CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Invasion by exotic species is one of the major causes for the loss of biodiversity (Richardson *et al*. 2000). However, introduced species by humans do not cause any damage to the native species or ecosystems. Some of the exotics can reproduce in the new regions after being introduced and may turn into a problem, competing with the native species and changing the structure and composition of local ecosystem (Coronado, *et al*. 2007). Plants used for economic (timber, forage, pulp production, etc.) or ornamental purposes, can spread as invasive plants beyond their cultivation area and become environmental pests (Reichard and White 2001; Zipperer, 2002).

Neem (*Azadirachta indica* A. Juss) is known to be an important source of triterpenoids (Mary Ndung'u, *et al*. 2004; ELteraifi, 2011) and multipurpose tree species native to the dry forest zones of the Asian subcontinent including india, Sri Lanca, Pakstan, Bangladesh, Malaysia, Myanmar and Thailand. The tree is found both cultivated and growing in its natural habitat in this zones and it has been transferred to many of the warmer parts of the world. It is a versatile tree with immense potential to protect the environment and developing sustainable agriculture in tropical Asia. Therefore, an urgent need to popularize its cultivation on marginal, degraded and wastelands through social forestry by involving rural people and also on fertile lands without affecting cropping i.e. through agroforestry. A part from its value as a timber tree, neem has antiseptic, medicinal, insecticidal properties and socio-cultural values for a long time and provides almost all the requirements of rural areas by the timber, fuel wood, fodder, oil, fertilizers and pest repellent. It is one of the most suitable and valuable tree species found in India. It can grow on wide range of soils up to pH 10 which makes it one of the most versatile and important trees in the Indian sub-content. Neem trees are often found growing scattered in the farmer's fields and on the boundaries of field without much effect on the crops due to its deep tap root system. (Kundu and Tigerstedt, 1997; Sacande, 2000; Scande, *et al*. 2001).

Azadirachta indica A. Juss (Meliaceae) is a fast-growing, small to medium-sized, ever-green tree, (5 to 20 m high), which sheds most of its leaves in the dry season and then blooms in full foliage. Two thirds of the height may be reached after 3 to 5 years. The first fruits can be harvested after 5 years and the first timber crop after 5 to 7 years (Kundu, 2000; Sacande, 2000). The tree has adaptability to a wide range of climatic and topographic conditions. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan. Neem tree requires little water and plenty of sunlight (Anonymous, 2006; Ogbuewu, *et al*. 2010).

In recent years, attention has been to breeding and improvement of agroforestry and multipurpose species, and especially tree species within the arid zone. Among those species, there has been special interest for neem (*Azadirachta indica* A. Juss). Neem can be utilized for a number of purposes such as production of timber and fuel wood, provision of shade, and for a number of medicinal purposes, as well as a biological insecticide. Oils and especially azarachterin can be extracted from leaves, fruits and other plant tissues (Khan *et al*., 2004). The products are used both locally, in small scales, by farmers and for household but also in large scale in industrial production (Khan *et al*., 2004). Neem seed is classified as intermediate between orthodox and recalcitrant (Scande, *et al*. 2001).

The neem trees are very well recognized worldwide. It is a native of Burma and India. It was first introduced in Sudan from India in 1916 at Shambat. It is now grown all over the country as a shade and avenue tree in villages and towns, in hospitals, schools, government houses, offices and railways stations (Elteraifi *et al*., 2001).

The neem trees have been grown successfully in all parts of Sudan. Neem has become naturalized species in various parts of the Sudan in quite diverse ecological and climatic conditions. Although the tree has been introduced to Sudan for several decades from its natural habitat in the Indian sub content but its use was mainly as a shade, ornamental avenue tree. Despite it is well recognized as the main ingredient in different industrial and pharmaceutical uses in India and other countries but so far in the Sudan its use has been restricted to the well know traditional areas mentioned above (Elteraifi *et al*., 2001).

In Sudan large scale neem plantations have not been tried and it is used for shade and amenity purposes. However, neem plantation was tried in Eltibon west Kordofan and in Debaibat South of El-Obied but the plantations failed and the trees died after the third year. The reason seems to be the water stress. The rains in both regions are between 300-400 mm/year and the water table is far below soil surface (Mahgoub, 2001). The number of neem trees in Sudan is estimated to be more than one million (Badi *et al*., 1989; Förster and Moser, 2000; El Shafie and Almahy 2012).

Seed size is known to affect various aspects of plant life (Milberg and Lamont, 1997); it influences the dispersal, seed water relations, emergence, establishment, survival and growth of seedlings (Wunderle, 1997).

Seed storage is very important to secure good quality seeds for planting programs whenever needed. Seed longevity depends on genetic and physiological factors as well as storage conditions (Govender, *et*. *al*., 2009). The most important factors that influence storage are temperature, moisture, seed characteristics, micro-organism geographical location and storage structure (Govender, *et*. *al*., 2009). It is necessary to improve methods that increase potential seed longevity in storage (Govender, *et*. *al*., 2009).

Seed viability can be extended by cold and dry storage at seed moisture content below 20% (Huang *et al*., 2003). Dormancy plays a major role in regulating germination in dry forest species. Dry environment is evidently characterized by the large variation but unpredictable seasonal changes in moisture and temperatures (Teketay, *et al*., 1997).

Variation in seed and seedling traits and its significance for seed source studies have been studied in a number of tree species. Various ecotypes of *Azadirachta indica* exhibit variation in several characters (Arora, 1993).

The synonyms of *Azadirachta indica* (*Antelaea azadirachta* (L.) Adelb, *Azadirachta fraxinifolia Moench, Melia azadirachta, M. fraxinifolia Adelb.,Melia indica (*A. juss*.),* Brandis, *M. pinnata* Stokes) (Schmidt, 2000).

The vernacular/common name (neem, neem tree, Indian lilac, white cedar (Eng.); margosa tree (Port.); nim (Urdu); indischer zedrac (Ger.)' azad-darakht-ihindi (Persian); tamaka, bowtamaka, tama, (Burmese); sadao india, sadao thai (Thai.))

(Kundu, 2000).

The neem tree is very similar in appearance to the China berry, all parts of which are extremely poisonous (Ganguli, 2002 and Siddig, 2009).

2.2 Ecology

The neem tree is noted for its drought resistance. Normally it thrives in areas with sub-humid conditions, with an annual rainfall between 400 and 1200 mm. it can grow in regions with an annual rainfall below 400 mm, but in such cases it depends largely on the ground water. Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils. It is a typical tropical/ subtropical tree and exists at annual mean temperatures between 21-32^0 C. It can tolerate temperatures below 4^0 C. In Indian, neem grows in the plains and in areas that reach an elevation of approximately 1850 m. In its introduced range, neem is cultivated from sea level to 1500 m elevation. Neem is tolerant to most soil types including dry, stony, shallow soils, lateritic crusts and highly leached sands and clay (Schmutterer. 1995).

It is well adapted to soil with pH of 5 to 8.5. But it grows best up to 8 m on deep and porous well-drained soils with pH of 6 to 6.5. It is moderately tolerant to high alkaline soils with high levels of sodium, carbonates and bicarbonates. It has been successfully established on deep highly eroded sites as well as on degraded soils with calcareous hardpans close to the surface. Neem growth is poor on seasonally waterlogged sites, salty sands, salty alluvial flats and poorly drained clays, as well as dry sands where the dry season water table is below 8 m in depth. On soils deficient in Zinc and Potassium, the growth of neem appears to be closely related to soil moisture availability. Growth is best on freely drained sites where the water table fluctuates between an approximately 3 to 5 m throughout the year (Schmutterer. 1995). Neem is a useful species for improving soil fertility on degraded dry sites due to the quality of its leaf litter and relatively rapid rate of leaf decomposition. Therefore, on fallow loam ferric acrisols in Togo, the top soil pH and calcium concentration under 5 years old neem stands increased at more rapid rate than those in adjacent stands of Acacia (Schmutterer. 1995). The seeds of neem do not live long and are usually within three months. To help the seed live longer the fruit pulp should be removed by hand and the seeds dried in the shade to level of 15 to 20 % moisture content. If the seeds have been properly air dried they should survive for up to twelve months in a refrigerator at 4^0 C (Schmutterer. 1995).

In India, neem is present in mixed forest with *Acacia* spp. and *Dalbergia sissoo*; in Indonesia, it is naturalized in lowland monsoon forest. In Africa, it is found in evergreen forest and in dry deciduous forest. Adult *A. indica* tolerates some frost; but seedlings are more sensitive. It quickly dies in waterlogged soils. *A. indica* requires large amounts of light, but it tolerates fairly heavy shade during the 1st few years (Orwa, *et al.,* 2009).

2.3 Propagation

Seed propagation is the usual method of regenerating neem. Seeds normally do not store well over 6 months. Seeds should be cleaned with water to remove the casing. No pre-treatment is necessary, commonly planted in nursery seedbeds for 8-11 months. Neem seedlings, once established, do not need frequent watering or fertilizing. Cutting is also possible. It can also regenerate by root suckers. Bird and fruit bats disperse the seeds. In India, the parrots eat the ripe neem fruits and pass the seeds through the digestive system. The bird drops disseminate the seeds; the seed germinate wherever the parrots drop them (Siddig, 2009). Micro propagation by micro-cuttings is carried out on more than twenty species including neem, *Azadirachta indica*, in Bangladesh (Sacande, 2000).

Neem seed lings can be produced vegetatively by air layering, cuttings, grafting and tissue culture; however, they are usually grown from seed in nurseries as bare-root stock or in polybags. Direct sowing is comparatively cost-effective, but may result in poor survival in drier zones. Although neem is a prolific seed producer, yet seed supply is frequently a problem. The viability of fresh seed decreases rapidly after two weeks and improperly stored seeds have low germination rates. Ripe seeds should be collected from the tree and processed immediately. First, the pulp is removed and the seeds are washed clean. Seeds are air dried for 3-7 days in the shade, or until the moisture content is about

30 %. They can then be stored for up to four months if kept at 15^0 C. seed will remain viable even longer if dried to 6-7 % moisture content and refrigerated in sealed containers at 4^0 C (Kureel, *et al,* 2009).

2.4 Botanical description

Medium sized tree, up to 15 m tall, rarely 25 m, with short, straight bole and long spreading branches, forming a dense, large, oval or rounded crown. Evergreen, or under extreme heat and drought, deciduous. Old bark turning dark grey, thick and furrowed. Leaves imparipinately compound with 7-17 pairs of leaflets, which are ovate or lanceolate, falcate with uneven base and dentate margins, 6-8 cm long, 1-3 cm wide. Inflorescence a 10-30 cm long panicle with many, small white to cream colored flowers. Neem is sometimes confused with the chinaberry, *Melia azedarach* L., but they are easily distinguished by the leaves. *Azadirachta spp.* has simple pinnate leaves, while those of *Melia spp.* are 2- to 3-pinnate (Schmidt, 2000).

Fruit ellipsoid drupe 1.2-2 cm long, green/yellow when ripe, with a thin hard cuticle and juicy fruit pulp. The seed pyrene contains one, rarely two, seeds weight varies with location and seed sources. Available information indicates from 1700 seeds per kg in the Sahel to 3500-9000 seeds per kg in India (Jensen *et al.*, 2004).

Flowers hermaphroditic or separate female and male. Pollination by insects. The tree starts flowering and fruiting at about 5 years of age. Flowering generally occurs in the dry season and fruit ripening during the early part of the rainy season. Season and duration of reproductive phenoperiods vary according to location and climate. In bi-modal climates there are sometimes two flowering and fruiting season (Jensen *et al*., 2004).

2.4.2 Distribution

Neem tree is native to the dry forest zones of the Asian subcontinent. The exact region of *Azadirachta indica* is not known. Some authors suggested that it may lie in Myanmar (Burma) and/or parts of southern India (Schumutterer, 1995; Chamberlain et al., 2000), while others consider large parts of southeastern and southern Asia from Indonesia to Iran as the origin, including of India, Pakistan, Sri Lanka, Malaya, Indonesia, Thailand and Burma (Shumutterer, 1995; Chamberlain et al., 2000). It has become widely distributed by introduction into dry, arid and semi-arid tropical and subtropical zones. In Africa different routes of spread have been documented between 1919 and 1927 and mainly from India (Anonymous, 2006). It has been grown well in plantations in the Sudan and Sahelian zones of Africa as well as in Sierra Leone, Malawi, Zimbabwe, Tanzania, Zanzibar and the non-Sahelian areas of Nigeria and Ghana. In Uganda it is also found around the Lake Victoria (ICRAF, 1995).Today neem is found throughout western, central and eastern Africa (Shumutterer, 1995 Chamberlain *et al*., 2000). One of the biggest plantations of neem is in the Arafat plain near Mecca in Saudi Arabia where it provides shade for pilgrims (Kundu *et al.,* 1997). It has recently been introduced in most of the warmer parts of the world including South and Central America and Australia (Shumutterer, 1995; Chamberlain, *et al,* 2000). The tree is now under cultivation in USA particularly Arizona, California, Florida and Oklahoma; and in Haiti, Colombia, Brazil, Honduras, Ecuador, the Dominican Republic and Argentina (Jacobson, 1986). It is one of the fastest spreading trees and has become pan-tropical.

Indian immigrants introduced neem to Mauritius and may also have taken it to continental Africa. It is now widely cultivated in Mauritania, Senegal, the Gambia, Guinea, Ivory Coast, Ghana, Burkina Faso, Mali, Benin. Niger, Nigeria, Togo, Cameroon, Chad, Ethiopia, Sudan, Somalia, Kenya, Tanzania and Mozambique.

2.5 Silviculture of neem

The simplest meaning of silviculture is the art and sciences of cultivating forest crop. It is the branch of forestry that deals with the establishment, development, care and reproduction of stand of timber. Neem can be artificially regenerated through direct sowing of seeds, and vegetative means i.e. rising through branch/stem cuttings. Planting of a container-grown seedling will be more successful for agroforestry, social forestry, afforestation and avenue planting and also for establishing plantations. However, if soil and environment are conducive, direct seedling may be cost-effective (Singh, 2002).

Azadirachta indica A.Juss trees may start flowering and fruiting at the age of 4-5 years, but economic quantities of seeds are produced only after 10-12 years. Pollination is by insects such as honeybees. Isolated trees may not set fruits, suggesting the occurrence of self-incompatibility. The flowering and fruiting season largely depend on location and habitat. In Sudan the peak of the flowering is in March to May, with fruiting in May to July (Hamza, 1990).

Fruits ripen in about 12 weeks and are eaten by bats and birds, which distribute the seeds. Yields of fresh fruits per tree range between 37 and 50 kg/year (Koul *et al.,* 1990). Neem seeds have a short viability in natural conditions (Anonymous, 2006); it is generally lost within 3 to 4 months after harvest and only 3-4 weeks in the dry areas (Sacande, 2000). Storage behavior of neem seeds is controversial; it has variously been described as recalcitrant, intermediate and orthodox seed (Elteraifi *et al*., 2001; Tompsett, 1994; Roederer and Bellefontaine, 1989; Maithani *et al*., 1989; Ezumah, 1986). In Sudan Elteraifi (1996) recommended short time storage of shade dried seeds in a ventilated container (cotton bags) at room temperature with high relative humidity.

A neem plantation can be raised by direct sowing seeds or by planting seedlings. Direct sowing of fresh seeds in the shelter of existing vegetation has also proved successful in Sudan (Elteraifi, 1996).

Mature fresh seeds germinate within 2-3 weeks with a germination percentage of 75-90 %. Neem can also be propagated vegetativly by air layering, root and shoot cuttings, grafting and cutting. Clonal propagation and micro propagation by somatic embryogenesis and organogenesis are also used (Allan, *et al*., 1999). Neem seedlings are usually propagated in a nursery and transplanted to the field although direct sowing has been successful under condition of adequate rainfall. Seedlings should be hardened off before being transplanted into the field. Newly transplanted seedlings may benefit from shading. A planting spacing of 1.8 x 1.8 m is recommended (De Jussieu, 1963). Planting density can be 100-300/ ha, but it is commonly 150/ha. Seedlings respond well to chemical and organic fertilizers, although they may not need fertilizers except on very infertile soils. Neem coppices well and produces root suckers, especially in dry localities. Early growth from coppice is faster than growth from seedlings. It withstands pollarding well, but seed production is adversely affected when trees are lopped for fodder. The practice of de-branching is very common in Sudan before the rainy season which affects seed production (Allan, *et al*.. 1999).

Weeding in neem plantations in dry areas is essential, as the tree cannot withstand competition, especially from grasses. The tree weeds and frost sensitive, especially in the seedling stage. It is not tolerant of extended flooding or fire (Allan, *et al*.. 1999).

Cuttings made in immature stands in order to stimulate the growth of the trees that remain and to increase the total yield of useful material from the stand are termed thinning. Surplus trees are removed for the purpose of concentrating the potential wood production of the stand on a limited number of selected trees. The total yield of the stand is augmented largely by the utilization of trees that would ultimately die of suppression; however, the value and utility of the final crop may be increased by virtue of the fact that the trees grow more rapidly than they would without thinning. The fundamental objectives of thinning are, in other words, to redistribute the growth potential of the stand to optimum advantage and to utilize all the merchantable material produced by the stand during the rotation (Smith and Whitman, 1992).

The general principles of thinning have been formulated for application in even –aged aggregations of trees. Although it is easiest to think in terms of even-aged stands, these principles also apply to the even-aged groups that make up the immature components of uneven-aged stands (Smith and Whitman, 1992).

2.6 Tree seed biology

2.6.1 Seed dormancy

In seeds of many tree species, maturation is unaccompanied by the induction of a state of dormancy. This is an advantage for seeds that mature in late summer to autumn early fall, since immediate germination would leave vulnerable seedlings exposed to harsh winter conditions. In nature, dormant seeds remain inactive until favorable growing conditions occur the following spring. Some may remain dormant for two growing seasons or more. Seeds can maintain viability for many years in a dormant state (Saad and Rao, 2001).

Seeds are released from dormancy through changes that occur during their exposure to cold, wet conditions over winter, and they usually germinate as temperatures rise in the following spring. Sometimes seeds do not germinate because water and gases cannot permeate the seed coats. In nature, such seed coat dormancy may be removed by chemical action in the soil solution, which breaks down resistant coats or leaches chemical inhibitors from the seeds. Dormancy may also be broken by seeds passing through the guts of birds or other animals (Saad and Rao, 2001).

2.6.2 Germination

Germination is the reactivation of physiological processes in the seed that result in the development of an embryo into independent seedlings. The three phases of germination are hydration, activation of growth processes and emergence of the embryo (Leadem, 1996). Dormancy plays a major role in regulating germination in dry forest species. Dry environment is evidently characterized by the large variation but unpredictable seasonal changes in moisture an temperatures (Teketay, 1997).

2.6.3 Hydration

When the moisture content of a mature seed falls below 10 %, it can survive extended storage periods. With this degree of dehydration, however, metabolic activity is virtually non-existent. Seeds must be rehydrated before germination can proceed (Saad and Rao, 2001).

Dry seed take up water rapidly, but early hydration is essentially a passive process. Thus dead and live seeds cannot be distinguished from one another on the basis of their initial water uptake; physical properties of the seed coat, such as wiriness, hairness and thickness, appear to be more important factors governing the entry of water into the seed (Saad and Rao, 2001).

Seed membranes are not fully operational during the early phase of hydration and substances are easily leached from the seeds. Within a few minutes to several hours, however, membranes resume full function. At this time, water is taken actively into the seed and respiration and other physiological processes increase to characteristic metabolic levels (Saad and Rao, 2001).

2.6.4 Environmental factors

Moisture, oxygen and favorable temperature are all essential for germination. Water is needed to activate physiological membranes; oxgen is required for respiration that fuels the germination process (Saad and Rao, 2001).

The environmental cues affecting germination operate in various and interrelated ways. Satisfaction of a single requirement is generally not sufficient to trigger germination (Leadem, 1996).

Temperature is one of the most important factors affecting seeds. Water uptake, gas diffusion, respiration and other metabolic processes all proceed faster at higher temperatures. Germination is dependent on all these processes and thus is strongly affected by temperature (Saad and Rao, 2001).

2.7 Seed collection and storage

Tree seeds are stored as important genetic resources for species biodiversity, ecosystem restoration, conservation and domestication (Berjak and Pammenter, 2004). The fruits are best collected from the tree since fallen fruits tend to have low viability. The optimum period for collection is when the colour of the fruit turns from green to yellowish-green. The easiest way of collection is to spread a tarpaulin under the trees and collect the fruits after they have been manually stripped of the branches or shed by shaking or beating the branches. At about 10-12 years of age a medium-sized tree may yield 35-55 kg of fresh fruit per year or some 25 kg of dry depulped fruit (Schmidt, 2000).

The fruit pulp must be removed before storage, either by hand or in a depulper and immediately to avoid fermentation. Green fruits are allowed to after-ripen in the shade 2-3 days before depulping. Depulping of green fruits is possible and does not affect viability but is more labour intensive (Schmidt, 2000).

Seed storage is the preservation of viable seed until their sowing/requirement. It is essential to offset the uncertainty of seed production/availability during bad seed years. It delays deterioration, maintains viability and protects seed from rodent and insect damage. The longevity of seeds is a species-specific characteristic (Bisht and Ahlawat, 1999). The seed of most of the species can be stored at low temperature and low moisture content in sealed containers. The seeds of many plant species are easy to handle and store for long periods of time, whilst others are very difficult and need special treatment to maintain viability. Easy maintenance of viability over extended periods of time depends on the seeds ability to withstand desiccation (Sacande, 2000). Seed viability can be extended by cold or dry storage at seed moisture content below 5 % (Huang *et al.*, 2003).

However, many species of moist tropical forests are so thoroughly adapted for germination that their seeds are almost impossible to store or even to transport. On the basis of storage behavior seeds can be divided into the following broad categories:

2.7.1 Orthodox seeds

These are seeds, which can withstand drying down to low moisture content of around 5% to 10% and successfully stored at low or subfreezing temperature for long periods. For example, *Acacia, Anthocephalus, Betula, Duabanga, Eucalyptus, Fraxinus, Pinus* and *Picea* (Bisht and Ahlawat, 1999).

2.7.2 Sub-orthodox seeds

Seeds of Abies, Juglans, Salix and Poplar loose viability within a few months in open air. These can be stored under same condition as true orthodox, but only for six months to a maximum of six years in some cases, loss of viability ranges from 0% to 34% when stored at -5 C^0 to -20 C^0 and moisture content between 5 to 10% (Bisht and Ahlawat, 1999).

2.7.3 Temperate-Recalcitrant seeds

Seeds are desiccation-sensitive and be dried to 35 to 50 % moisture content of fresh weight. Storage temperature varies from 3^0 C to -3^0 C e.g. Acer, Aesculus and Quercus (Bisht and Ahlawat, 1999).

2.7.4 Recalcitrant seeds

Seeds which cannot tolerate drying below a relatively high moisture content (often in the range of 20% to 50% net basis) and which cannot be stored successfully for long periods, e.g. Hollong, Mekai and other dipterocarps, cane, champ, neem, rubber and members of family lauraceae. Their seeds are sensitive to low temperature chilling damage and death may occur if stored in low temperature. These are most difficult group to store even for short period (Bisht and Ahlawat, 1999).

2.8 Seed morphometric characteristics

The developmental stage of seed at harvest plays an important role in postharvest storage longevity. Furthermore, this longevity depends on plant species and external factors such as moisture content temperature and the composition of the gaseous atmosphere during storage. Seed eventually deteriorate, even under optimal storage condition (Sacande, 2000).

Marked genetic variations may exist among the metric measurements (length, width, thickness and their ratios) on the seeds (Sacande, 2000).

The various metric measurements on seeds are very important quantitative variables for determining size and shape of seeds (Wyllie-echeverria *et al*., 2003).

Seed size and shape are important determinations of seed dispersal and probable loss, moisture imbibition and germination of seeds and grain grading quality (Cerda and Garcia-Fayos, 2002).

2.9 Seed testing

Seed testing is used for control of quality parameters during seed handling and test results are submitted to customers as documentation on seed quality. Standard parameters such as seed weight, purity and germination or viability enter as factors in the calculation of seed demand. Moisture content in seeds is particularly important during storage (Schmidt, 2000).

Seed testing is essential to assess the physical and biological aspects of seed. Seed tests are commonly done immediately after extraction and shortly before actual sowing. It is also done periodically on seed lots kept in long storage. For small nurseries, common sense, clean hands, is enough for testing tasks. Some of the common terms and methods have been described below:

2.9.1 Seed lot

A seed lot is defined as a specified quantity of seeds of reasonably uniform quality from a particular geographic source (Bisht and Ahlawat, 1999).

2.9.2 Purity test

It determines what proportion of the seed sample by weight has pure seed and what proportion is other material. The four recognized components of a seed lot are pure seeds other damaged seeds and inert matter such as seed wings, twigs, stone soil and other non-seed materials. The separation is done manually by placing seeds on a working table. (Bisht and Ahlawat, 1999).

2.9.3 Seed weight

It is normally expressed for 1000 pure and full seeds. Factors affecting seed weight are size, moisture content and proportion of full seeds in the lot. It is generally calculated by taking 10 random samples of 100 seeds from a pure lot. If the difference between two replicates exceeds 10% of the mean weight, additional replicates should be drawn (Bisht and Ahlawat, 1999; Schmidt, 2000).

`2.9.4 Seed moisture content

Knowledge of seed moisture content is essential to determine the viability and storage conditions. Seeds of high content cannot be stored and over drying can make them non-viable. It can be determined by drying of 10 g sample in oven at 103^0 C for 17 hours (or at 130^0C for 1 to 4 hours) (Bisht and Ahlawat, 1999; Schmidt, 2000).

2.9.5 Germination test

The most reliable test for seed viability is to germinate a representative sample (four replicates of 100 seeds each) under laboratory conditions. Under field conditions cutting the seeds into two equal parts can test viability. Seeds having full growing, firm and undamaged embryo can be presumed to be good.

However, this is not a reliable test for stored seeds because loss of viability in storage may not produce immediate visible changes (Bisht and Ahlawat, 1999; Schmidt, 2000).

2.9.6 Laboratory germination counts (LGC)

One hundred seed are placed on moist blotting paper or cotton-wool in a petridish after giving the necessary pre-treatment in case of very small seeds, e.g. khokan and kadam, one-gram seed is taken. The petridishes are placed in a warm (not hot) place and kept moist regularly. The number of seeds, which germinate, is counted every day and after four weeks or more. It is expressed as the numbers of seeds germinate per kilogram (Bisht and Ahlawat, 1999; Schmidt, 2000).

2.10 Variation in seed characteristics

Fruit and seed characteristics like weight, length, width, diameter, azadirachtin and oil contents are highly variable both within and between provenances of neem (Veerendra, 1995; Kundu *et al.*, 1998). The weight, length, diameter of neem seeds from four populations (two from Bangladesh, one from Thailand and one from Kenya) were measured and analyzed (Kundu *et al.*, 1998). There were significant differences between populations for all seed parameters and the populations from Thailand and Kenya were differentiated both from one another and the material from Bangladesh. This study had similar results to that carried out by Kramer (1983) who also found significances between populations of neem from Tamil Nadu, India. The is also found seed length and seed oil content to be highly heritable and the weight of 100 seeds to be robust selection index by virtue of its high genotypic coefficient of variation, high genetic advance and moderate heritability.

The variation in fruit yield per tree, 100-fruit weight and 100-kernel weight and oil content in 13-year-old neem trees from Jodhpur, India was measured and analyzed (Jindal *et al.*, 1983). Fruit yield per tree was highly variable and positively correlated with tree height, collar diameter, dbh and canopy diameter. 100-fruit weight was positively correlated with 100-seed weight and 100-kernal weight but there were no correlations between kernel oil content and other fruit characteristics.

Variation in oil and protein content of neem seeds from Laos, Nepal, Ghana, Bangladesh, Myanmar and three areas of India was found to be highly significant (Rathore *et al.,* 1998). Neem seeds were collected from 12 different locations in Tamil Nadu and their azadirachtin and oil contents were determined (Sridharan *et al.*, 1998). Both seed characters were highly variable, but showed some correlation with climatic factors. There was a significant positive correlation between oil content and the number of sunshine hours from September to March and a negative correlation between azadirachtin content and the total number of rainy days during the fruiting season (April to August) (Kundu and Tigerstedt, 1997).

2.11 Variation in growth characteristics

Variation in reproductive output and quality may exist within and between individuals, families and provenances due to their differential life history, ability and behavior. The variation may occur in flowers and fruit production, seed characteristics and viability as well as growth of seedling in the nursery and after field planting (Kundu and Tigerstedt, 1997). Variation in the seedling growth characteristics of ten provenances of neem from Myanmar, Bangladesh, India, Pakistan and the Sudan were studied under growth chamber condition (Kundu and Tigerstedt, 1997).

Principal component and cluster analysis revealed three distinct groups of populations with provenances from the high rainfall regions being separated from those from the low rainfall areas. Shoot/root ratio and leaf number were proportional to mean annual rainfall and suggested neem employs an adaptive strategy in response to water deficit at the initial place of seedling growth. Significant provenance variation was also established for net photosynthesis, stomata conductance, stomata density and total guard cell length (Kundu and Tigerstedt, 1997).

Variation in plant height, collar diameter and survival rate of six neem provenances (three from Thailand, one from Myanmar, one from Nepal and one from Ghana) were measured at three test sites in Bangladesh and India they were classed as optimum, intermediate and stress environments for neem (Kundu *et al.*, 1998). There were significant differences between provenances for height and collar diameter and suggest that genotype x environment effect for height. The authors suggest that rainfall and temperature may be the environmental factors affecting variation in growth characteristics and note the importance of provenance testing for a given site.

The height, collar diameter, dbh, clear bole length and canopy diameter of 13-year-old trees grown at Cazri, Jodhpur, India were also assessed for variation in growth characteristics (Jindal *et al.*, 1983). All characters were found to be highly variable and tree height was positively and significantly correlated with collar diameter, dbh, canopy diameter and fruit yield per tree.

2.12 Drought and water stress

Drought is one of the major abiotic stress factors that affect all living organisms including human in terms of health and food. Water absence from the soil solutions affects the natural evaporative cycle between earth and atmosphere that contribute to the amount of rainfall. Drought occurs when soil moisture level and relative humidity in air is low while temperature is high. UN reports (2006) estimate that one third of world population has been living in areas where the water sources are poor. Water stress resulting from the withholding of water, also changes the physical environment for plant growth as well as crop physiology (Kramer, 1980). Almost every plant process is affected directly or indirectly by water supply (Akınc, 1997). Plants, as one of basic food sources, either in nature or cultivations, in their growing period, require water or at least moisture for germination. Certainly, most land plants are exposed to short or long term water stress at some times in their life cycle and have tended to develop some adaptive mechanisms for adapting to changing environmental conditions. Some plants may adapt to changing environment more easily than others giving them an advantage over competitors. Water stress may range from moderate, and of short duration, to extremely severe and prolonged summer drought that has strongly influenced evolution and plant life (Bottner *et al.*, 1995).

An adverse effect of increased frequency and intensity of drought on plant growth and productivity had been predicted in the semi-arid and arid areas due to climate warming change (Gallé *et al.*, 2007; IPCC, 2007; Elmagboul *et al.,* 2012).

On a global scale, primary productivity, plant species distribution and the diversity of plant communities are correlated with rainfall gradients and moisture availability (Lieth, 1975; Currie and Paquin, 1987; O'Brien, 1993). Even within the moist and wet tropics, species' distributions and diversity gradients strongly correlate with annual rainfall (Gentry, 1988; Condit, 1998; Bongers *et al.,* 1999) and soil moisture availability may be one of the main factors influencing habitat associations of tropical trees, shrubs and herbs (Whitmore 1984; Richards 1998; Sollins 1998; Webb and Peart 2000; Elmagboul *et al.,* 2012). Nevertheless, the responses of tropical rainforest plants to soil water availability and drought have received little attention, perhaps because they were assumed to experience little drought stress. In tropical forests, annual rainfall varies from about 800 mm to >10,000 mm (Walsh, 1998). In most of the tropics, dry season droughts occur regularly once or twice per year (Windsor 1990; Walsh and Newbery 1999) and, even in many a seasonal equatorial areas, dry periods of 15–35 days occur at least every other year (Becker 1992; Burslem *et al*., 1996; Walsh and Newberry 1999). Especially severe droughts in tropical regions occur in association with El Nino climatic events (Toma *et al*., 2000; Elmagboul *et al.,* 2012).

During the dry season, plants in tropical forests can be exposed to considerable drought stress. Wilting has been observed, and pre-dawn water potentials of down to 3.5 MPa have been measured (Becker and Wong 1993; Tobin *et al*., 1999). Drought has been associated with increased mortality and decreased growth rates in tropical plants (Turner, 1990; Fisher *et al*., 1991; Condit *et al*., 1995; Elmagboul *et al.,* 2012).

2.12.1 Water stress

Plants experience water stress either when the water supply to their roots becomes limiting or when the transpiration rate becomes intense. Water stress is primarily caused by the water deficit, i.e. drought or high soil salinity. In case of high soil salinity and also in other conditions like flooding and low soil temperature, water exists in soil solution but plants cannot uptake it – a situation commonly known as ‘physiological drought’. Drought occurs in many parts of the world every year, frequently experienced in the field grown plants under arid and semi-arid climates. Regions with adequate but non-uniform precipitation also experience water (Seyed *et al*., 2012; Elmagboul *et al.,* 2012).

Since the dawn of agriculture, mild to severe drought has been one of the major production limiting factors. Consequently, the ability of plants to withstand such stress is of immense economic importance. The general effects of drought on plant growth are fairly well known. However, the primary effect of water deficit at the biochemical and molecular levels are not considerably understood yet and such understanding is crucial. All plants have tolerance to water stress, but the extent varies from species to species. Knowledge of the biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water limited conditions in higher plants (Seyed *et al*., 2012; Elmagboul *et al.,* 2012).

2.12.2 Effects of water stress on plants

Drought, as an abiotic stress, is multi dimensional in nature, and it affects plants at various levels of their organization. In fact, under prolonged drought, many plants will dehydrate and die. Water stress in plants reduces the plant-cell’s water potential and turgor, which elevate the solutes’ concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases leading to growth inhibition and reproductive failure. This is followed by accumulation of Abssic acid (ABA) and compatible osmolytes like proline, which cause wilting. At this stage, overproduction of reactive oxygen species (ROS) and formation of radical scavenging compounds such as ascorbate and glutathione further aggravate the adverse influence. Drought not only affects plant water relations through the reduction of water content, turgor and total water, it also affects stomata closure, limits gaseous exchange, reduces transpiration and arrests carbon assimilation rates. Negative effects on mineral nutrition and metabolism leads to a decrease in the leaf area and alteration in assimilate partitioning among the organs. Alteration in plant cell wall elasticity and disruption of homeostasis and ion distribution in the cell has also been reported. Synthesis of new protein and mRNAs associated with the drought response is another outcome of water stress on plants. Under the water stress cell expansion slows down or ceases, and plant growth is retarded. However, water stress influences cell enlargement more than cell division. Plant growth under drought is influenced by altered photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones (Seyed *et al.*, 2012; Elmagboul *et al.,* 2012). In moist tropical forests, mecha-nisms of desiccation tolerance are of major importance for seedling survival through a dry season (Elmagboul *et al.,* 2012).

2.12.3 Morphological, anatomical and cytological changes

In the majority of the plant species, water stress is linked to changes in leaf anatomy and ultra structure. Shrinkage in the size of leaves, decrease in the number of stomata; thickening of leaf cell walls, cutinization of leaf surface, and underdevelopment of the conductive system. Increase in the number of large vessels, submersion of stomata in succulent plants and in xerophytes, formation of tube leaves in cereals and induction of early senescence are the other reported morphological changes (Elmagboul *et al.,* 2012).

The root-to-shoot ratio increases under water-stress conditions to facilitate water absorption and to maintain osmotic pressure, although the root dry weight and length decrease as reported in some plants like sugar beet and *Populus*. Higher root-to-shoot ratio under the drought conditions has been linked to the ABA content of roots and shoots. Water stress is linked to decrease in stem length in plants such as *Albizzia*, *Erythrina*, *Eucalyptus*. Decreased leaf growth, total leaf area and leaf-area plasticity were observed under the drought conditions in many plant species, such as peanut and Oryza *sativa*. Although water saving is the important outcome of lower leaf area, it causes reduced crop yield through reduction in photosynthesis. However, in some plants, higher yield was reported under-water deficit condition (Seyed *et al*., 2012; Elmagboul *et al.,* 2012).

2.12.4 Plant responses to water stress

Plants adapt themselves to drought conditions by various physiological, biochemicals, anatomical, and morphological changes, including transitions in gene expression. The physiology of plants' response to drought at the whole plant level is highly complex and involves deleterious and/or adaptive changes. This complexity is due to some factors such as plant species and variety, the dynamics, duration and intensity of soil water depletion, changes in water demand from the atmosphere, environmental conditions, as well as plant growth and the phenological state in which water deficit is developed (Seyed *et al*., 2012; Elmagboul *et al.,* 2012).

Plants' strategies to cope with drought normally involve a mixture of stress avoidance and tolerance strategies. Early responses of plants to drought stress usually help the plant to survive for some time. The acclimation of the plant to drought is indicated by the accumulation of certain new metabolites associated with the structural capabilities to improve plant functioning under drought stress. The main aspects of plant responses to water involve the maintenance of homeostasis (ionic balance and osmotic adjustment), counter action to resulted damages and their quick repair such as scavenging of ROS, decrease oxidative stress, the regulation and recovery of growth (Seyed *et al*., 2012; Elmagboul *et al.,* 2012).

2.12.5 Biotechnology and water stress

Various approaches have so far been tested to produce stress tolerant plants using classical genetic methods as well as improved plant breeding techniques. One approach to improve plant resistance and crop performance in water-limited environments is to select genotypes that have improved yield in dry environments. The approach is proven partially successful, but it is difficult to accomplish due to the variability of rainfall and the polygenic nature of drought tolerance. The strategy of gene transfer to crop plants from their more tolerant wild relatives using classical genetic methods has also been of limited success. A partial list of potentially important traits for plant breeding might include water-extraction efficiency, water-use efficiency, hydraulic conductance, osmotic and elastic adjustments, and modulation of leaf area (Seyed *et al*., 2012; Elmagboul *et al.,* 2012).

2.13 Provenance variation

Studies on natural variation for better utilization as the correct choice of seed sources may be critical to successful establishment and long term productivity (Boyle *et al*., 1997). To study the natural variation of a given species is important to determine the geographical differences and the variation that exist between sites, within provenance, stands and sites (Elfeel, 1996).

Several dry tropical species have a wide geographical distribution and have genetically adapted to different environmental conditions found within their historic range. Success in establishment and productivity of forest tree plantations is determined largely by species used and the seed sources (Lacaze, 1978). The interaction of the environmental factors with genetic systems leads to the developments of patterns of geographical variation, the environment factors and their regional variation which gives observed patterns of variation in forest trees (Morgensterm, 1996).

2.14 Seeds and seedling provenance variation

Analysis of juvenile characters is an important aspect of variation and selection (Jindal *et al.*, 1983). Conservation of genetic resources of the species depends on a wide range of technical and scientific efforts. The first step involves defining geographical zones of the species and determining the genetic variation among and between these zones. The second step involves assessing conservation status, which is the present stage of the genetic resources (Gradual *et al.*, 1997). The variation between and within populations can be assessed in seeds germination and seedling growth and morphological and metric characters in the field (Hamrick, 1994 and Frankhan *et al.*, 2002). Variation in percentage germination and rate of seedling growth among provenance have been observed in several Indian dry tropical species such as *prosopis cineraria* (Arya *et al*., 1995), *Acacia nilotica* (Krishnan and Toky, 1995), *Alebiziz lebbeck* (Roy, 1985) and *Azadirachta indica* (Kundu and Tigerstedt, 1997).

Variation in seed and seedling traits and its significance for seed source studies have been studied in a number of tree species. Various ecotypes of *Azadirachta indica* exhibit variation in several characters (Arora, 1993). Significant provenance variation in height growth and survival rates of neem was reported among 39 seed sources from India by (Rajawat *et al*., 1994). Geographical variation in seed size, oil content and tree growth related characters has also been reported (Veerendra, 1995; Surendran *et al*., 1993; Dwivedi, 1993).

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