data obtained from the field were tabulated and subjected to statistical analysis using SPSS software version 17. The variation in fruit characteristics were analyzed between the three seed sources. The variations in the seed characteristics, germination characteristics, seedling growth characteristics and biomass were analyzed in Factorial Completely Randomized Block Design with three factors (Seed sources) and four levels (Seed grades) constituted in six replications. Both the comparisons, among the seed sources and the seed grades were done using the least significant difference (LSD) test. One way analysis of variance was done in the case of the fruit characteristics and the quantity of alkaloids. Two way ANOVA was carried out for the seed characteristics, germination characteristics and biomass characteristics. Factorial Randomized Block Design was used for analyzing the seedling growth characteristics. The final germination percentage and energy values were arcsine transformed before being statistically analyzed (Wartidiningsih et al., 1994). Duncan's Multiple Range Test was used in the analysis by which, the means of the similar treatments were put into the same subgroups.

Linear regression was carried out for the fruit parameters and seed characteristics. For the fruit parameters, number of seeds per fruit was taken as the dependent parameter and fruit volume was taken as the independent variable. In the case of the seed characteristics, seed density and seed weight were taken as the dependent variables and seed size and seed thickness were taken as the independent variables. Correlation coefficients were found out for the fruit characteristics and seed characteristics.

Results

RESULTS

The present study on the evaluation of seed source variation in *Strychnos nux-vomica* L. from different forest types of Kerala was carried out during the period 2009-2011 at the College of Forestry, Vellanikkara, Kerala, India. The results obtained are presented below.

4.1 Fruit parameters

The influence of the three forest types on fruit parameters like fruit weight, fruit volume and number of seeds per fruit were assessed in the present experiment. Data obtained are shown in the Table 7. The data revealed that the three seed sources differed significantly with respect to the mean fruit weights ($P<0.01$). The largest fruit weight of 47.3 g was observed in moist deciduous forest type (Pattikkad – SS 2) and the lowest of 27.4 g was observed in the case of dry deciduous forest type of Chinnar. The fruit weight in the case of riparian forest type of Peechi was intermediate (38.8 g). The increase in the fruit weight of moist deciduous forest compared to the dry deciduous forest type of Chinnar was 73 per cent and that of riparian forest type was 42 percent. Similar trend was observed with respect to fruit volume also with values of 33.1, 26.7 and 18.9 cm^3 in the case of moist deciduous (SS 2), riparian (SS 3) and dry deciduous (SS 1) forests respectively ($p<0.01$). The increase in fruit volume for the moist deciduous seed source when compared to dry deciduous was 75 per cent and that of riparian forest type was 24 per cent. The data on number of seeds per fruit revealed that the two seed sources SS 2 and SS 3 are similar with the values 2.2 and 2.4, respectively, and are superior to SS 1 which have the mean value of 1.5 ($p<0.01$). The percentage increase in the number of seeds of moist deciduous and riparian sources when compared to dry deciduous source were 47 percent and 60 percent respectively. Number of seeds per fruit was significantly correlated to the fruit weight and fruit volume (Table 8).

Table 7. Comparison of fruit parameters among the seed sources

Seed sources	Weight (g)	Volume (cm^3)	Number of seeds/ fruit
Dry deciduous SS 1	27.4^{a} (±1.23)	18.9^{a} (±0.95)	1.5^{a} (±0.09)
Moist deciduous SS 2	47.3^{c} (±1.89)	33.1^{c} (±1.39)	2.4^{b} (±0.13)
Riparian SS 3	38.8^{b} (±1.27)	26.7^{b} (±1.23)	2.2^{b} (±0.10)
Mean	37.8	26.2	2
F value	35.58**	26.5**	14.89**
P Level	<0.01	<0.01	<0.01

Table 8. Correlation coefficients for number of seeds per fruit with the fruit weight and volume for the three seed sources

	Seed sources	Fruit weight	Fruit volume
Number of seeds per fruit	SS 1	0.830**	0.769**
	SS 2	0.800**	0.763**
	SS 3	0.814**	0.837**

Values with similar superscript within a column do not differ significantly.
Standard deviation of respective mean values are given in parenthesis
** Significant at 1% level

4.2 Seed traits

The results obtained on seed length and seed width are given in Table 9. Seeds from riparian source had more length (24.6 mm) followed by moist deciduous forest and the shortest seeds were obtained from dry deciduous seed source. Seed length differed significantly ($p<0.01$) among the different seed size grades as revealed through main effects. The largest seed length of 28.4 mm was observed in the case of largest size grade of seeds. This was 41% more when compared to the length of smallest size grade of seeds. The seed length of 24-27 mm and 21-24 mm size grades showed a decreasing trend and was intermediate between the largest and smallest size grades.

Significant differences were observed in the width of seeds, as affected by the seed sources, size grade of the seeds and their interactions. Invariably, width of seeds was largest in the case of larger sized seeds and was smallest in the smaller sized seeds, among all seed sources. The 24-27 mm and 21-24 mm size grade seeds were intermediate. On an average, width of seeds of largest size grade seeds was 41 percent more compared to the smallest size grade seeds. The moist deciduous forests and riparian forests had largest width of seeds compared to the dry deciduous forests.

Data pertaining to the average of the seed sizes and seed thickness as affected by variations in the seed source, size grade, and their interactions are shown in the Table 10. Significant differences ($p<0.01$) were observed in the case of seed size and the trends were more or less similar to the width of seed. Thickness of the seeds was largest (5.3 mm) in the case of dry deciduous forests and was significantly superior to that of moist deciduous and riparian forests. And this increase was 10 per cent more.

Table 9: Variations in seed length and seed width (mm) of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Seed length (mm)					Seed width (mm)				
Seed sources	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS1 Dry deciduous	28.6 (± 1.42)	26.1 (± 0.89)	21.5 (± 1.17)	19.6 (± 1.09)	24^{A} (± 3.6)	26.2 (±1.38)	25.1 (±0.87)	23.1 (±1.35)	19 (±1.15)	23.4^{A} (±3.1)
SS 2 Moist deciduous	28.4 (± 0.91)	25.6 (± 0.92)	22.8 (± 0.82)	20.2 (± 1.09)	24.3^{AB} (±2.4)	27.9 (±1.18)	24.8 (±1.03)	22.5 (±1.02)	19.7 (±1.1)	23.7^{B} (±2.3)
SS 3 Riparian	28.3 (± 1.22)	26.1 (± 0.83)	23.3 (± 1.14)	20.7 (± 1.9)	24.6^{B} (± 2.6)	27.8 (±0.94)	24.9 (±1.08)	22.1 (±0.88)	19.4 (±0.77)	23.6^{B} (±2.4)
Mean	28.4^{D} (± 1.2)	26^{C} (± 0.9)	22.5^{B} (± 1.1)	20.2^{A} (± 1.5)	24.3 (± 3.3)	27.3^{D} (±1.4)	25^{C} (±1)	22.6^{B} (±1.2)	19.4^{A} (±1.0)	23.6 (±3.2)
	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	5.211**		815.32**	0.918		14.66**		766.79**	4.153**	
Pr level	0.006		0.000	0.483		0.00		0.00	0.00	
± SEm	1.342					1.157				

Values with similar superscript within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

Table 10: Variations in seed size and seed thickness (mm) of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Seed size (mm)					Seed thickness (mm)				
Seed sources	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS1 Dry deciduous	27.4 (±0.9)	25.6 (±0.6)	22.3 (±0.7)	19.3 (±0.7)	23.7^{A} (±3.2)	5.3 (±0.45)	5.6 (±0.89)	5.1 (±0.32)	5.2 (±0.64)	5.3^{B} (±0.7)
SS 2 Moist deciduous	28.1 (±1)	25.5 (±0.6)	22.7 (±0.8)	20.2 (±1.3)	24.2^{C} (±3.1)	5.1 (±0.49)	4.9 (±0.48)	5 (±0.55)	4.2 (±0.52)	4.8^{A} (±0.6)
SS 3 Riparian	27 (±0.9)	25.3 (±0.8)	22.5 (±0.7)	19.8 (±0.9)	23.9^{B} (±3.2)	4.6 (±0.62)	5.1 (±0.44)	4.5 (±0.59)	5 (±0.59)	4.8^{A} (±0.5)
Mean	27.5^{D} (±1)	25.5^{C} (±0.7)	22.5^{B} (±0.8)	19.8^{A} (±1.1)	23.8 (±3.2)	5^{A} (±0.6)	5.2^{B} (±0.7)	4.9^{A} (±0.7)	4.8^{A} (±0.6)	4.6 (±0.7)
	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	8.765**		1246.4**	2.520*		25.41**		8.90**	13.55**	
Pr level	0.00		0.00	0.022		0.00		0.00	0.00	
± SEm	0.739					0.307				

Values with similar superscript within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

The average seed weight and seed density as affected by the size grades, seed sources and their interaction were significantly different ($p<0.01$) and are depicted in Table 11. Interestingly, the lowest seed weight was observed for the dry deciduous seed source. This was 12 per cent less when compared to the highest seed weight (2.5 g) observed for the riparian forests. Significant differences were observed in the seed density due to the variations in seed sources, seed size grades and their interactions ($p<0.01$). Similar to the seed weight, seed density was also highest for the riparian forests. In spite of the highest fruit weight obtained (Table 8), the moist deciduous forest possessed the lowest seed weight and was 15 per cent less compared to the riparian forest.

The data pertaining to the regression of seed characters like seed size or thickness on seed weight or density belonging to different seed sources and also when the seed sources are pooled together are given the Table 12. It was found that density or weight of seeds was significantly correlated to the size of seeds belonging to all the three seed sources. Largest regression (r^2= 0.792 and 0.801) was in the case of dry deciduous forests. When regression was done on pooled data of the three sources, significant correlations were found between the seed size or thickness Vs seed density or seed weight.

Table 11: Variation in seed weight (g) and seed density of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Seed weight (g)					Seed density				
Seed sources	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS1 Dry deciduous	2.8 (±0.24)	2.5 (±0.28)	1.9 (±0.30)	1.4 (±0.20)	2.2^{A} (±0.6)	0.6 (±0.07)	0.6 (±0.07)	0.4 (±0.07)	0.3 (±0.06)	0.48^{A} (±0.2)
SS 2 Moist deciduous	3.4 (±0.31)	2.4 (±0.60)	2.0 (±0.55)	1.6 (±0.28)	2.4^{B} (±0.6)	0.8 (±0.05)	0.4 (±0.09)	0.3 (±0.14)	0.3 (±0.08)	0.45^{A} (±0.1)
SS 3 Riparian	3.3 (±0.28)	2.6 (±0.22)	2.1 (±0.43)	1.9 (±0.40)	2.5^{B} (±0.5)	0.8 (±0.04)	0.5 (±0.10)	0.4 (±0.10)	0.4 (±0.10)	0.53^{B} (±0.1)
Mean	3.2^{D} (±0.4)	2.5^{C} (±0.4)	2^{B} (±0.4)	1.6^{A} (±0.4)	2.4 (±0.7)	0.73^{C} (±0.1)	0.5^{B} (±0.1)	0.37^{A} (±0.1)	0.33^{A} (±0.1)	0.49 (±0.2)
	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	19.18**		248.72**	5.89**		22.67**		352.97**	16.89**	
Pr level	0.00		0.00	0.00		0.00		0.00	0.00	
± SEm	0.134					0.007				

Values with similar superscript within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

Table 12. Regression equations for the seed traits (seed weight or seed density as dependent variables and seed size or seed thickness as independent variables).

Seed sources	Regression Equations	N	r^2
Dry deciduous	Density= 0.890 x size (mm) + 0.043	100	0.792 **
	Weight(g)= 0.891 x size (mm) + 0.164	100	0.801 **
Moist deciduous	Density= 0.803 x size (mm) + 0.044	100	0.652 **
	Weight(g)= 0.808 x size (mm) + 0.171	100	0.654 **
Riparian	Density= 0.802 x size (mm) + 0.056	100	0.653 **
	Weight(g)= 0.726 x size (mm) + 0.187	100	0.632 **
Seed sources altogether	Density= 0.811 x size (mm) + 0.048	300	0.665 **
	Weight(g)= 0.796 x size (mm) + 0.178	300	0.670 **
	Weight(g)= 0.111 x thickness (mm) + 0.117	300	0.670 **

** Significant at 1% level

4.3 Germination studies

Each seed grade belonging to each seed source was replicated six times. Germination commenced after 35 days of sowing and the observations were continued for the subsequent 45 days. It was recorded on daily basis. Different growth stages are shown in the Plates 7a, 7b, 7c and 7d. A total of 225 (39%) seeds were germinated in Chinnar dry deciduous source, SS 1, 92 seeds (16%) in Pattikkad moist deciduous source, SS 2 and 214 seeds (37%) in Peechi riparian source, SS 3, out of 576 seeds sown for each seed source.

Germination percentage and germination energy as affected by the size grades, seed sources and their interactions are given in the Table 13. Significant differences were found in the germination percentage due to the influence of seed sources ($p<0.01$), size grades of seeds ($p<0.01$) and their interactions ($p<0.01$). In general, irrespective of the seed sources, germination percentage of larger seed grades was significantly superior to the germination percentage of the lower size seed grades. Germination percentage decreased significantly with the subsequent stepwise reductions in the size grade of seeds. Compared to lower sized seed grade, germination percentage of largest size grade was 2.44 times higher. Germination percentage of seeds of dry deciduous and riparian forest was on par and were significantly superior to that of moist deciduous forest by 2.38 times ($p<0.01$). Interaction effects also showed similar trends. The largest sized seed grades belonging to dry deciduous source has given the highest germination of 58 per cent which was on par with that of riparian forests (54%); larger sized seed grade of moist deciduous forest gave lower ($p<0.01$) germination percentage (20). Similar trend was observed in the case of size grade 2, 3 and 4 among the different seed sources. The similar trend as for the germination percentage among the seed sources and grades was repeated in the case of germination energy also. Seeds of dry deciduous source (49 per cent) and riparian source had higher germination energy. This was 2.2 times more compared to the

Plate 7a. Seed of *Strychnos nux-vomica*

Plate 7b. Three day old germinant of *Strychnos nux-vomica*

Plate 7c. Seven days old seedling of *Strychnos nux-vomica*

Plate 7d. 10 days old germinant of *Strychnos nux-vomica*

lowest germination energy of seeds of the moist deciduous source. The germination energy of largest size grade seeds was 2.3 times more compared to that of smallest size grade seeds. With declining size grade of seeds, the germination energy showed a declining trend except for the moist deciduous source.

Data pertaining to the speed of germination and mean daily germination as influenced by seed sources and size of seeds and their interactions are given in Table 14 ($p<0.01$). It was found that seed source only had significant influence on the speed of germination. The speed of germination of dry deciduous and riparian forests was at par and was significantly superior to that of moist deciduous forests. On an average, this increase was equal to 136 per cent.

Data showed that MDG was significantly influenced by the seed sources, size grades, and their interactions ($p<0.01$). The increasing trend of the interaction effect of MDG was clearly observed in accordance with the ascending order of seed sizes. Compared to the smallest size grade, the average increase in the MDG of the largest size grade was 116 per cent. Similar to other germination parameters, the dry deciduous and riparian seed sources showed their superiority over the moist deciduous seed source, with an increase of 107 per cent.

The variations in peak value of germination and germination value as affected by the seed sources, size grades and their interaction are given in the Table 15. The peak values of germination for the seeds collected from dry deciduous source and the riparian source were on par and were 108 per cent more when compared to seeds of moist deciduous forest. The peak value of germination in the largest size grade seed was 121 per cent higher when compared with that of smallest grade seed.

As germination value is the product of mean daily germination and peak value of germination, it also showed the similar trend. As that of other germination traits, the germination studies revealed the inferiority of the moist deciduous forest as seed source. The dry deciduous and riparian sources were on par in the case of germination value and were 327 per cent higher when compared to the moist deciduous source. As in the case of MDG and PV, germination value was also higher for the largest size grade which was about 415 per cent more when compared to that of smallest size grade.

Table 13: Variations in Germination Percentage and Germination Energy of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Germination Percentage					Germination Energy				
	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
Seed sources	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS1 Dry deciduous	0.62 [58] (±0.15)	0.45 [44] (±0.05)	0.33 [32] (±0.10)	0.23 [22] (±0.04)	0.41^{B} [39] (±0.19)	0.94 [78] (±0.26)	55 [52] (±0.16)	0.38 [37] (±0.09)	0.28 [28] (±0.09)	0.54^{B} [49] (±0.30)
SS 2 Moist deciduous	0.20 [20] (± 0.02)	0.18 [18] (± 0.10)	0.17 [17] (± 0.10)	0.11 [11] (± 0.05)	0.16^{A} [16] (± 0.09)	0.26 [26] (±0.13)	0.21 [21] (±0.13)	0.21 [21] (±0.17)	0.40 [38] (±0.21)	0.27^{A} [26] (±0.17)
SS 3 Riparian	0.58 [54] (± 0.16)	0.40 [39] (± 0.04)	0.34 [34] (± 0.08)	0.21 [22] (± 0.05)	0.38^{B} [37] (± 15)	0.75 [67] (±0.18)	0.49 [47] (±0.11)	0.39 [38] (±0.13)	0.23 [23] (±0.05)	0.47^{B} [44] (±0.23)
Mean	0.47^{D} [44] (± 0.23)	0.35^{C} [33] (± 0.13)	0.28^{B} [27] (± 0.12)	0.18^{A} [18] (± 0.09)	0.32 [31] (± 0.18)	0.65^{C} [57] (±0.35)	0.41^{B} [40] (±0.20)	0.33^{A} [32] (±0.15)	0.30^{A} [30] (±0.15)	0.42 [40] (±0.26)
-	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	61.78**		36.11**	3.26**		20.043**		19.648**	8.465**	
Pr level	0.000		0.000	0.008		0.000		0.000	0.000	
± SEm	0.008					0.023				

Values with similar superscript within a column or row do not differ significantly. Germination percentages are given in square brackets and arcsine values (in radians) are given without brackets. Standard deviations of respective mean values are given in parenthesis. ** Significant at 1% level

Table 14: Variations in Speed of Germination and Mean Daily Germination of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Speed of Germination					Mean Daily Germination				
Seed sources	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
1 Dry deciduous	0.27 (±0.05)	0.18 (±0.02)	0.14 (±0.04)	0.10 (±0.02)	0.17^{B} (±0.08)	0.795 (±0.152)	0.613 (±0.060)	0.418 (±0.194)	0.332 (±0.075)	0.540^{B} (±0.221)
SS 2 Moist deciduous	0.09 (±0.02)	0.08 (±0.04)	0.08 (±0.03)	0.06 (±0.01)	0.07^{A} (±0.03)	0.305 (±0.022)	0.275 (±0.155)	0.243 (±0.139)	0.205 (±0.031)	0.257^{A} (±0.106)
SS 3 Riparian	0.24 (±0.07)	0.16 (±0.01)	0.15 (±0.05)	0.10 (±0.02)	0.16^{B} (±0.08)	0.757 (±0.153)	0.542 (±0.028)	0.485 (±0.111)	0.323 (±0.084)	0.527^{B} (±0.186)
Mean	0.20^{C} (±0.09)	0.14^{B} (±0.05)	0.12^{B} (±0.05)	0.09^{A} (±0.03)	0.14 (±0.07)	0.619^{D} (±0.258)	0.477^{C} (±0.175)	0.382^{B} (±0.177)	0.287^{A} (±0.087)	0.441 (±0.219)
	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	51.06**		29.13	4.59		45.97**		27.19**	3.746**	
Pr level	0.000		0.920	0.782		0.000		0.000	0.003	
± SEm	0.001					0.013				

Values with similar superscript and similar case within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

Table 15: Variations in Peak Value of Germination and Germination Value of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Peak Value of Germination					Germination Value				
Seed sources	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS1 Dry deciduous	0.825 (±0.157)	0.607 (±0.060)	0.418 (±0.195)	0.332 (±0.075)	0.545^{B} (±0.230)	0.6 (±0.07)	0.6 (±0.07)	0.4 (±0.07)	0.3 (±0.06)	0.48^{A} (±0.2)
SS 2 Moist deciduous	0.307 (±0.020)	0.278 (±0.157)	0.243 (±0.139)	0.205 (±0.031)	0.258^{A} (±0.107)	0.8 (±0.05)	0.4 (±0.09)	0.3 (±0.14)	0.3 (±0.08)	0.45^{A} (±0.1)
SS 3 Riparian	0.770 (±0.168)	0.542 (±0.028)	0.485 (±0.111)	0.323 (±0.084)	0.530^{B} (±0.193)	0.8 (±0.04)	0.5 (±0.10)	0.4 (±0.10)	0.4 (±0.10)	0.53^{B} (±0.1)
Mean	0.634^{D} (±0.271)	0.476^{C} (±0.173)	0.382^{B} (±0.178)	0.287^{A} (±0.087)	0.444 (±0.226)	0.73^{C} (±0.1)	0.5^{B} (±0.1)	0.37^{A} (±0.1)	0.33^{A} (±0.1)	0.49 (±0.2)
	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	45.289**		28.493**	3.992**		33.925**		29.573**	6.150**	
Pr level	0.00		0.00	0.002		0.00		0.00	0.00	
± SEm	0.014					0.015				

Values with similar superscript and similar case within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

4.4 Seedling growth

Observations on seedlings' growth characters like height, collar diameter, number of leaves and number of branches were taken at thirty days' interval for six months (180 days) starting from 15th May. The whole seedlings representing the four seed grades and the three seed sources were considered for the measurements. The results for the seedling growth are presented here.

4.4.1 Height growth

The variations in the height growth of the seedlings as affected by the seed sources, seed grades, time and source-grade interactions are shown in the Table 16. On an overall basis, seedlings raised from the seeds of moist deciduous source were the tallest (18.1 cm) and the seedlings obtained from seeds of dry deciduous source were the shortest (16.3 cm). The seedlings obtained from riparian source were intermediate in height. The differences in seedling height among the three seed sources were significantly ($p<0.01$).

The seedlings obtained from largest sized seeds (SG 1) were taller (17.6 cm) and were superior to the seedlings obtained from seeds of other grades which were on par.

Even though interactions in seedling height between seed sources and seed grades were found to be significant ($p<0.01$), definite trend could not be delineated. Interactions between seed source and time of observation, seed grade and time of observation and also three factor interactions among seed source, seed size and time of observations were found to be non-significant.

4.4.2 Collar diameter growth

The variations in the collar diameter growth as affected by the seed sources, seed grades, time and source-grade interactions are shown in Table 17. The differences in

Table 16. Variations in height of *Strychnos nux-vomica* seedlings as affected by the seed sources, seed grades, durations and their interactions.

	Dry deciduous source					Moist deciduous source					Riparian source				
	Seed grades					Seed grades					Seed grades				
Days	**SG 1**	**SG 2**	**SG 3**	**SG 4**	**Mean**	**SG 1**	**SG 2**	**SG 3**	**SG 4**	**Mean**	**SG 1**	**SG 2**	**SG 3**	**SG 4**	**Mean**
30	14.37 (±3.91)	11.27 (±4.69)	13.18 (±5.02)	12.36 (±5.59)	12.791 (±4.90)	14.26 (±2.68)	13.33 (±4.83)	13.80 (±3.76)	15.06 (±3.98)	14.111 (±3.85)	14.73 (±4.41)	14.56 (±3.59)	14.55 (±4.23)	14.16 (±4.41)	14.498 (±4.17)
60	16.73 (±2.81)	14.84 (±4.60)	16.37 (±4.49)	15.65 (±4.53)	15.898 (±4.36)	16.59 (±2.17)	16.39 (±3.94)	15.27 (±3.64)	17.44 (±3.72)	16.425 (±3.47)	18.02 (±3.37)	17.87 (±3.31)	17.67 (±3.77)	16.99 (±4.31)	17.636 (±3.87)
90	17.20 (±2.72)	15.24 (±4.77)	16.78 (±4.57)	16.15 (±4.80)	16.341 (±4.51)	16.86 (±2.28)	16.59 (±3.91)	15.60 (±3.67)	18.05 (±4.07)	16.775 (±3.59)	18.44 (±3.77)	18.31 (±3.29)	18.17 (±3.82)	17.44 (±4.45)	18.091 (±3.96)
120	17.77 (±2.91)	15.82 (±5.04)	17.44 (±4.89)	16.73 (±5.08)	16.942 (±4.78)	17.66 (±2.12)	16.98 (±3.94)	16.29 (±3.90)	18.66 (±3.99)	17.399 (±3.62)	19.25 (±4.39)	18.92 (±3.50)	18.75 (±4.01)	18.11 (±4.70)	18.759 (±4.17)
150	18.48 (±2.85)	16.43 (±5.33)	18.06 (±5.09)	17.49 (±5.51)	17.614 (±5.07)	18.12 (±2.22)	17.37 (±4.01)	16.85 (±4.08)	19.54 (±4.52)	17.970 (±3.86)	19.92 (±4.99)	19.62 (±3.61)	19.51 (±4.22)	18.82 (±4.98)	19.468 (±4.42)
180	19.14 (±3.05)	17.07 (±5.71)	18.81 (±5.51)	18.16 (±5.94)	18.292 (±5.46)	18.99 (±2.20)	17.78 (±4.10)	17.62 (±4.45)	20.22 (±4.55)	18.654 (±4.01)	20.83 (±5.82)	20.31 (±3.98)	20.16 (±4.52)	19.54 (±5.32)	20.208 (±4.76)
Mean	17.28 (±4.30)	15.11 (±5.34)	16.77 (±5.23)	16.09 (±5.54)	16.31^{A} (±5.16)	17.08 (±2.69)	16.41 (±4.29)	15.91 (±4.58)	18.16 (±4.37)	16.89^{B} (±3.98)	18.53 (±4.84)	18.26 (±3.98)	18.14 (±404)	17.51 (±4.98)	18.11^{C} (±4.60)

Means on specific grades	SG 1	SG 2	SG 3	SG 4
	17.63^{c} (±4.30)	16.60^{a} (±4.89)	16.94^{b} (±4.80)	17.25^{b} (±5.17)

Means on specific durations/ time	30 Days	60 Days	90 Days	120 Days	150 Days	180 Days
	13.80^{a} (±4.51)	16.65^{b} (±4.09)	17.07^{b} (±4.22)	17.70^{c} (±4.44)	18.35^{d} (±4.71)	19.05^{e} (±5.04)

	Source	**Grade**	**Time**	**Source-Grade**	**Source-Time**	**Grade-Time**	**Source-Grade-Time**
F value	53.475	66.734	5.703	6.925	0.268	0.097	0.090
P level	0.000	0.000	0.001	0.000	0.988	1.000	1.000
±SEm	19.650						

Values with similar superscript and similar case within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

seedling collar diameter among the three seed sources were significantly different ($p<0.01$). Contrary to the variations in seedling height, seedlings raised from the seeds of dry deciduous source had greatest collar diameter (3.6 mm) and the seedlings obtained from seeds of moist deciduous source had the lowest collar diameter (3.2 mm). The seedlings obtained from riparian source were having intermediate in collar diameter.

Significant differences were found in the collar diameter of seedlings obtained from the seeds of different size grades, irrespective of the seed sources. In general, seedlings obtained from largest sized seeds (SG 1) had greatest collar diameter (3.5 mm) and was significantly superior to the seedlings obtained from seeds of the rest of the grades which were at par among themselves.

Even though interactions in seedling collar diameter between seed sources and seed grades were found to be significant ($p<0.01$), no definite trend could be delineated. Interactions between seed source and time of observation, seed grade and time of observation and also three factor interactions among seed source, seed size and time of observations were found to be non-significant.

4.4.3 Number of leaves and branches

The variations in the data pertaining to the number of leaves as affected by the seed sources, seed grades, time and source-grade interactions are shown in Table 18. Seedlings raised from all three seed sources did not show any variation with respect to number of leaves.

Significant differences were found in the number of leaves on the seedlings obtained from the seeds of different size grades, irrespective of the seed sources. In general, seedlings obtained from smallest sized seeds (SG 4) had greatest number of leaves (9.9) and seedlings obtained from seeds of the rest of the grades were at par.

Table 17. Variations in collar diameter of *Strychnos nux-vomica* seedlings as affected by the seed sources, seed grades, durations and their interactions.

	Dry deciduous source					Moist deciduous source					Riparian source				
	Seed grades					Seed grades					Seed grades				
Days	SG 1	SG 2	SG 3	SG 4	Mean	SG 1	SG 2	SG 3	SG 4	Mean	SG 1	SG 2	SG 3	SG 4	Mean
30	2.69 (±0.72)	2.46 (±0.80)	2.57 (±0.88)	2.53 (±1.05)	2.56 (±0.85)	2.50 (±0.76)	2.27 (±0.62)	2.32 (±0.69)	2.23 (±1.43)	2.33 (±0.60)	2.35 (±0.56)	2.50 (±0.69)	2.19 (±0.59)	2.26 (±0.57)	2.33 (±0.95)
60	3.64 (±0.72)	3.42 (±0.71)	3.54 (±0.76)	3.58 (±0.72)	3.54 (±0.73)	3.11 (±0.34)	3.32 (±0.62)	3.10 (±0.62)	3.31 (±0.63)	3.21 (±0.50)	3.29 (±0.45)	3.23 (±0.39)	3.04 (±0.63)	2.89 (±0.45)	3.11 (±0.64)
90	3.77 (±0.70)	3.54 (±0.75)	3.67 (±0.81)	3.73 (±0.78)	3.68 (±0.76)	3.20 (±0.34)	3.40 (±0.64)	3.20 (±0.63)	3.45 (±0.65)	3.31 (±0.53)	3.40 (±0.48)	3.35 (±0.42)	3.16 (±0.69)	3.00 (±0.44)	3.23 (±0.66)
120	3.90 (±0.71)	3.68 (±0.80)	3.80 (±0.84)	3.87 (±0.83)	3.81 (±0.81)	3.35 (±0.33)	3.48 (±0.67)	3.35 (±0.63)	3.57 (±0.70)	3.44 (±0.56)	3.54 (±0.44)	3.46 (±0.44)	3.26 (±0.78)	3.12 (±0.42)	3.34 (±0.68)
150	4.05 (±0.69)	3.82 (±0.86)	3.95 (±0.90)	4.04 (±0.92)	3.97 (±0.87)	3.43 (±0.34)	3.57 (±0.70)	3.47 (±0.66)	3.72 (±0.74)	3.55 (±0.62)	3.66 (±0.46)	3.59 (±0.39)	3.39 (±0.87)	3.24 (±0.46)	3.47 (±0.72)
180	4.19 (±0.71)	3.97 (±0.94)	4.10 (±0.96)	4.19 (±1.01)	4.11 (±0.94)	3.60 (±0.36)	3.66 (±0.76)	3.63 (±0.67)	3.86 (±0.82)	3.69 (±0.68)	3.82 (±0.46)	3.71 (±0.55)	3.49 (±0.99)	3.37 (±0.48)	3.60 (±0.76)
Mean	3.71 (±0.91)	3.48 (±0.95)	3.60 (±0.99)	3.66 (±0.71)	3.61^C (±0.97)	3.20 (±0.58)	3.28 (±0.68)	3.18 (±0.87)	3.36 (±1.04)	3.25^B (±0.73)	3.34 (±0.84)	3.31 (±0.77)	3.09 (±0.84)	2.98 (±0.94)	3.16^A (±0.77)

Means on specific grades	SG 1	SG 2	SG 3	SG 4
	3.49^c (±0.90)	3.38^b (±0.85)	3.31^{ab} (±0.92)	3.28^a (±1.02)

Means on specific durations/ time	30 days	60 days	90 days	120 days	150 days	180 days
	2.42^a (±0.87)	3.29^b (±0.70)	3.42^c (±0.73)	3.54^d (±0.76)	3.68^e (±0.82)	3.82^f (±0.87)

	Source	Grade	Time	Source-Grade	Source-Time	Grade-Time	Source-Grade-Time
F value	109.424	165.695	2.964	7.959	0.976	0.085	0.328
P level	0.000	0.000	0.031	0.000	0.462	1.000	1.000
±SEm	0.579						

Values with similar superscript and similar case within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

Interactions between seed source and time of observation, seed grade and time of observation and also three factor interactions among seed source, seed size and time of observations were found to be non-significant.

4.5 Biomass accumulation

Dry weights of stem, leaves and roots were not significantly influenced by seed sources and seed grades. Hence, the variations in root-shoot ratio were also non-significant (Table 19, 20).

4.6 Seedling vigour index

Seedling vigour index was significantly influenced by the seed sources, size grades, and their interactions ($p<0.05$). The results are given in the Table 21. Both dry deciduous and riparian sources showed similar values for seedling vigour index (1.0) whereas moist deciduous forests showed its inferiority with the lowest average (0.4). In the case of size grades, the largest size grade showed its superiority in seedling vigour index with a value of 1.2 over the other size grades. The smallest size grade had the lowest seedling vigour index (0.45). The vigour index of largest seed size was 167 per cent more compared to that of smallest seed size.

Table 18. Variations in number of leave of *Strychnos nux-vomica* seedlings as affected by the seed sources, seed grades, durations and their interactions.

	Dry deciduous source					**Moist deciduous source**					**Riparian source**				
	Seed grades					**Seed grades**					**Seed grades**				
Days	**SG 1**	**SG 2**	**SG 3**	**SG 4**	**Mean**	**SG 1**	**SG 2**	**SG 3**	**SG 4**	**Mean**	**SG 1**	**SG 2**	**SG 3**	**SG 4**	**Mean**
30	6.65 (±2.44)	5.15 (±2.81)	6.07 (±2.73)	5.96 (±3.04)	5.96 (±2.79)	6.67 (±3.54)	5.56 (±2.54)	7.36 (±2.33)	6.31 (±2.34)	6.48 (±2.05)	6.61 (±1.91)	6.20 (±2.36)	5.94 (±1.83)	6.23 (±1.74)	6.25 (±2.40)
60	7.58 (±2.88)	6.93 (±2.43)	7.25 (±2.43)	7.25 (±2.23)	7.25 (±2.42)	7.00 (±2.67)	6.61 (±2.16)	7.32 (±2.04)	7.13 (±2.41)	7.01 (±1.60)	7.78 (±1.68)	7.31 (±1.61)	6.79 (±1.83)	7.05 (±1.15)	7.23 (±2.19)
90	9.19 (±3.00)	8.63 (±2.63)	8.83 (±2.84)	8.93 (±2.60)	8.90 (±2.77)	8.61 (±2.00)	8.39 (±2.60)	9.00 (±2.41)	8.94 (±2.71)	8.73 (±2.19)	9.46 (±2.22)	8.96 (±2.06)	8.48 (±2.50)	8.72 (±1.98)	8.90 (±2.47)
120	10.54 (±3.07)	9.94 (±2.96)	10.20 (±3.34)	10.41 (±2.86)	10.28 (±3.13)	9.89 (±1.64)	9.89 (±2.75)	10.41 (±2.69)	10.00 (±2.92)	10.05 (±2.38)	10.70 (±2.59)	10.29 (±2.37)	9.87 (±2.13)	10.00 (±2.63)	10.21 (±2.67)
150	12.16 (±3.73)	11.65 (±3.48)	11.78 (±4.06)	12.09 (±3.52)	11.92 (±3.78)	11.50 (±1.56)	11.67 (±3.60)	12.09 (±3.42)	11.81 (±3.54)	11.77 (±3.14)	12.37 (±3.26)	11.94 (±3.06)	11.56 (±2.92)	11.67 (±3.62)	11.88 (±3.32)
180	13.51 (±4.12)	12.96 (±4.07)	13.15 (±4.78)	13.57 (±4.03)	13.30 (±4.38)	12.78 (±2.13)	13.17 (±4.13)	13.50 (±3.99)	12.88 (±4.03)	13.08 (±3.58)	13.61 (±3.75)	13.27 (±3.65)	12.95 (±2.86)	12.95 (±4.41)	13.19 (±3.83)
Mean	9.94 (±4.15)	9.21 (±4.08)	9.55 (±4.23)	9.70 (±3.97)	9.60^{A} (±4.14)	9.41 (±3.44)	9.21 (±3.70)	9.95 (±3.30)	9.51 (±4.05)	9.54^{A} (±3.50)	10.09 (±3.41)	9.66 (±3.90)	9.27 (±3.49)	9.44 (±3.62)	9.57^{A} (±3.77)

Means on specific grades	SG 1	SG 2	SG 3	SG 4
	9.39^{a}	9.48^{a}	9.54^{a}	9.92^{b}

Means on specific durations/ time	30 Days	60 Days	90 Days	120 Days	150 Days	**180 Days**
	6.16^{a}	7.19^{b}	8.86^{c}	10.20^{d}	11.86^{e}	13.21^{f}

	Source	**Grade**	**Time**	**Source-Grade**	**Source-Time**	**Grade-Time**	**Source-Grade-Time**
F value	0.157	308.273	2.125	2.357	0.292	0.224	0.102
P level	0.854	0.000	0.095	0.028	0.983	0.999	1.000
±SEm	9.153						

Values with similar superscript and similar case within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

Table 19: Variations in Stem Dry Weight and Leaf Dry Weight of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Stem Dry Weight					Leaf Dry Weight				
Seed sources	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS1 Dry deciduous	0.64 (±0.10)	0.73 (±0.11)	0.61 (±0.15)	0.66 (±0.16)	0.66^{A} (±0.13)	0.19 (±0.03)	0.25 (±0.06)	0.21 (±0.06)	0.20 (±0.08)	0.21^{A} (±0.06)
SS 2 Moist deciduous	0.74 (±0.17)	0.65 (±0.13)	0.82 (±0.24)	0.59 (±0.25)	0.7^{A} (±0.21)	0.19 (±0.03)	0.17 (±0.08)	0.19 (±0.11)	0.15 (±0.11)	0.18^{A} (±0.11)
SS 3 Riparian	0.83 (±0.15)	0.79 (±0.18)	0.78 (±0.24)	0.67 (±0.29)	0.76^{A} (±0.21)	0.17 (±0.17)	0.12 (±0.08)	0.24 (±0.06)	0.14 (±0.06)	0.17^{A} (±0.06)
Mean	0.74^{A} (±0.16)	0.72^{A} (±0.10)	0.74^{A} (±0.22)	0.64^{A} (±0.23)	0.71 (±0.21)	0.18^{A} (±0.08)	0.18^{A} (±0.08)	0.21^{A} (±0.07)	0.16^{A} (±0.08)	0.19 (±0.09)
	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	1.930		1.053	0.907		1.838		1.188	1.185	
Pr level	0.154		0.376	0.496		0.168		0.322	0.326	
± SEm	0.036					0.006				

Values with similar superscript and similar case within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level

Table 20 : Variations in Root Dry Weight and Root: Shoot Ratio of *Strychnos nux-vomica* as affected by seed source and seed grade.

Seed parameters	Root Dry Weight					Root: Shoot Ratio				
Seed sources	Seed size grades (mm)				Mean	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21		SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS1 Dry deciduous	0.64 (±0.10)	0.73 (±0.11)	0.61 (±0.15)	0.66 (±0.16)	0.66^{A} (±0.13)	0.19 (±0.03)	0.25 (±0.06)	0.21 (±0.06)	0.20 (±0.08)	0.21^{A} (±0.06)
SS 2 Moist deciduous	0.74 (±0.17)	0.65 (±0.13)	0.82 (±0.24)	0.59 (±0.25)	0.7^{A} (±0.21)	0.19 (±0.03)	0.17 (±0.08)	0.19 (±0.11)	0.15 (±0.11)	0.18^{A} (±0.11)
SS 3 Riparian	0.83 (±0.15)	0.79 (±0.18)	0.78 (±0.24)	0.67 (±0.29)	0.76^{A} (±0.21)	0.17 (±0.17)	0.12 (±0.08)	0.24 (±0.06)	0.14 (±0.06)	0.17^{A} (±0.06)
Mean	0.74^{A} (±0.16)	0.72^{A} (±0.10)	0.74^{A} (±0.22)	0.64^{A} (±0.23)	0.71 (±0.21)	0.18^{A} (±0.08)	0.18^{A} (±0.08)	0.21^{A} (±0.07)	0.16^{A} (±0.08)	0.19 (±0.09)
	Seed sources		Seed grades	Source-grade Interactions		Seed sources		Seed grades	Source-grade Interactions	
F value	1.930		1.053	0.907		1.838		1.188	1.185	
Pr level	0.154		0.376	0.496		0.168		0.322	0.326	
± SEm	0.036					0.006				

Values with similar superscript and similar case within a column or row do not differ significantly. Standard deviation of respective mean values are given in parenthesis. ** Significant at 1% level.

Table 21. Variation in seedling vigour index of *Strychnos nux-vomica* as affected by the seed source and seed grade

Seed sources	Seed size grades (mm)				Mean
	SG 1 27-30	SG 2 24-27	SG 3 21-24	SG 4 18-21	
SS 1 (Dry deciduous)	1.46 (±0.3)	1.23 (±0.4)	0.83 (±0.4)	0.61 (±0.2)	1.03^{B} (±0.5)
SS 2 (Moist deciduous)	0.52 (±0.2)	0.40 (±0.1)	0.41 (±0.1)	0.26 (±0.5)	0.40^{A} (±0.5)
SS 3 (Riparian)	1.63 (±0.3)	1.00 (±0.3)	0.89 (±0.4)	0.48 (±0.2)	1.00^{B} (±0.5)
Mean	1.20^{C} (±0.2)	0.88^{B} (±0.2)	0.71^{B} (±0.3)	0.45^{A} (±0.3)	0.90 (±0.5)

	Seed sources	Seed grades	Source-grade Interactions
F value	36.083**	20.943**	3.024*
P level	0.000	0.000	0.012
±SEm	0.053		

Values with similar superscript within a column or row do not differ significantly.
Standard deviations of respective mean values are given in parenthesis.
** Significant at 1% level and * significant at 5% level

4.7 Alkaloid content

The major alkaloids of *Strychnos nux-vomica* seeds were estimated by High Performance Liquid Chromatograph. The alkaloids tested were strychnine and brucine. The area swapped by the liquid chromatogram was converted to quantity in ppm and the actual quantity of the alkaloids from each sample was estimated. The results are shown in the Table 22. Both strychnine and brucine estimations were done in the three seed sources without considering the size grades of the seeds. The variations in the strychnine and brucine content were significant ($p<0.01$) among the seed sources. The quantity of strychnine was highest in the moist deciduous seed source (3683 ppm) and was followed by dry deciduous seed source (2788 ppm). When compared to the strychnine content of Peechi riparian source, SS 3 (1439 ppm), Pattikkad moist deciduous source had 156 per cent higher strychnine content and Chinnar dry deciduous source had 94 per cent higher strychnine content. Results on brucine estimation showed that the quantity of brucine was highest in the dry deciduous seed source, SS 1 (1691 ppm) which was on par with that of moist deciduous seed sources SS 2 (1408 ppm). The riparian seed source SS 3 had lowest brucine content (562 ppm) which was 60 per cent and 67 per cent lower when compared to the brucine content of SS 1 and SS 2, respectively.

4.8 Isozyme content

The presence of the isozymes were observed in the leaf samples of the seedlings raised from the three seed sources. Clear bands were obtained for esterase in all the three seed sources (Plate 8). Seed sample did not give any bands for the isozymes.

Table 22. Variation in the strychnine and brucine content from the seed sample of *Strychnos nux-vomica* as affected by the seed sources

Seed sources	Alkaloid content in ppm	
	Strychnine	Brucine
SS 1 (Dry deciduous)	2787.50^{ab}	1691.67^{b}
SS 2 (Moist deciduous)	3683.13^{b}	1408.23^{ab}
SS 3 (Riparian)	1438.89^{a}	562.12^{a}
F value	28.456**	13.497**
P level	0.000	0.000

Values with similar superscript within a column or row do not differ significantly.

** Significant at 1% level

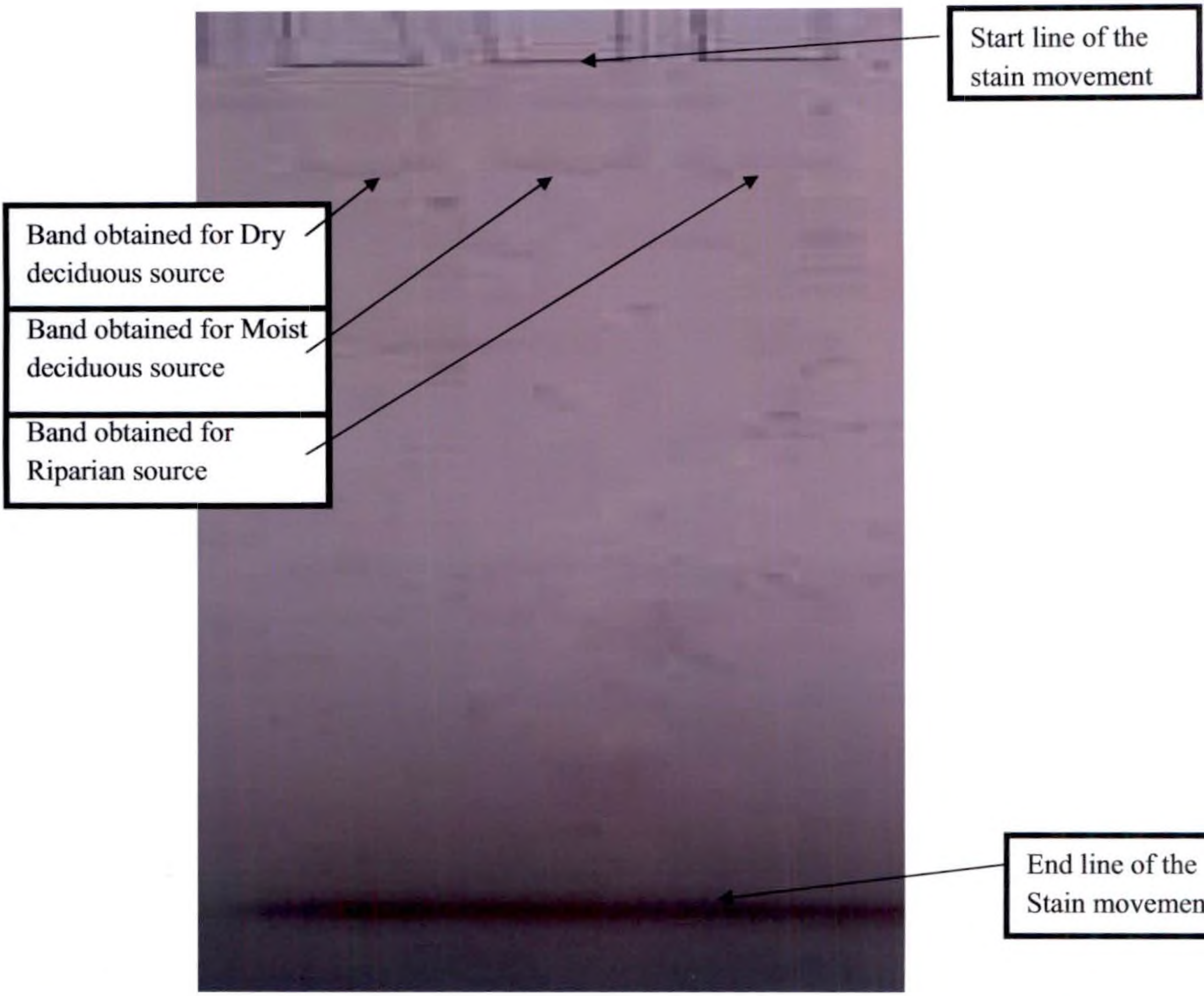


Plate 8. Esterase bands obtained for the leaf extract of *Strychnos nux-vomica* from the three seed sources

Discussion

DISCUSSION

Strychnos nux-vomica is an important medicinal tree and is being over- exploited in its natural habitats without any conscious replanting of the seedlings. Heavy removal of fruits from the forests for medicinal purposes without any consideration for natural regeneration is major cause for the depletion of its natural population in the forests. The alkaloids from the plant is used in various medical preparations in Ayurveda, Homoeopathy, Unani etc. and are suitable against almost all nervous disorders, cancerous conditions etc (Rathi et al. 2008). Many researches are being done to exploit its value in medicines against various diseases and disorders (Howes, 1904, Chopra, 1958; Sukul et al., 2001; Yin, 2003; Mā Lian et al. 2009; Rao et al., 2009; Vijayakumar et al., 2009; Singh et al. 2010; Bonamin et al., 2011). The tree is disappearing from our homesteads also. Pure plantations of *S. nux-vomica* are totally absent in our country and are required to be raised to reduce the over-exploitation from the natural forests. The tree is highly tolerant to stress conditions like flood and drought and is suitable for planting in the wastelands of the country (Ramachandra and Kumar, 2003). No studies have so far been carried out on identification of the superior seed sources of this species. Because of all these reasons, it is necessary to identify the superior seed sources from among the natural sources of *S. nux-vomica* with regard to their growth characters, yield and medicinal properties so as to collect seeds for raising healthier seedlings for the future domestication programmes. The present study aims to find out the best seed sources among the dry deciduous, moist deciduous and riparian forests of Kerala based on the fruit and seed quality, seedling growth characteristics, alkaloid content, and isozyme content.

The tree is found naturally growing in the dry deciduous forests, moist deciduous forests and riparian forests. The dry deciduous forests of Chinnar WLS was taken as the first seed source. The second seed source was moist deciduous forests at Pattikad Range of Thrissur Forest division. The riparian patches of forests at the dam site (flood plains) of Peechi-Vazhani WLS, was taken as the third seed source. The variations in fruit parameters, seed traits, germination attributes, seedling growth characteristics, biomass accumulation, alkaloid content and isozyme analysis among the three seed sources are discussed below.

5.1 Fruit parameters

Various fruit traits like fruit weight, fruit volume and number of seeds per fruit are very valuable for checking the superiority of the mother plant. Fruit weight and fruit volume are directly related to the seed size, seed health and number of seeds per fruit, which reflect in the germination aspects and seedling growth aspects (Ponnammal et al., 1993). Plants having higher number of fruits with maximum fruit weight and fruit volume are advisable for the food and medicinal uses. Most genetically variable traits are related to the fruits and seeds that are transmitted from generation to generation (Gabriel, 1978).

From a study conducted in Ghana by Peprah et al. (2009), it was found that total seed weight per fruit was found to be positively correlated with fruit size in *Allanblankia parviflora*, hence, selections based on fruit size suggest a substantial potential for high seed yield. As per the present study, maximum mean fruit weight, mean fruit volume and mean number of seeds per fruit were observed for the moist deciduous source and which were minimum for the dry deciduous seed source (Fig. 2, 3 and 4). The variations in size of fruits are also depicted in Plate 9. As per preliminary observations in the fruit collection sites, the number of fruits per tree were more for the dry deciduous source (data not shown) whereas fruit size and weight were lower. Fruit weight and fruit volume were highly correlated with the number of seeds per fruit (Table 8). It was also found that the fruits having larger seeds, yield less number of seeds and was directly correlated to the fruit volume. The fruits belonging to dry deciduous source, having more than three seeds per fruit were very rare. Single seeded fruits were having comparatively larger seeds in terms of the average diameter for all the three seed sources in *Strychnos nux-vomica* as per the observations of the present study.

The fruit weight, fruit volume and number of seeds per fruit, for the three seed sources were significantly different. The results have shown that largest fruit weight of 47.3 g was observed in the moist deciduous forest type of Pattikkad and the lowest was in the case of dry deciduous forests of Chinnar. Fruit weight in the case of riparian forest type was intermediate. Fruit volume and number of seeds per fruit also showed similar trend. Strychnos trees in the dry deciduous forest type were characterized by thinner crown density, wider branches and spreading crown shape. The fruits produced by these trees were smallest in size (fruit volume= 19 cm^3), more uniformly distributed in the crown and largest in number per tree (data not shown); number of seeds per fruit were lowest in trees from dry deciduous forests (1.5). Strychnos trees in the moist deciduous forests were comparatively larger in size, dense crown, less spreading and more or less columnar in shape. The fruits were largest in size (33 cm^3) and more concentrated in certain parts of the crown and number of seeds per fruit were also more. In the riparian site, trees were smaller in size leaning towards water and greater portion of the crown was concentrated towards the water body and with intermediate values for all the fruit traits under consideration. The largest fruit weight, fruit volume and number of seeds per fruit in the case of moist deciduous forests, may be because of the crown and site characteristics. The lower values obtained with respect to these characteristics in the case of dry deciduous forests may be because of the reasons already given above and also due to the reduced site quality as a result of lesser rainfall (Scovsgaard and Vanclay, 2008). Sivaprasad and Channabasappa (2011) have also observed greater fruit weight in *Terminalia alata* growing in moist deciduous forests compared to semi-evergreen and dry deciduous forest types. Kaushik et al. (1996) also obtained similar results in the case of *Acacia nilotica*. Apart from age, vigour, crown exposure and genotype of mother tree, soil and climate of the place of seed origin are

Plate 9. Ripe fruits of Strychnos nux-vomica with varying sizes

important factors affecting the seed traits (Salazar and Quesada, 1987). The fertility status and high rainfall availability in the moist deciduous and riparian forests may be contributing to the increase in fruit weight, fruit volume and number of seeds per fruit. Study conducted on the phenotypic variations of the fruits of *Adansonia digitata* from 10 different provenances over the rainfall gradient at Mali have also shown that fruit weight has significant difference among the provenances (De Smedt et al., 2011). In another study conducted on the same species (*A. digitata*) from five land use types of South Africa, it was found that both individual fruit size and total volume production were significantly different among the fruit sources (Venter and Witkowski, 2010).

The number of seeds per fruit ranged from one to thirteen as per the observations of the present study. As the seeds are widely used plant part for alkaloid extraction in *Strychnos nux-vomica*, more number of seeds per fruit is preferred. Moreover, *Strychnos nux-vomica* is mainly a seed propagated plant species. From the results on the fruit traits, the moist deciduous and riparian seed sources can be considered as superior seed sources.

5.2 Seed traits

S. nux-vomica is a tree on the verge of extinction from the wild without much regeneration in the forests of Kerala. Present study has attempted to depict the variations among the wild seed sources of *S. nux-vomica* through different seed traits like length, width, thickness, weight and density. All seeds were categorized into four size classes based on the preliminary measurements on the seed size. Further experiments were done with the seed grades for each seed sources, as seed grading is an important process for the testing of the superiority of the seeds (Willan, 1985). The superiority of seed traits can be directly correlated to the better growth of the seedlings in future (Manonmani and Vanangamudi, 2002). The results obtained for different seed traits of *S. nux-vomica* have been discussed hereunder.

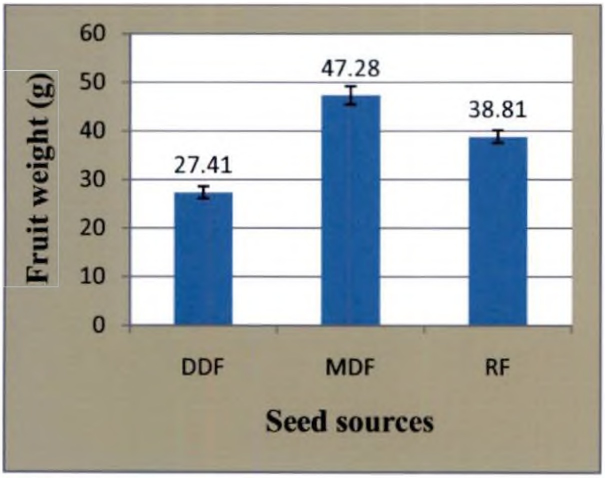


Fig. 2. Variations in fruit weight *in Strychnos nux-vomica* as affected by the seed sources

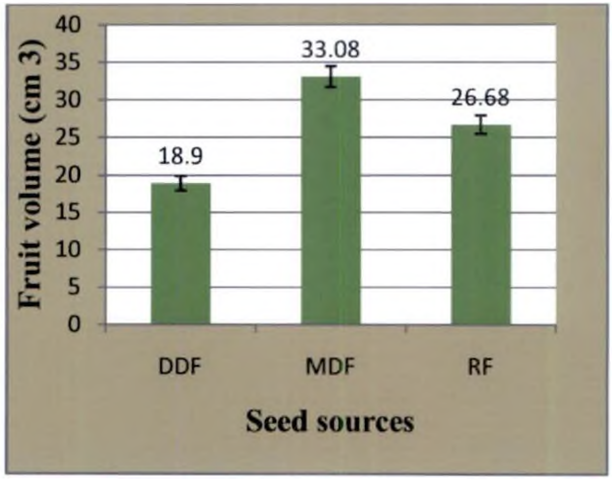


Fig. 3. Variations in fruit volume *in Strychnos nux-vomica* as affected by the seed sources

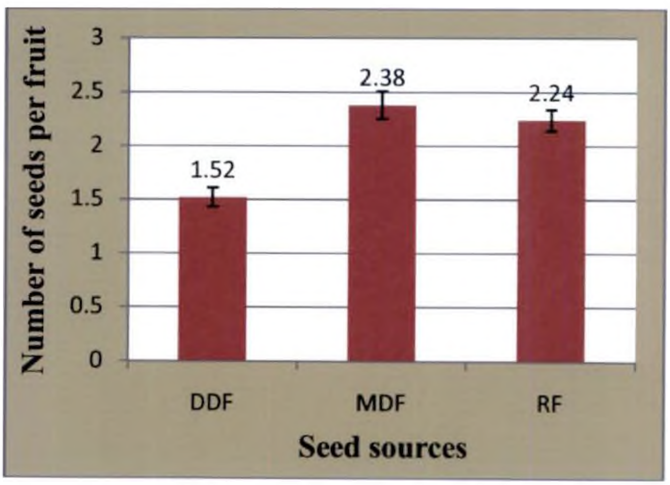


Fig. 4. Variations in number of seeds per fruit in *Strychnos nux-vomica* as affected by the seed sources

(DDF= Dry Deciduous Forests, MDF= Moist Deciduous Forests and RF= Riparian Forests)

As the seeds of *S. nux-vomica* are almost round in shape, the largest diameter of the seed was considered as the seed length and its orthogonal diameter was taken as the seed breadth. The average of seed length and seed width was considered as the seed size. Seed length was greatest for the riparian seed source and lowest for the dry deciduous seed source (Fig. 5). Seed width was similar in both moist deciduous and riparian seed sources and was the lowest in the dry deciduous seed source (Fig. 6). As the fruits from the dry deciduous source were smaller in size, the seed size was also lowest (Fig. 7). Jijeesh and Sudhakara (2007) determined the natural variability of teak (*Tectona grandis* Linn.f.) fruits belonging to different plantations in Nilambur Division, Kerala and the influence of fruit size on the number of seeds and significant variations were found. Vanangamudi et al. (1999), in their study on the effect of seed source on physiological and physical qualities of *Acacia nilotica* seeds could find significant results among the seed sources with respect to seed breadth. Studies in this regard suggest that seedlings derived from larger seeds have a greater competitive ability than those emerged from smaller seeds, which result in lesser growth (Dolan, 1984). The maximum germination percentage and germination energy were given by the larger seeds belonging to all the three seed sources of *S. nux-vomica.* The present study also showed that the growth characteristics are also better for the seedlings obtained from the larger seeds. Hence, larger seeds may be used for the planting programmes of *S. nux-vomica.* Deepa (2010) could observe that the seeds having maximum seed width as well as seed length can contribute to maximum seedling growth in *Saraca asoca.* But Sujith et al. (1994) found that seed size did not have any influence on seed germination of *Ceiba pentandra.* In general, larger seed grades of *S. nux-vomica* have given greater values for the germination attributes.

It was found that mean seed thickness was similar for moist deciduous and riparian seed sources (Fig. 8). The dry deciduous seed source (5.3 mm) showed the highest seed thickness. Plate 10 shows the variation in the seed thickness among the three seed sources.

Higher seed and leaf thickness are the general characters for the dry deciduous plant species. Similar characters were observed in *S. nux-vomica* also. Seed thickness is an indication of the nutrient reserve in the seeds. It is a general phenomenon regarding the seeds from low rainfall areas (Soriano et al., 2011). Either cotyledon thickness or the pericarp thickness, could be contributing to the total seed thickness. Significant variation was observed with respect to pericarp thickness among the seed sources of *Dalbergia sissoo* (Singh and Pokhriyal, 2001). Seed thickness could be considered as another parameter which can contribute to the superiority of the plant, but it is more or less environment dependent. Therefore, a larger sized seed with more thickness may be advisable for use in the propagation of *S. nux-vomica* and these characters may be adopted for future breeding strategies in this species.

Like fruit weight, seed weight is also an indication of the superiority of the plant. Aslan (1975) observed that larger and heavier seeds produced better quality seedlings in *Pinus brutia.* Ahlawat et al. (2007) observed that improvement in seed weight will improve other associated traits in *Acacia nilotica*. Manga and Sen (1996) found that in *Prosopis cineraria*, seed weight, seed volume and seed thickness was found to have significant and positive correlations with germination percentage. Seed weight of *Strychnos nux-vomica* from dry deciduous seed source was significantly different from the moist deciduous and riparian seed sources (Fig. 9). The differences in seed weight may be due to environmental influences (Harper 1977). Despite the higher seed thickness, seed weight was lowest for the dry deciduous source; this may be due to high oil content in the seeds of dry deciduous forest. Seed density was highest for the riparian source (Fig. 10) and may be due to the higher moisture content. Jones and Miller (1991), found that a drier climate is supposed to improve the oil yield in *Jatropha curcas* seeds. Since the seeds were

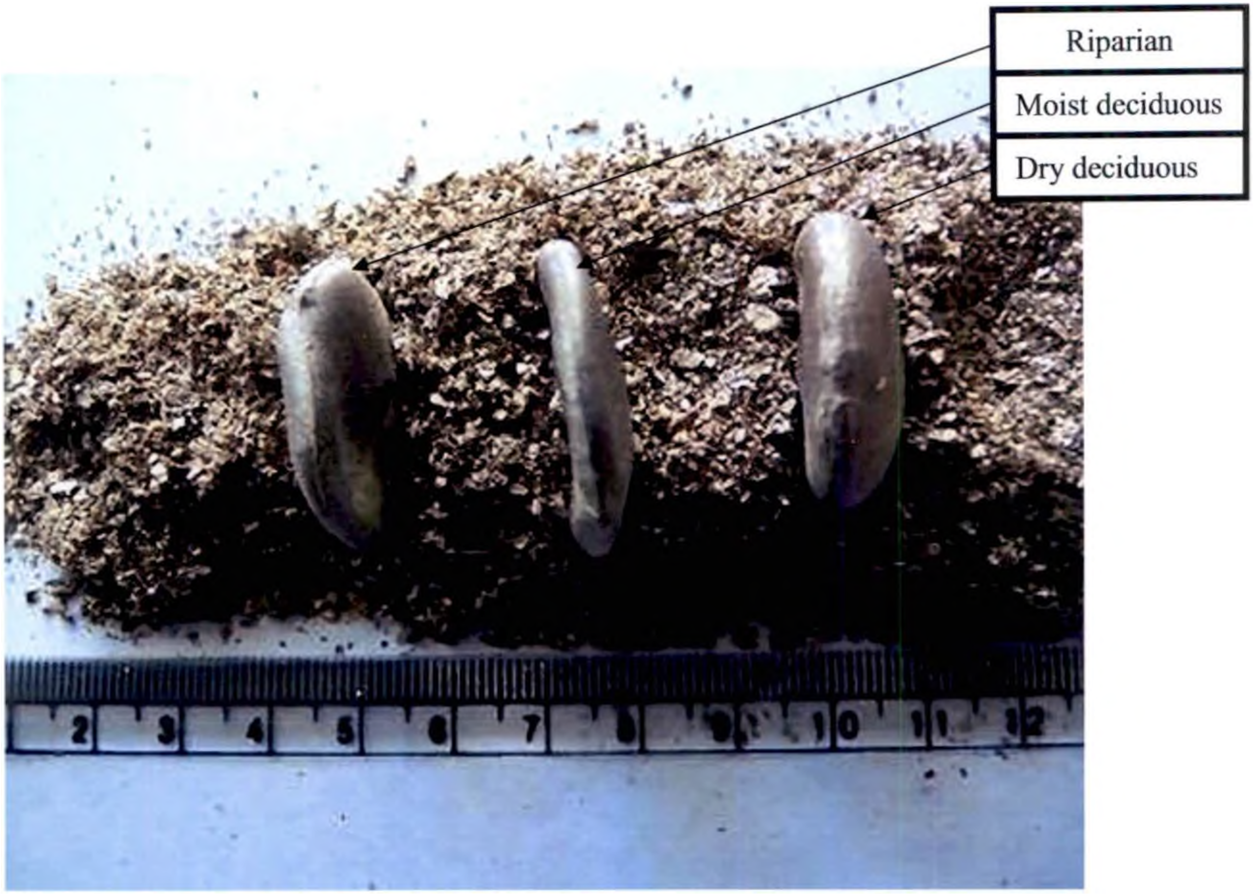


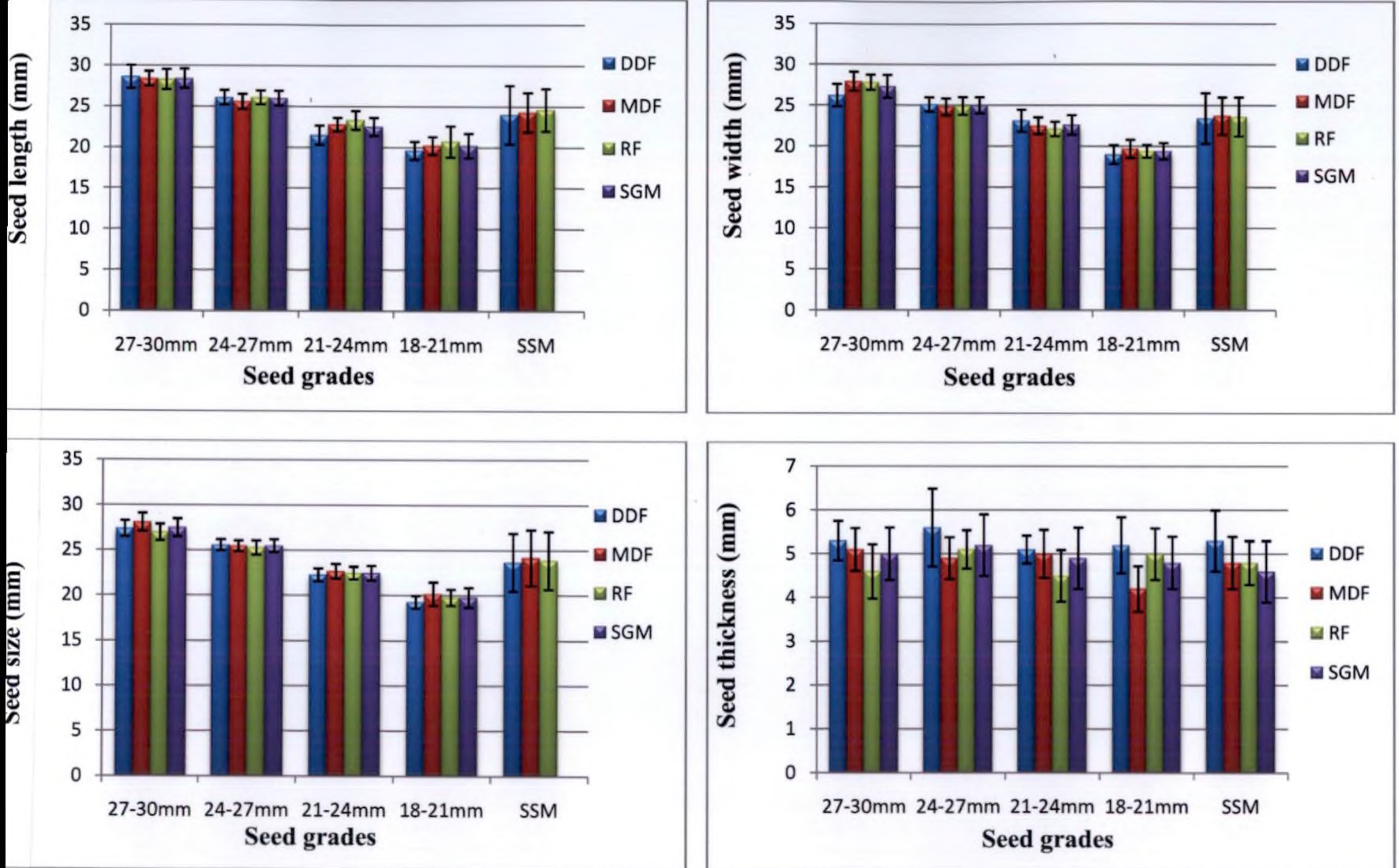
Plate 10. Variation in seed thickness of *Strychnos nux-vomica* from three seed sources Riparian (SS 3), Moist deciduous (SS 2) and Dry deciduous (SS 1)

collected from different locations, from trees approximately of the same age, differences observed in seed parameters may be attributed to genetic nature as a result of adaptation to diverse environmental condition prevailing throughout the distributional range of the plants (Mathur et al., 1984).

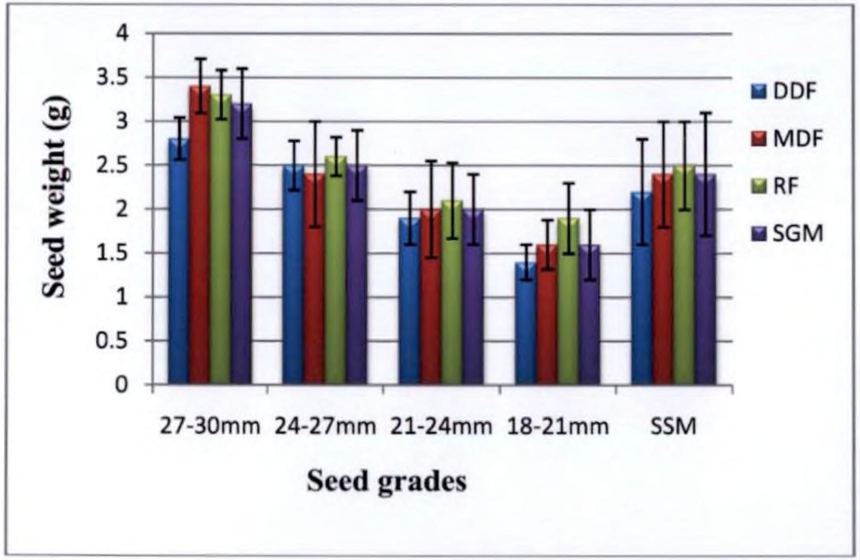
5.3 Germination attributes

Germination percentage showed marked differences among the seed sources. Germination percentage was highest for the seeds collected from the dry deciduous seed source which was similar to that of riparian seed source (Fig. 11). The moist deciduous seed source (16 %) showed a significant difference from dry deciduous (39 %) and riparian (37 %) seed sources and was exceptionally low. Germination percentages calculated for different seed grades showed that the larger seeds had better germination percentage for all the seed sources. Germination percentage was directly correlated to the seed size in *S. nux-vomica.* Sudhakara and Jijeesh (2008), also reported that *Tectona grandis* drupes belonging to largest size grade gave higher germination percentage but the size variation did not affect the germination value. Gera et al. (1999) in the study on source variation in *Albizzia procera*, have found that seed length is significantly correlated with germination percentage as well as seedling collar diameter. Manomani and Vanangamudi (2002) showed that, it is best to collect sandal seeds from Coimbatore and select only big sized seeds in order to obtain maximum germination percentage and increased seedling vigour.

Speed of germination was similar for seeds collected from dry deciduous and riparian seed sources. Speed of germination is also an indicator of superiority of the seed lot. Speed of germination was highest for the largest seed grade and was lowest for the smallest seed grade (Fig. 12). Since germination energy is a measure of speed of germination, it gives an idea of the vigour of the seed and of the seedling, which it produces (Willan, 1985).



g. 5, 6, 7 and 8. Variations in seed length, seed width, seed size and seed thickness (mm) in *Strychnos nux-vomica* as affected by the ed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means. DDF= Dry eciduous Forests, MDF= Moist deciduous Forests, RF= Riparian Forests, SSM= Seed Source Mean and SGM= Seed Grade Mean



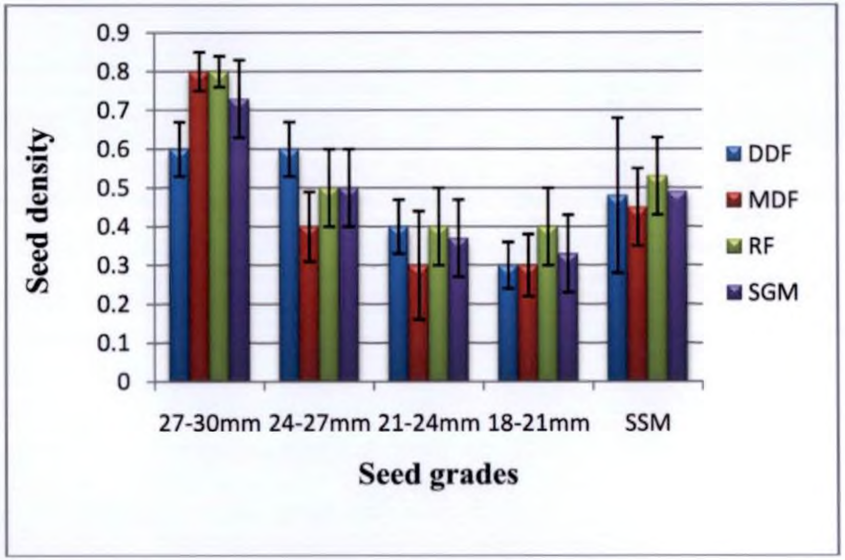


Fig. 9 and 10. Variations in seed weight (g) and seed density in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests, RF= Riparian Forests, SSM= Seed Source Mean and SGM= Seed Grade Mean

Germination energy was regarded as a measure of velocity of germination and hence assumed as a measure of seedling vigour (Hossain et al., 2005). Considerable differences have been obtained for germination energy among the seed sources and the seed grades. Germination energy for the three seed sources was different and was highest for the dry deciduous seed source (Fig. 13). Germination energy was significantly different for the four seed grades. Highest germination energy was obtained for the largest seed grade of the dry deciduous seed source. The interest in germination energy is based on the theory that only those seeds which germinate rapidly and vigorously under favourable conditions are likely to be capable of producing vigorous seedling in field conditions, whereas weak or delayed germination is often fatal (Aldhous, 1972).

Mean daily germination is a measure of number of seeds germinated per day. Mean daily germination for the moist deciduous source was exceptionally low when compared to the other seed sources (Fig. 14). It was highest for the dry deciduous seed source which was similar to the riparian seed source. Mean daily germination was different for the four seed grades. Peak value of germination in dry deciduous and riparian forests were significantly different from the moist deciduous source. Peak value of germination was highest for the largest seed grade of the dry deciduous seed source and lowest for the smallest seed grade of moist deciduous seed source (Fig. 15).

As the product of mean daily germination and peak value gives the germination value, it was highest for the dry deciduous and then for the riparian and least for the moist deciduous seed source (Fig. 16). In a study on *Pinus roxburgii,* Thapliyal and Dhiman (1997) found that germination value was significantly correlated with seedling height. Germination value can therefore be regarded as an integrated measure of seed quality, which has been used by several tropical seed workers e.g. for *Terminalia ivorensis* (Okoro, 1976) and for *Pinus kesiya* (Costales and Veracion, 1978). Germination value calculated for different seed grades showed significant differences among the four seed grades.

While considering the germination aspects, the dry deciduous and the riparian sources of *Strychnos nux-vomica* showed the superiority. Both of these seed sources have great potential for giving more seedlings in domestication. As the aim is the selection of seeds for the domestication programmes, the dry deciduous and the riparian seed sources are advisable.

5.4 Seedling growth characteristics

The performance of the seedlings can be considered as a tool for identifying the best seed source for a particular species. Influence of seed source variability on seedling attributes have been studied in a number of species and significant variations have been found with respect to the growth attributes of the seedlings (Ginwal et al., 1996; Maideen et al., 1997; Thapliyal and Dhiman, 1997; Jalil et al., 2000; Kundu and Tigerstedt, 2007). The characters that showed greater genetic influence can be directly screened or selected for the improvement of this potential tree-crop. Studies have revealed the presence of a positive correlation between the performance of seed sources and their seedling attributes. It is well known that the early vigour of the best performing seedlings will be reflected in superior performance during subsequent growth stages.

In the present study, it was found that the seedling height among the three seed sources were significantly different. Seedlings raised from the seeds of moist deciduous source produced taller seedlings (18.1 cm) and the seedlings obtained from seeds of dry deciduous source had the shortest height (16.3 cm) (Fig. 17-20). The seedlings obtained from the riparian source were intermediate in height. The existence of variation among seed sources for some seed and seedling traits have been demonstrated in *Acacia nilotica* by Ginwal et al. (1996). Height variation was

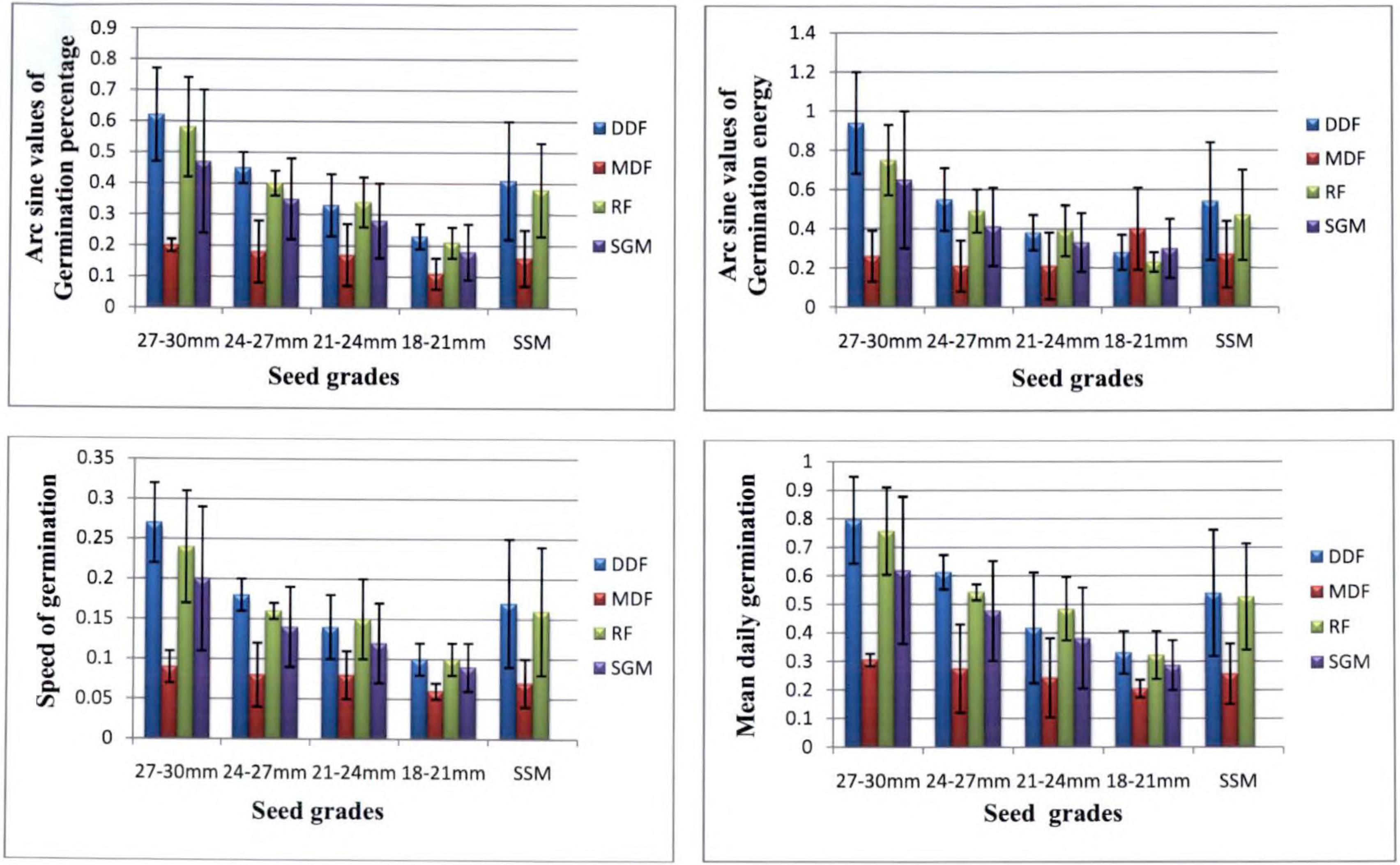
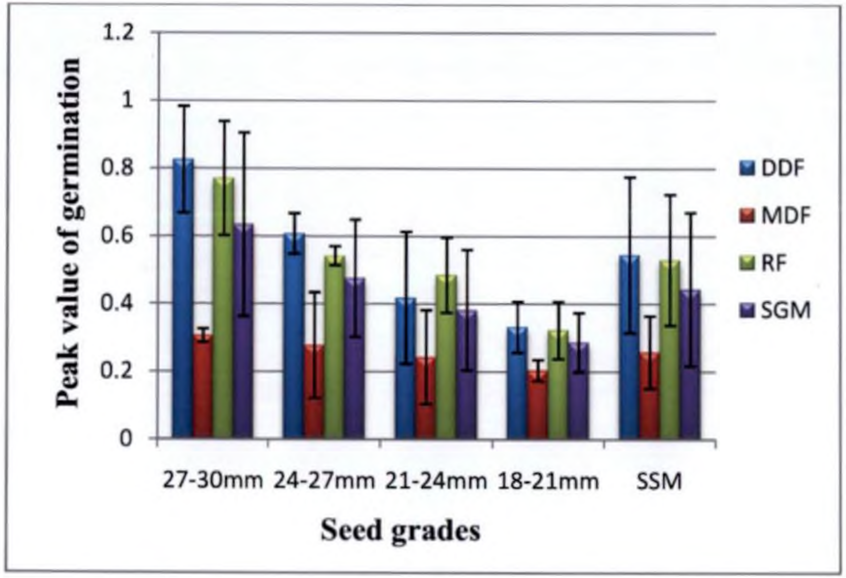


Fig. 11, 12, 13 and 14. Variations in germination percentage, germination energy, speed of germination and mean daily germination in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests, RF= Riparian Forests, SSM= Seed Source Mean and SGM= Seed Grade Mean



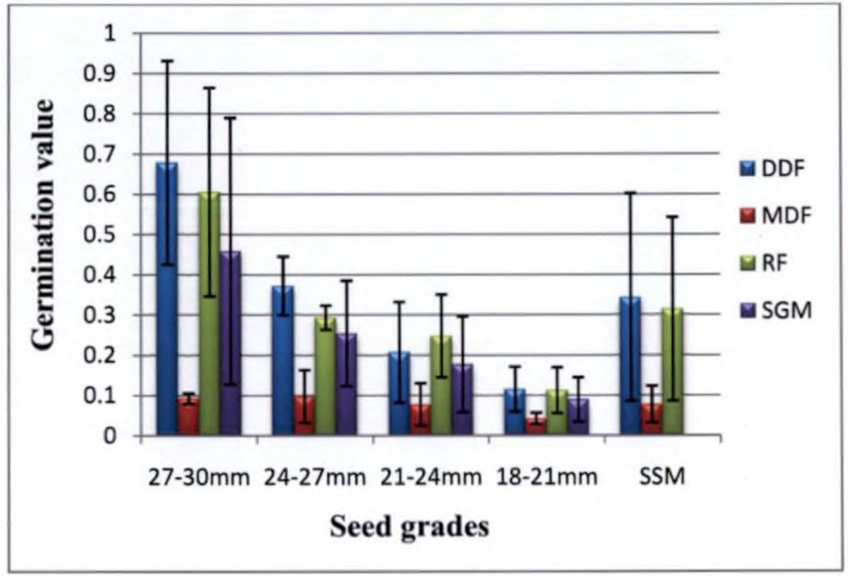


Fig. 15 and 16. Variations in peak value of germination and germination value in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests, RF= Riparian Forests, SSM= Seed Source Mean and SGM= Seed Grade Mean also observed significantly in *Azadirachta indica* (Kundu, 1998; Jaleel et al., 2000). The present study has shown that, even though, the germination attributes were considerably lower in *S. nux-vomica* from the moist deciduous source, the seedling height was more.

Seedlings obtained from larger sized seeds (SG 1) were taller (17.6 cm) and were superior to the seedlings obtained from seeds of the rest of the grades. Howe and Ritcher (1982) have observed significant variations in seedling height based on the seed sizes in *Virola surinamensis.*

Grading of seedlings based on their height and collar diameter at an early age is recommended for selection of best performing seedlings in the field (Jijeesh et al., 2007). Seedlings with high collar diameter and large number of leaves are known to have good yield potential. Collar diameter was highest for the dry deciduous source. It may be due to the water storage potential of the seedlings raised from the seeds of dry deciduous forests. Minimum collar diameter was observed for the riparian seed source and may be because of the proximity of the water body in the natural habitats. It was revealed that rapid changes in the stem diameter occurred throughout the day and were closely related to plant water content in *Pinus persica* (Simonneau et al., 1992).

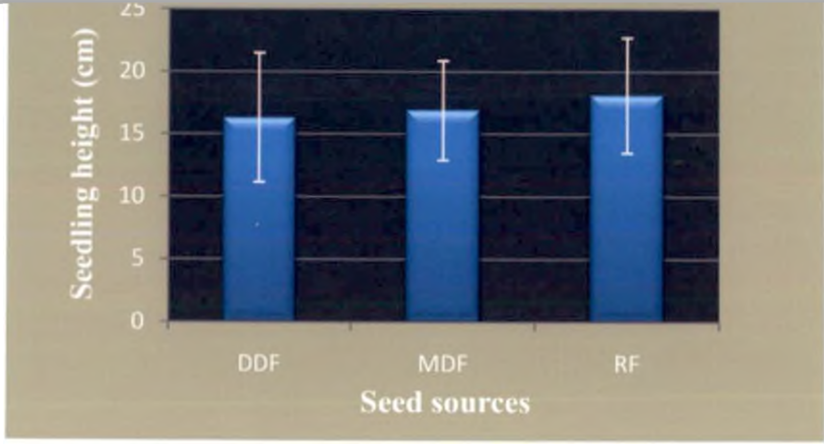
The collar diameter of seedlings among the three seed sources were significantly different. Contrary to the variations in seedling height, seedlings raised from the seeds of dry deciduous source had greatest collar diameter (3.6 mm) and the seedlings obtained from seeds of moist deciduous source had the lowest collar diameter (3.2 mm). The seedlings obtained from riparian source were intermediate in collar diameter as in the case of seedling height (Fig. 21-24).

Seedlings obtained from largest sized seeds (SG 1) had greatest collar diameter (3.5 mm) and were significantly superior to the seedlings obtained from seeds of the rest of the grades. Interactions between seed source and time of observation, seed grade and time of observation and also three factor interactions among seed source, seed size and time of observations were found to be non-significant. It may be inferred that the collar diameter growth in *Strychnos nux-vomica* seedlings may be an ecological adaptation and the seedling will be more stout and sturdy for the dry deciduous seed source as an adaptation to resist the ground dwelling winds during the dry periods.

In the case of number of leaves per seedling, all the seed sources were similar. Leaf number is an important criterion in determining the best seedlings. More number of leaves indicate better photosynthesizing ability, which ensure better growth of seedlings in future. Riparian seed source had slightly higher number of leaves (Fig. 25-28) and it may be an adaption for high degree of transpiration in waterlogged conditions. It was observed that the branched seedlings possessed more number of leaves than the unbranched seedlings in *S. nux-vomica*. *S. nux-vomica* seedlings showed early branching at the seedling stage itself and no new branches were produced after the initial branching by any of the seedling during the study period. The number of branches varied from 0 to 1 only and the data have not been presented since statistical analysis has not been done. The seedlings obtained from smallest sized seeds (SG 4) had greatest number of leaves (9.9) and the superiority of larger sized seeds could not be found in the case of number of leaves. Even though interaction effects of seed sources and seed grades on the number of leaves were found to be significant, no definite trend could be delineated.

5.5 Biomass accumulation

Greater dry matter accumulation during the seedlings stage may be an indication of the genetic superiority of the plant. Biomass estimation enables to analyze the photosynthetic efficiency of the plant. Findings of Kundu and Tigerstedt (1999) in neem confirmed a positive correlation between net photosynthesis with whole plant



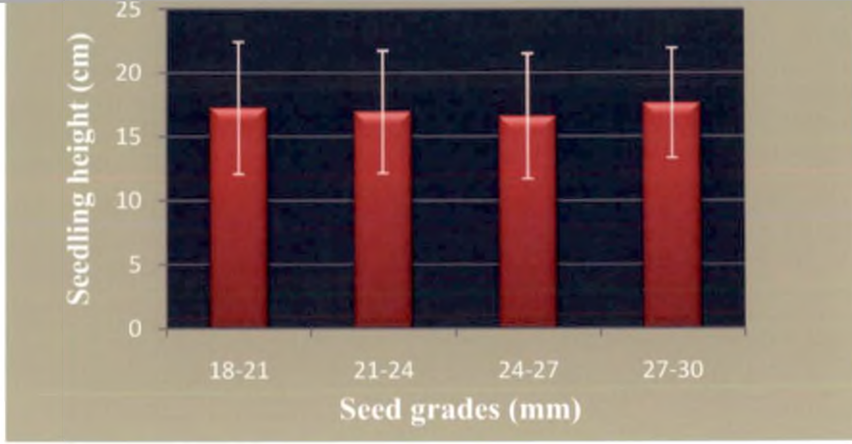
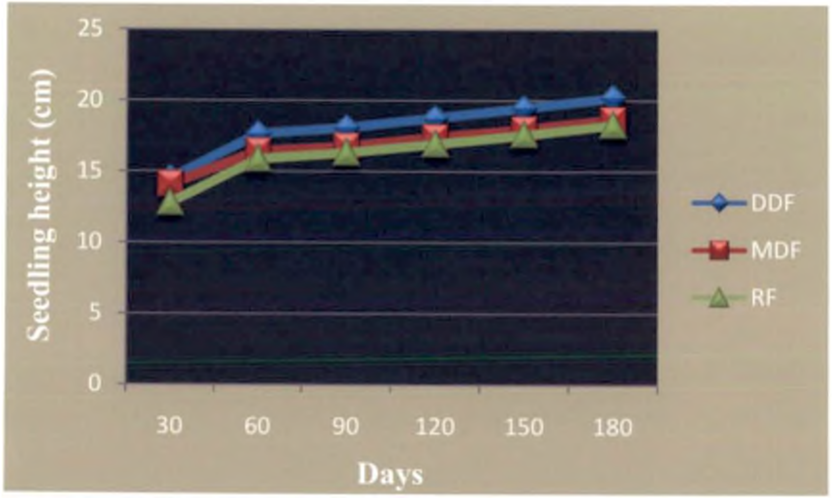


Fig.17 and 18. Variations in seedling height in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means



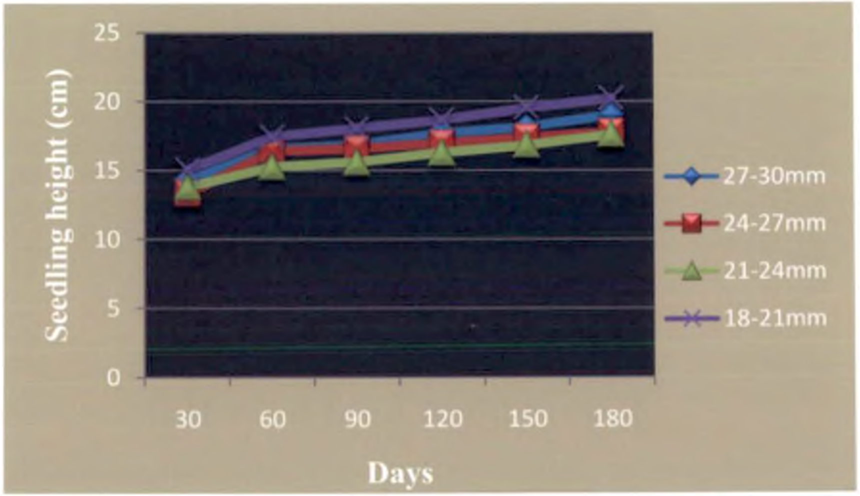


Fig. 19 and 20. Variations in seedling height in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively on different time interval. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests and RF= Riparian Forests

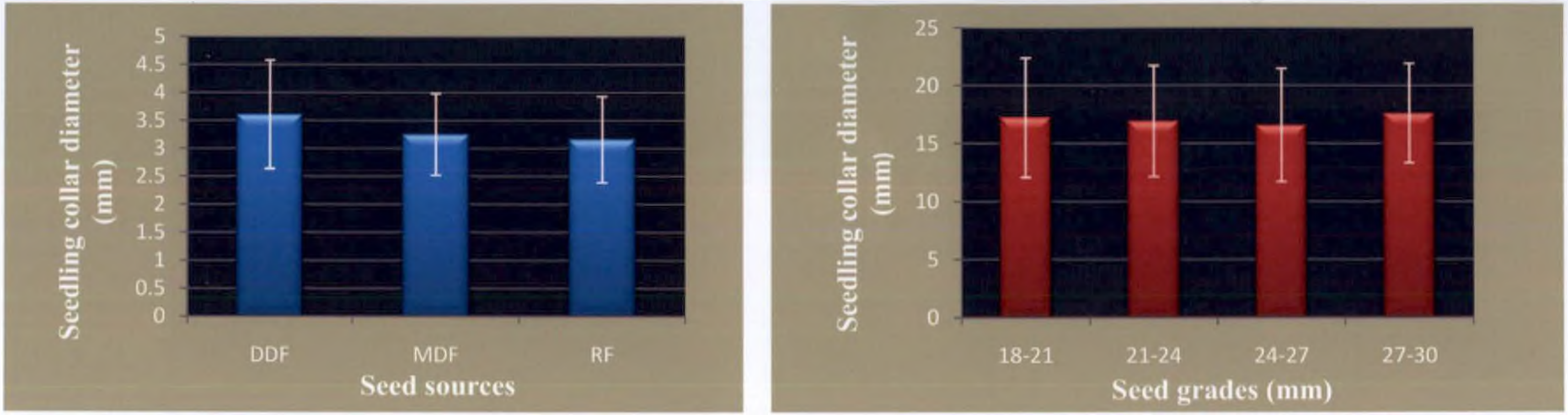


Fig.21 and 22. Variations in seedling collar diameter in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means

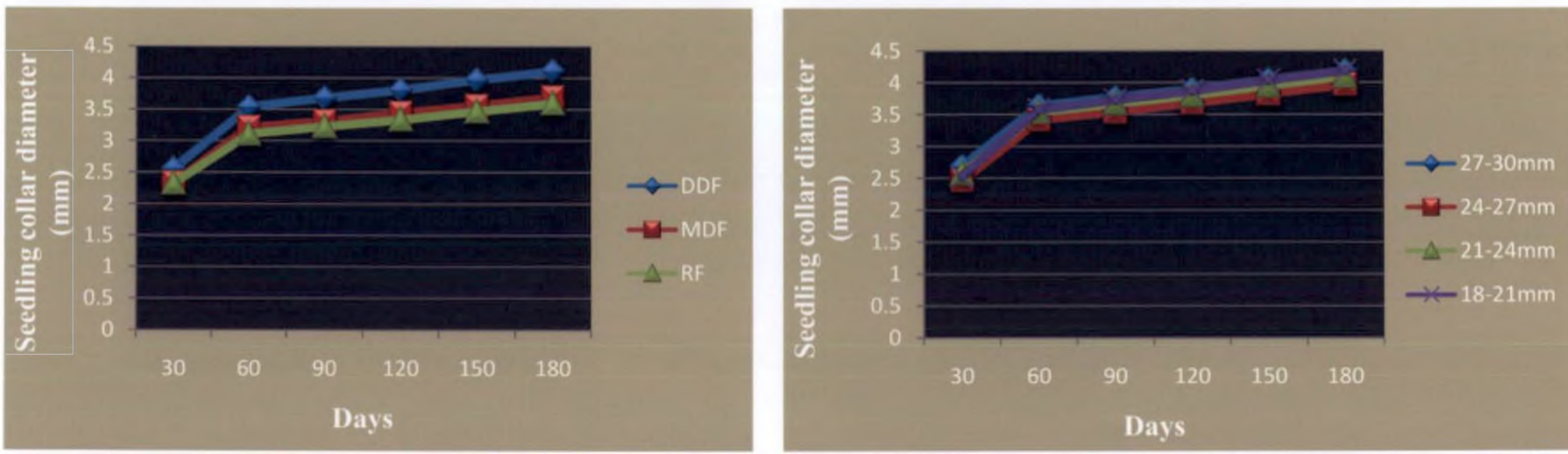


Fig. 23 and 24. Variations in seedling collar diameter in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively on different time interval. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests and RF= Riparian Forests

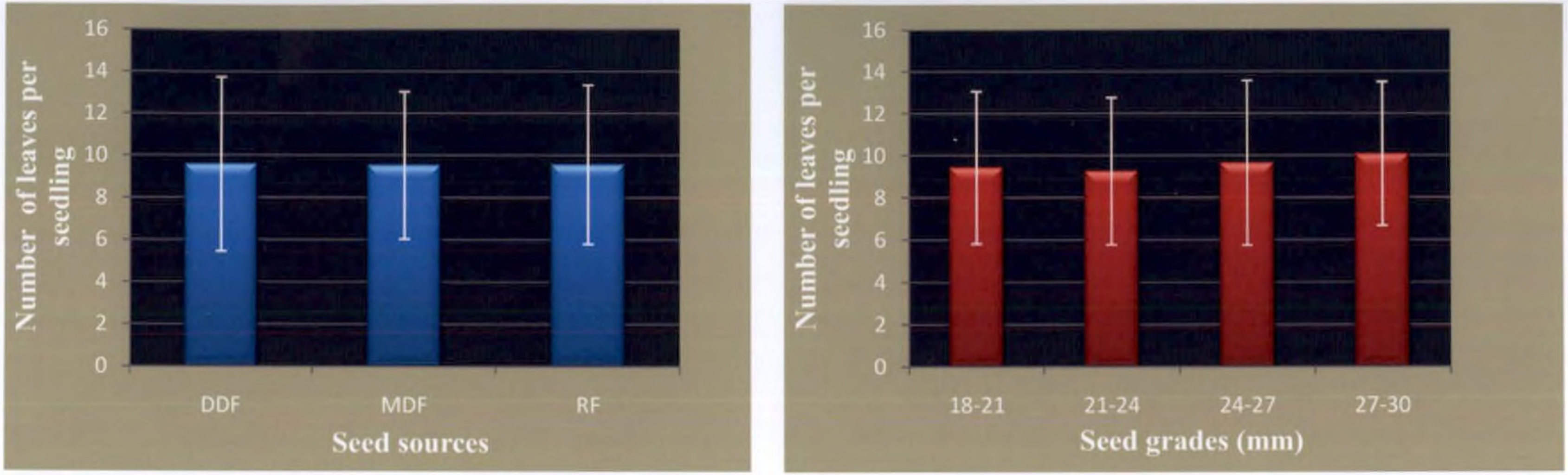


Fig.25 and 26. Variations in number of leaves per seedling in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means

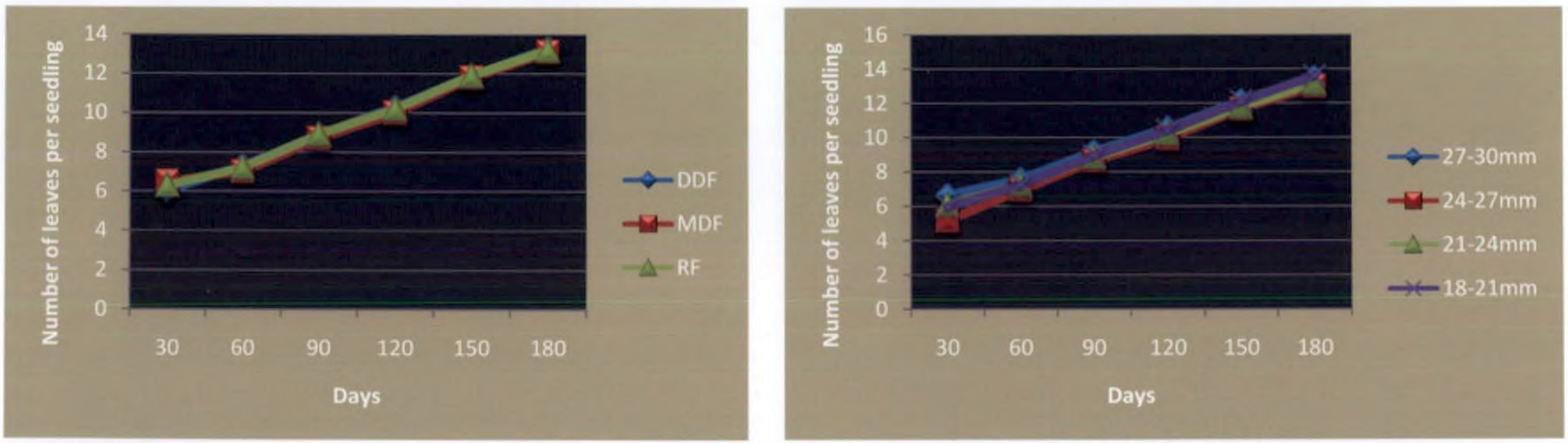


Fig. 27 and 28. Variations in number of leaves per seedling in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively on different time interval. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests and RF= Riparian Forests dry weight and leaf number. Significant variation was also observed by Singh and Pokhriyal (2001) in terms of biomass production in *Dalbergia sissoo* seedlings.

The dry weights of the stems, leaves and roots were not significantly different (Fig. 29-31). Also the root: shoot dry weight ratio was insignificant (Fig. 32). In a study conducted by Wulff (1986) in *Desmodium paniculatum*, it was found that the seed size is directly correlated to the total biomass of the seedlings. Vigour index for the seedlings were similar for the dry deciduous and riparian seed sources which differ from the moist deciduous seed source (Fig. 33). High value for seedling vigour index advocates the better seed source because vigour index is a function of germination percentage and the seedling dry weight. As per the present study, the greater variations in the germination percentage are reflected in the variations in seedling vigour index.

Plate 11 shows an uprooted seedling. The leaf phyllotaxi and the branching pattern of the seedling are showed in the Plate 12. *S. nux-vomica* possesses opposite leaves and opposite branches during the seedling stage. The root architecture of root trainer seedling of *S. nux-vomica* is also shown in the Plate 13. The roots have undergone aerial pruning and end with more than two finger like projections at the base of the main root.

5.6 Alkaloid estimation

The major alkaloids, strychnine and brucine are active than the minor alkaloids (a-colubrine, b-colubrine, icajine, 3-methoxyicajine, proto-strychnine, vomicine, novacine, N-oxystrychnine, pseudo-strychnine and iso-strychnine). Strychnine and brucine although being toxic in nature have remarkable therapeutic action. Medically, small doses of strychnine act as stimulant, laxative and as treatment for other stomach ailments, whereas brucine is primarily used in the regulation of high blood pressure and other comparatively benign cardiac ailments (Rathi et al., 2008).

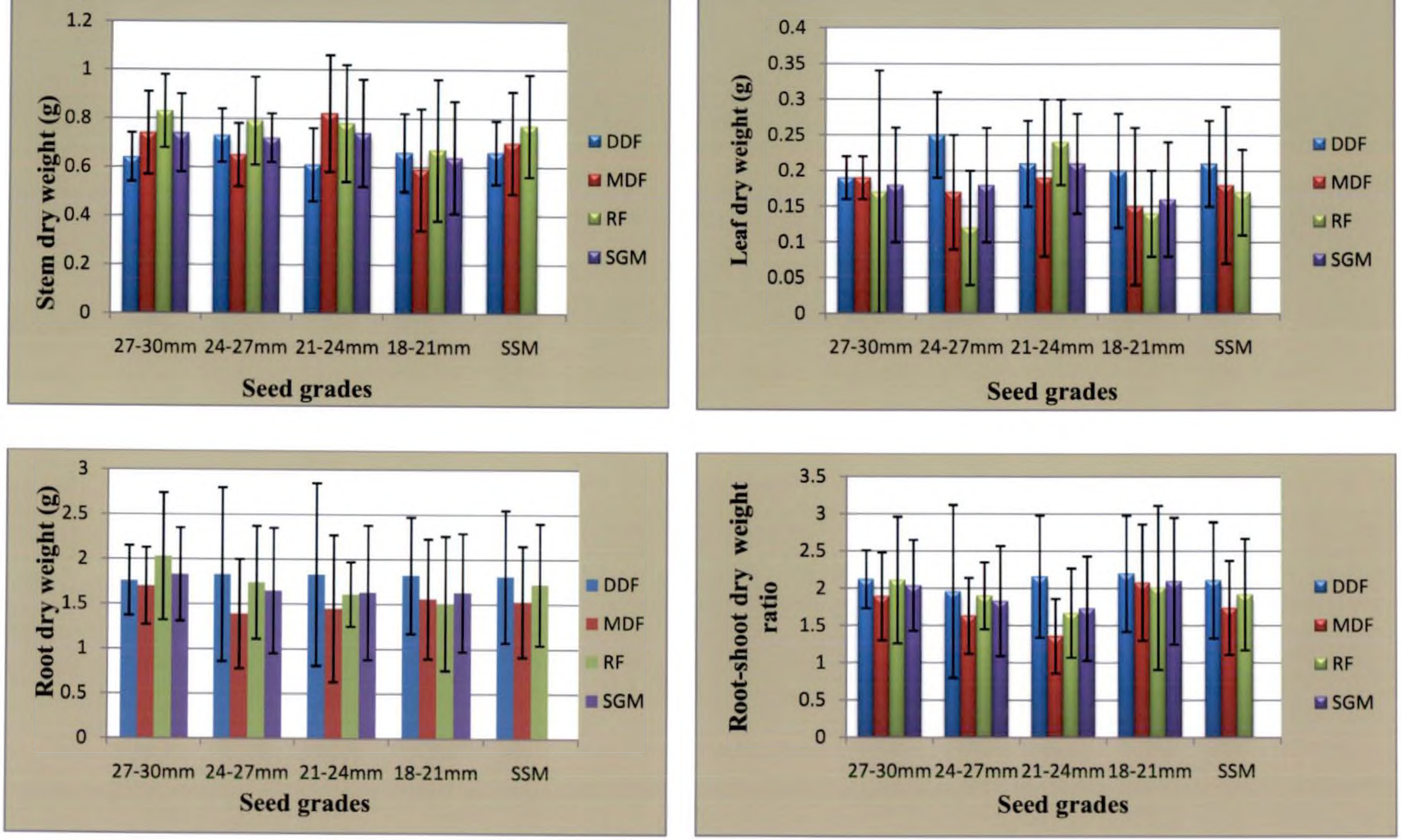


Fig. 29, 30, 31 and 32. Variations in seedling stem dry weight, leaf dry weight, root dry weight and root: shoot dry weight ratio in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively. Error bar indicates the standard deviation from the respective means. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests, RF= Riparian Forests, SSM= Seed Source Mean and SGM= Seed Grade Mean

Strychnine estimation done among the three seed sources showed that the quantity of strychnine was higher for moist deciduous seed source of *Strychnos nux-vomica*. The area swapped by High Performance Liquid Chromatogram (Fig. 35) have given the quantity of alkaloids (in ppm) present in the seed sample. Riparian seed source was significantly different from the moist deciduous seed source. Brucine content is also higher in moist deciduous seed source and was on par with dry deciduous seed source (Fig. 34). The riparian seed source was significantly different from the other seed sources. It was observed that the riparian seed source was having very low quantity of brucine. So in terms of the alkaloid content, the better seed source was the moist deciduous seed source and the poor seed source was the riparian. Mean strychnine and brucine content of the seeds from these seed sources were similar whereas it was different and minimum for the riparian seed source. The traditional knowledge do support this statement. It has been observed that the tuber crops grown near *Strychnos nux-vomica* yield the tubers with bitter taste. Seasonal variations in the quantity of strychnine and brucine, have been reported in *Strychnos nux-vomica* (Bandopadhyay and De, 1997).

Plate 11. Six month old root trainer seedling of *Strychnos nux-vomica*

Plate 12. Leaf phyllotaxi and branching pattern of *Strychnos nux-vomica* seedlings

Plate 13: Root architecture of the *Strychnos nux-vomica* seedling grown in root trainer

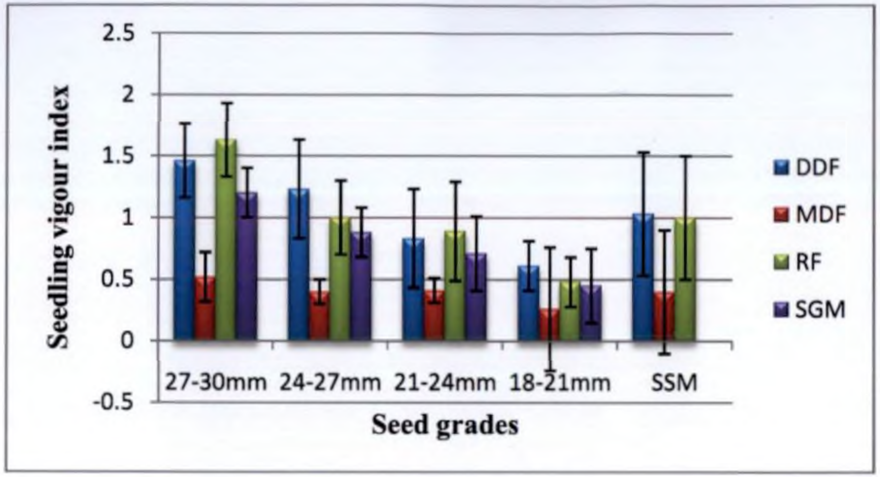


Fig. 33. Variations in seedling vigour index in *Strychnos nux-vomica* as affected by the seed sources and seed grades respectively.

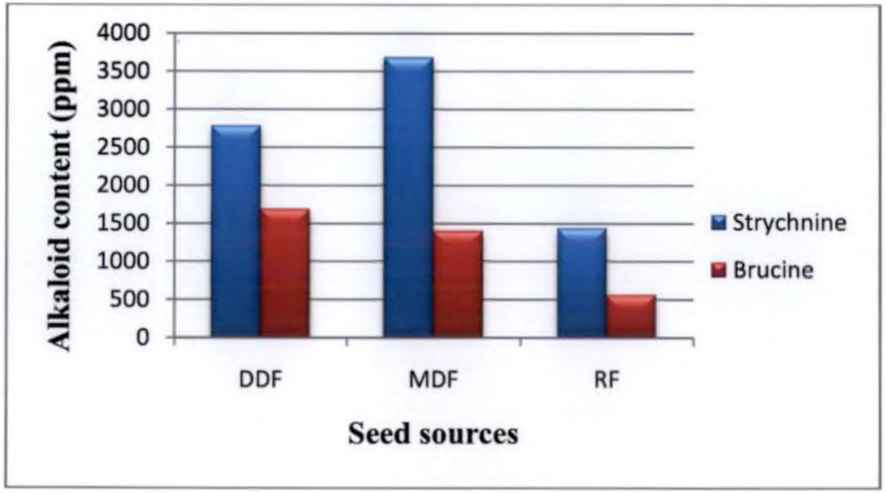


Fig. 34. Variations in strychnine and brucine content in the seeds of *Strychnos nux-vomica* as affected by the seed sources.
(Error bar indicates the standard deviation from the respective means. DDF= Dry Deciduous Forests, MDF= Moist deciduous Forests, RF= Riparian Forests, SSM= Seed Source Mean and SGM= Seed Grade Mean.)

5.7 Isozyme analysis

Seed sources differ significantly among species with different geographic ranges, life forms, and taxonomic affinities. Various methods using different molecular markers, are employed for drawing the genetic variations in plants. Isozyme is one of the commonly used molecular marker for finding out the genetic differences between the seed sources and many isozymes are present in the forest trees (Lundkvist and Rudin, 1977). Suma and Balasundaran (2003) have found low degree of genetic variability between five provenances of *Santalum album* in India through the isolation and evaluation of five isozymes. In the present study, the presence of isozymes, esterase and peroxidase were tested for the three seed sources in *S. nux-vomica*. The seed sample did not give the isozyme bands and may be because of the quiescence developed in the seeds (Tomas and Gupta, 1981). But the leaf sample from the seedlings has given the clear bands for esterase whereas peroxidase has given the smeared bands. The presences of isozymes were observed in all the three seed sources in similar pattern.

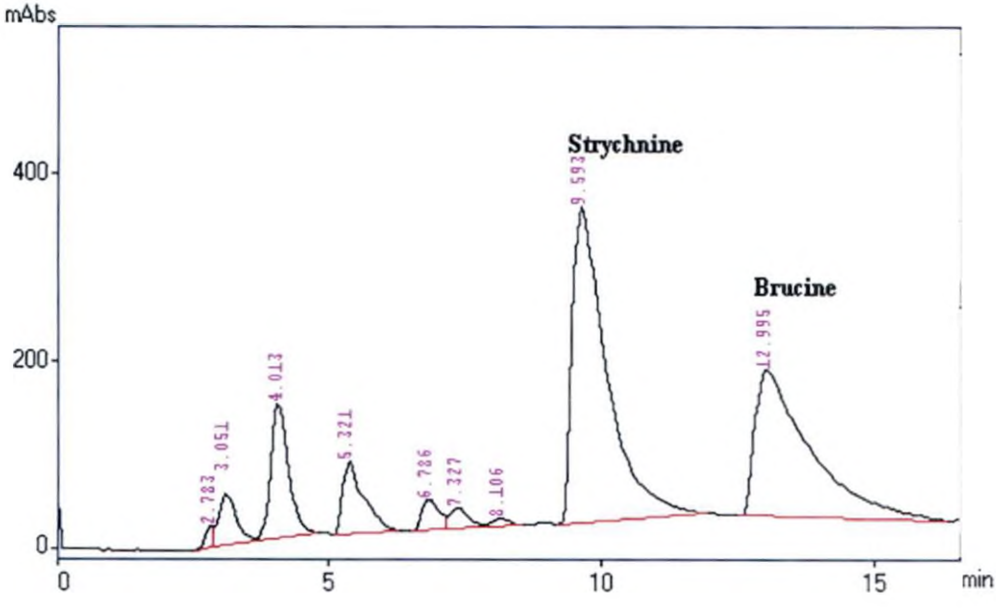


Fig. 35. High Performance Liquid Chromatogram

Summary

SUMMARY

An investigation entitled "Evaluation of seed source variation in *Strychnos nux-vomica* Linn. (Kanjiram) from different forest types of Kerala" through the estimation of variation in fruit traits, seed traits, germination attributes, seedling growth characteristics, biomass estimation and alkaloid content was conducted at the College of Forestry, Vellanikkara. The first seed source was taken from dry deciduous forests of Chinnar wildlife sanctuary and denoted as seed source one (SS 1). The second seed was from the moist deciduous forests of Pattikkad range of Thrissur forest division. The third seed source was from the degraded riparian patches of the dam site of Peechi-Vazhani wildlife sanctuary. Fruit collection was done after noting the geographic locations and tree dimensions. The germination and other trials were done in the nursery of College of Forestry. The salient findings of the study are summarized below.

6.1 Fruit parameters

Fruit parameters like fruit weight, fruit volume and number of seeds per fruit were determined for all the fruits which are collected. Fruit weight is measured by using electronic weighing balance and fruit volume is measured by water displacement method. It was found that the fruit weight, fruit volume and number of seeds per fruit vary significantly among the three seed sources and the greatest values were observed for the moist deciduous seed source. The least values were observed for the dry deciduous source, but the number of fruits per tree was largest for the same. Significant correlations were found between the fruit weight, fruit volume and number of seeds per fruit.

6.2 Seed extraction, storage and grading

Seeds were extracted manually from the fruits after taking the observations on the fruit traits. An over-night soaking has been done for extracting the seeds from the

pulp around the seeds after breaking the fruit wall. The seeds were shade dried and stored. The entire seeds were graded based on the seed size. For categorizing the seed size grades, each seed was measured for its length and width and the average was calculated. Based on this, the seed grades were of the size grades viz. 27mm-30mm (SG 1), 24mm-27mm (SG 2), 21mm-24mm (SG 3) and 18mm-21mm (SG 4). Further observations were done based on these seed size grades.

6.3 Seed traits

Seed parameters like seed length, seed width, seed thickness, seed weight and seed density were measured for 25 sample seeds from the four seed size grades. Seed length, seed width and seed thickness were measured in millimeters by using a digital vernier caliper. Seed weight was measured by using an electronic weighing balance and seed density was measured by using electronic specific gravity module.

The seed length of riparian seed source was significantly higher to dry deciduous. Seed length of moist deciduous forest was intermediate. Significant differences were observed in the width of seeds, as affected by the seed sources, size grade of the seeds and their interactions. The moist deciduous forests and riparian forests had largest width of seeds compared to the dry deciduous forests. Significant differences were observed in the case of seed size and the trends were more or less similar to the width of seed. Surprisingly thickness of the seeds was largest (5.3 mm) in the case of dry deciduous forests and was significantly higher to that of moist deciduous and riparian forests. Interestingly, the lowest seed weight was observed for the dry deciduous seed source which was 12 per cent less when compared to the highest seed weight (2.5 g) observed for the riparian forests. The largest seed weight was given by the largest size grade (2.8 g). Similar to the seed weight, seed density was also highest for the riparian forests. In spite of the highest fruit weight obtained the moist deciduous forest possessed the lowest seed weight and was 15 per cent less compared to the riparian forest. It could be found that density or weight of seeds was significantly correlated to the size of seeds belonging to all the three seed sources. Larger regression coefficients (r^2= 0.792 and 0.801) were in the case of dry deciduous forests.

6.4 Germination attributes

Germination started after 30 days of sowing and continued for 45 days. The daily germination was noted in each replication for 45 days. Germination percentage, speed of germination, germination energy, mean daily germination, peak value of germination and germination value were estimated as the germination attributes among the seed sources and seed grades. Significant differences were found in the germination percentage due to the influence of seed sources, size grades of seeds and their interactions. Germination percentage of seeds of dry deciduous and riparian forest was on par and were significantly superior to that of moist deciduous forest by 2.38 times. Germination percentage of larger seed grades was significantly superior to the germination percentage of the lower size seed grades with the subsequent stepwise reductions. The main effect of seed source only had significant influence on the speed of germination. The speed of germination of dry deciduous and riparian forests were at par and were significantly superior to that of moist deciduous forests. Seeds of dry deciduous source have given the highest germination energy (49 percent) and the germination energy of largest size grade seeds was 2.3 times more compared to that of smallest size grade seeds with a declining trend except for the moist deciduous source. Compared to the smallest size grade, the average increase in the MDG of the largest size grade was 116 per cent. Again, dry deciduous and riparian seed sources indicated their superiority over the moist seed source, with an increase of 107 per cent. The peak values of germination for the dry deciduous source and the riparian source were on par and were 108 per cent more when compared to that of moist deciduous forest. When compared to the peak value of germination in the smallest size grade, the peak value of germination for the largest size grade was 121 per cent higher. As germination value is the product of mean daily germination and peak value of germination, it also showed the similar trend. In general, the germination studies revealed the inferiority (possessed lowest values) of the moist deciduous seed source. The dry deciduous and riparian sources were on par in the case of germination value and were 327 per cent higher when compared to the moist deciduous source. As in the case of MDG and PV, germination value was also higher for the largest size grade which was about 415 per cent more when compared to that of smallest size grade.

6.5 Seedling growth characteristics

On an overall basis, seedlings raised from the seeds of moist deciduous source had more height (18.1 cm) and the seedlings obtained from seeds of dry deciduous source had the least height (16.3 cm). The seedlings obtained from largest sized seeds (SG 1) had greater height (17.6 cm) and was in general, significantly superior to the height of the seedlings obtained from seeds of the rest of the grades which were on par among themselves. Contrary to the variations in seedling height, seedlings raised from the seeds of dry deciduous source had greatest collar diameter (3.6 mm) and the seedlings obtained from seeds of moist deciduous source had the least collar diameter (3.2 mm). In general, seedlings obtained from largest sized seeds (SG 1) had greatest collar diameter (3.5 mm) and were significantly superior to the seedlings obtained from seeds of the rest of the grades which were at par among themselves. Contrary to the variations in seedling height and collar diameter, seedlings raised from the seeds of the riparian source had greatest number of leaves (9.6) which were on par with the moist deciduous source and dry deciduous source. The seedlings obtained from smallest sized seeds (SG 4) had more number of leaves (9.9) and seedlings obtained from seeds of the rest of the grades were also similar and were on par. In the case of the seedling height, seedling collar diameter and number of leaves as affected by the seed sources and seed grades, no definite trend could be delineated.

6.6 Biomass accumulation and seedling vigour index

The variations on the data pertaining to the seedling stem dry weight, leaf dry weight and root dry weight as affected by the seed sources, seed size grades and interactions were not significant. The highest stem and leaf dry weights were given by the riparian source. Whereas highest root dry weight was given by the dry deciduous source. Both dry deciduous and riparian sources showed same value for seedling vigour index (1.0) whereas moist deciduous forests showed inferiority with the lowest average (0.4). In the case of size grades, the largest size grade showed its superiority in seedling vigour index with a value of 1.2 over the other size grades. The vigour index of largest seed size was 167 per cent more compared to that of smallest seed size.

6.7 Biochemical characteristics

The major alkaloids of *Strychnos nux-vomica* seeds were estimated by High Performance Liquid Chromatograph. The alkaloids tested were strychnine and brucine. The area swapped by the liquid chromatogram was converted to quantity in ppm and the actual quantity of the alkaloids from each sample was estimated. Both strychnine and brucine estimations were done in the three seed sources without considering the size grades of the seeds. The quantity of strychnine was found to be highest in the moist deciduous seed source (3683 ppm) and was followed by dry deciduous seed source (2788 ppm). The quantity of brucine was highest in the dry deciduous SS1 seed source (1691 ppm) which was on par with that of moist deciduous seed sources SS 2 (1408 ppm). The riparian source was the inferior source regarding the alkaloid content. The isozyme esterase gave the similar bands for the leaf extract of the seedlings for all the three seed sources, by Polyacrylamide Gel Electrophoresis and indicated the genetic similarity.

Conclusions

CONCLUSION

The present study on "Evaluation of seed source variation in *Strychnos nux-vomica* L. (Kanjiram) from different forest types of Kerala" was successful in identifying the best natural seed source. The fruits were collected from three seed sources representing three forest types viz., dry deciduous forests of Chinnar wildlife sanctuary, moist deciduous forests of Pattikkad forest range and riparian forests fringing Peechi reservoir. The present study showed significant differences in the fruit traits, seed traits, germination attributes, seedling attributes and alkaloid content among the three seed sources. Largest sized fruits were obtained from the moist deciduous forests and smallest were from the dry deciduous forests. A positive correlation could be observed with the seed size to the germination aspects and the seedling growth characteristics in *S. nux-vomica* and the larger seeds showed their superiority over the small ones. Despite the higher values obtained for the fruit parameters (Fruit weight, fruit volume and number of seeds per fruit) for the moist deciduous forest source, dry deciduous and riparian seed sources were superior, especially with respect to the germination aspects and biochemical characteristics. The procedure for the estimation of isozyme, esterase and peroxidase were standardized and their presence in *S. nux-vomica* seedling leaves was confirmed by this study. The clear bands obtained for the esterase confirmed that the seed sources do not vary genetically and the variations found in the above characteristics under study were due to the differences in site characteristics. The study suggested that the seed source from the dry deciduous forest of Chinnar wildlife sanctuary was the best seed source of *S. nux-vomica.*

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APENDIX I

Abstracts of the ANOVA tables for the fruit parameters of *Strychnos nux-vomica* as affected by the seed sources

Parameters	Df	Mean square
Fruit weight	2	25741.599
Fruit volume	2	13137.811
Number of seeds per fruit	2	54.164

APPENDIX II

Abstracts of the ANOVA tables for the seed parameters of *Strychnos nux-vomica* as affected by the seed sources and seed grades

Source	df	Mean squares					
		Seed length	Seed breadth	Seed size	Seed thickness	Seed weight	Seed density
Seed source	2	6.091	19.638	6.477	7.801	2.577	0.160
Grade	3	952.886	888.177	921.095	2.733	33.411	2.489
Seed source*Grade	6	1.072	4.488	1.863	4.160	0.792	0.119
Error	264	1.169	0.947	0.739	0.308	0.134	0.007

APPENDIX III

Abstracts of the ANOVA tables for the germination attributes of *Strychnos nux-vomica* as affected by the seed sources and seed grades

ource	df	Mean squares					
		Germination percentage	Germination energy	Speed of germination	Mean daily germination	Peak value of germination	Germination value
eed source	2	0.543	0.926	0.070	2.375	3.550	6.268
rade	3	0.299	0.620	0.040	1.240	2.097	5.224
ource*Grade	6	0.026	0.075	0.006	0.096	0.182	0.996
rror	60	0.007	0.017	0.001	0.025	0.046	0.151

APPENDIX IV

Abstracts of the ANOVA tables for the seedling characteristics of *Strychnos nux-vomica* as affected by the seed sources and seed grades

Source	df	Mean squares		
		Seedling height	Seedling collar diameter	Number of leaves per seedling
Seed source	2	1050.772	63.406	1.440
Time	5	1311.307	96.013	2821.631
Seed grade	3	112.056	1.718	19.446
Source * Time	10	5.274	0.565	2.675
Source * Grade	6	136.082	4.612	21.578
Time * Grade	15	1.908	0.049	2.051
Source * Time * Grade	30	1.778	0.190	0.937
Error	2964	19.650	0.579	9.153

APPENDIX V

Abstracts of the ANOVA tables for the seedling biomass of *Strychnos nux-vomica* as affected by the seed sources and seed grades

Source	df	Mean squares			
		Stem dry weight	Leaf dry weight	Root dry weight	Seedling vigour index
Seed source	2	0.069	0.608	0.514	3.563
Grade	3	0.038	0.017	0.166	2.089
Seed source*Grade	6	0.032	0.008	0.130	0.139
Error	60	0.036	0.011	0.485	0.111

EVALUATION OF SEED SOURCE VARIATION IN *Strychnos nux-vomica* L. (KANJIRAM) FROM DIFFERENT FOREST TYPES OF KERALA

by

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ABSTRACT OF THE THESIS

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ABSTRACT

A detailed investigation was conducted to evaluate the seed source variation in *Strychnos nux-vomica* L. (Kanjiram) from different forest types of Kerala through the estimation of variation in fruit traits, seed traits, germination attributes, seedling attributes, biomass estimation, alkaloid content and isozyme analysis. Identification of seed source variation of the species will be a valuable tool for its use in tree improvement programmes and by the cultivation of the tree on a commercial scale, pressure on natural stands can be reduced. The seed sources selected for the study were the dry deciduous forests of Chinnar Wildlife Sanctuary, moist deciduous forests of Pattikkad Forest Range and the riparian forests of Peechi Dam Site. The fruit parameters were tested with completely randomized design with unequal replications. The fruit traits viz. weight, volume and number of seeds per fruit, varied significantly ($p<0.05$) among the three seed sources and the highest values for these parameters were observed for the moist deciduous source and lowest were observed for the fruits of dry deciduous forests. The fruit weight and fruit volume were significantly correlated ($p<0.01$) to the number seeds per fruit. After the extraction, the seeds were categorized into four grades based on the seed size (average of the longest and its orthogonal diameter for each seed) viz., 27-30 mm, 24-27 mm, 21-24 mm and 18-21 mm. The subsequent tests on seed traits, germination attributes, seedling biomass and vigour index were done in factorial CRD and the seedling growth characteristics were tested in factorial RBD. The seeds from the dry deciduous source possessed the lowest length, width, size and weight. Seed thickness (5.3 mm) and seed density (0.53) were highest for the dry deciduous source and riparian source respectively. The regression equations were fitted for the density and weight by taking the seed size as the independent variable and were significant ($p<0.05$). The large sized seeds were significantly superior to the smaller ones with respect to the seed traits.

The germination percentage and germination energy were similar for the dry deciduous (39%; 49%) and the riparian (38%; 44%) seed sources and were the lowest for the moist deciduous seed source (16%; 26%). Speed of germination (0.17), mean daily germination (0.54), peak value of germination (0.55) and germination value (0.34) for the dry deciduous source did not vary significantly ($p<0.05$) with that of riparian source; the moist deciduous source showed the lowest values. Germination attributes of larger seed grades were significantly superior compared to that of smaller seeds. From the final growth measurements it could be delineated that the seedling height (18.1 cm) was highest for the moist deciduous source and seedling collar diameter (3.6 mm) was highest for the dry deciduous source; but the riparian source produced the highest number of leaves per seedling. The seedlings from the larger seeds showed the greater growth. Seedling biomass production did not vary significantly among the seed sources and grades. Seedling vigour indices for the dry deciduous and riparian sources were significantly ($p<0.05$) higher to that of moist deciduous source. Highest strychnine content (3683 ppm) was obtained from the moist deciduous source and the highest brucine content (1692 ppm) was obtained from dry deciduous source. The riparian source possessed significantly ($p<0.05$) lowest alkaloid content. The presence of esterase was conformed in the seedling leaf extracts of all the three seed sources without any variations. Studies on different characteristics on the fruits, seeds and seedlings of *Strychnos nux-vomica*, revealed that the best performed seed source was the dry deciduous forest source and the poorest was the moist deciduous forest source. The results of this study will be useful in *Strychnos nux-vomica* for increasing the germination, seedling growth and to get higher quantities of alkaloids through breeding programmes.