SEED PRODUCTION POTENTIAL, DORMANCY AND SEED STORAGE BEHAVIOUR IN *Sida* spp.

by

VEERESH NETEKAL

(2012-12-120)

DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"SEED PRODUCTION POTENTIAL, DORMANCY AND SEED STORAGE BEHAVIOUR IN *Sida* spp."** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara, **Veeresh Netekal**

Date: (2012-12-120)

CERTIFICATE

Certified that this thesis entitled **"SEED PRODUCTION POTENTIAL, DORMANCY AND SEED STORAGE BEHAVIOUR IN *Sida* spp."** is a record of research work done independently by Mr. Veeresh Netekal under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellanikkara,

Date:

Dr. Alice Kurian
(Major Advisor, Advisory Committee)
Professor (Horticulture)
College of Horticulture
Vellanikkara.

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Veeresh Netekal,** a candidate for the degree of **Master of Science in Horticulture** with major in Plantation Crops and Spices, agree that the thesis entitled **"SEED PRODUCTION POTENTIAL, DORMANCY AND SEED STORAGE BEHAVIOUR IN *Sida* spp."** may be submitted by Mr. Veeresh Netekal, in partial fulfilment of the requirement for the degree.

Dr. Alice Kurian
(Chairman, Advisory Committee)
Professor (Plantation Crops and Spices)
College of Horticulture, Vellanikkara.

Dr. E. V. Nybe
(Member, advisory committee)
Professor and Head **(**Plantation Crops & Spices)
College of Horticulture, Vellanikkara.

Dr. M. Asha Sankar
(Member, advisory committee)
Professor **(**Plantation Crops & Spices)
College of Horticulture, Vellanikkara.

Dr. T. Girija
(Member, advisory committee)
Professor (Plant Physiology)
College of Horticulture**,** Vellanikkara.

Dr. E. V. Anoop
(Member, advisory committee)
Associate Professor (Wood Science)
College of Forestry, Vellanikkara

EXTERNAL EXAMINAR

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Veeresh Netekal

(2012-12-120)


Dedicated
to my
nation
INDIA

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LIST OF ABBREVIATIONS

%	Percentage
µm	Micrometer
µM	Micro Molar
a.m.	Anti-meridian
cm	Centimeter
cm^3	Cubic centimeter
dS m^{-1}	Decisiemen per meter
g	Gram
h	Hour
ha	Hectare
kg	Kilogram
mg	Milligram
min	Minute
m	Meter
ml	Millilitre
N	Normal
nm	Nanometer
^oC	Degree Celsius
pm	Post-meridian
ppm	Parts Per Million
rpm	Rotations Per Minute
spp.	Species

Introduction

INTRODUCTION

The two main global challenges currently attracting popular interest are climatic changes and biodiversity loss. According to Dasgupta (1995), the utilization value of biodiversity is to be located in the potential future uses of genetic materials especially for pharmaceutical purposes. Medicinal plants are the complex chemical storehouses that contain many undiscovered compounds with unknown medicinal properties.

Plants have been a major source of therapeutic agents since time immemorial. The increasing acceptance of traditional herbal systems of medicine, like Ayurveda, within India and outside, has resulted in the revival of ancient traditions of medicine. Medicinal plants and their derivatives are thus looked upon not only as a source of affordable health care, but also as an important commodity item of international trade and commerce (Kurian and Sankar, 2007).

In view of the phenomenal increase in the demand of herbal drugs, medicinal plants have been indiscriminately exploited leading to rarity and endangerment of many valued plant species. In India, more than 90.00 per cent plant species, utilized by user industry are collected from wild and over 70.00 per cent of the collection involves destructive harvesting of officinal parts like root, bark, wood, stem and whole plant. This possesses immense threat to the genetic stock of these medicinal species. As per IUCN (International Union for Conservation of Nature and Natural Resources) guidelines, around 200 species have become rare, endangered and threatened (Kurian and Sankar, 2007).

The medicinal plant known as ‘Bala’ in Sanskrit belongs to the genus *Sida* of the family Malvaceae. The genus is of great importance in the Indian traditional systems of medicine and is one of the most widely used raw drug in the production of different Ayurvedic formulations. It is reputed as a remedy for curing neurological

disorders, as an anti-rheumatic and anti-pyretic agent. It is also reported to possess anti-tumor, anti-HIV, hepatoprotective, abortifacient, antimicrobial and immunostimulant properties (Khare *et al*., 2002). *Sida* species are a source of indoloquinoline alkaloids, principally cryptolepine, which produce many pharmacological effects such as anti-microbial, anti-hyperglycemic and cytotoxic effects and as leads in the design of new anticancer drugs (Karou *et al*., 2005).

In recent years, the growing market demand for *Sida* species makes it difficult to rely on harvesting the plant material from the wild for its supply. *Sida alnifolia* (Syn. *Sida rhombifolia* ssp. *retusa*), the principal source of Bala in Kerala, has reached a stage of rarity due to habitat destruction, over exploitation and destructive harvesting for collection of roots, the officinal part. Low reproductive capacity, seed output and seed viability (Lissy, 2004) also adds to its rarity. Out of 230 species, *Sida alnifolia* ranks top in procurements of raw drugs by Ayurvedic industries, with an annual consumption of 1193.47 tons per year (Sasidharan and Muraleedharan, 2009). *Sida alnifolia* is one among the 35 (short duration) medicinal plants promoted for cultivation by National Medicinal Plant Board, New Delhi. Market surveys have indicated that roots of other species of *Sida* and a few other Malvaceous plants and even plants belonging to other genera's including, *Abutilon* species, two species of *Urena*, two species of *Pavonia* and *Grewia*, *Triumfetta rhomhoidea*, *Malvastrum coromandalianum* and even *Anisomeles indica* (Lamiaceae) are considered as sound substitutes, used to adulterate Bala (Aiyer & Kolammal, 1993). In Kerala *Sida alnifolia* is used as the drug while North Indian preparations mainly contain *Sida cordifolia* (Shylaja *et al.,* 2006).

Seeds of *Sida* species are reported to exhibit seed coat imposed dormancy posing problems in germination (Egley, 1989; Seal and Gupta, 1998). Though few attempts have been made to elucidate the reasons for dormancy in *Sida* species (Egley, 1976; Chauhan and Johnson, 2008), detail studies are warranted to identify

the factors responsible for inducing dormancy and to establish germination requirements to bypass natural seed dormancy mechanisms. Available literature on seed treatments to improve germination in *Sida* species (Seal and Gupta, 2000; Lissy, 2004; Chauhan and Johnson, 2008) have focused on germination percentage alone and effect of treatments on the growth and vigour of the seedling is little studied. Storage behaviour of seeds is important from the conservation and domestication point. Specific information on seed production potential, dormancy, germination and response to storage are needed to plan and execute a conservation strategies and domestication programmes in genus *Sida*, which are at present limited. In this context, the study was taken up with the following objectives:

1. To assess seed production potential in *Sida* spp.
2. To characterize dormancy behaviour in *Sida* spp.
3. To standardize ideal pre-treatments for improving germination in *Sida alnifolia*
4. To study seed storage behaviour in *Sida alnifolia*

Review of Literature

REVIEW OF LITERATURE

The genus *Sida* belonging to Malvaceae family, constitute a large number of herbs and shrubs distributed throughout the tropics. Held in repute by Ayurvedic physicians, this genus constitutes the medicinally acclaimed group of plants, balas, which find application as a panaceae for rheumatism and neurological complaints. Four varieties namely, bala, atibala, nagabala and mahabala are mentioned in bhavaprakasa nighantu, which constitute the group of balacatustyam. The other two varieties, rajabala mentioned in rajaninghantu and bhomibala in oushadhinighantu are not in vogue in practice. Of the four types of bala mentioned above as accepted by Ayurvedic formulary of India, bala is the most widely used. This has been equated with *Sida cordifolia and* is the widely used source of bala in northern parts of India. Kerala physicians have adopted *Sida alnifolia* (*Sida rhombifolia* ssp. *retusa*) for this drug (Sivarajan and Balachandran, 1994). Various other species like *Sida acuta*, *S. rhombifolia*, *S. humilis* and *S. veronicaefolia* are also used in Ayurvedic system (Kirtikar *et al*., 2003). The natural occurrence of *Sida* species especially *Sida alnifolia* is getting reduced due to over exploitation, unscrupulous collection and destruction of habitat. The literature available on seed production, dormancy, germination and storage of seeds in members of Malvaceae with special reference to *Sida* species and similar species which exhibit dormancy are reviewed in this chapter.

2.1. COMMON *Sida* spp.

2.1.1. *Sida acuta* Burm. (Common wireweed)

These are erect shrubs or subshrubs upto one meter tall. Branches are distichous, green, terete, pubescent with minute stellate hairs or glabrescent. Leaf blades on flowering and fruiting shoots are 3-6 × 1-2 cm lanceolate to ovate, truncate at base, serrate, entire towards base; leaves on young plants are obovate, suborbicular or elliptic-rhomboid, 3 nerved at base, in lateral nerves in 5-6 pairs and sparsely

hirsute to glabrate on both surfaces. Petioles are 1-5 mm long, pulvinate on both the ends. Stipules are 6-10 × 1-1.5 mm, unequal, one lanceolate or falcate, 1-3 nerved, the other linear to filiform. Flowers are solitary or paired in the leaf axils, pedicels are 3-10 mm long, sparsely stellate-pubescent. Calyx is 5-6 cm diameter, 6-8 mm long, campunalate, ciliate on margins, divided to the middle, basally 10-costate, corolla 8-10 mm in diameter, glandular and hairy. Staminal column is 2-2.5 mm long, glabrous or slightly pubescent; anthers pale yellow; or white; petals 7-10 mm long, obliquely obovate, outside sparsely glandular hairy. Staminal column is 2-2.5 mm long, glabrous or minutely pubescent; anthers pale yellow measuring 1-1.5 mm long. Ovary is 1.5 mm long, ovoid, sparsely hairy towards apex; styles 6-8; stigma globose and yellow. Shizocarp is 5 mm long, glabrous; mericarps 6-8, 3 × 2 mm, trigonous with acute angles, pale when mature, prominently reticulate on the sides below, reticulate or transversly rugose on the back, apically 2-awned, awns are divergent, linear with dilated base, glabrous, almost equaling to the calyx, 1-1.5 mm long. Seeds are 2 mm long trigonous with rounded angles, glabrous or shortly hairy at hilum (Sivarajan and Pradeep, 1996).

2.1.2. *Sida cordifolia* L. (Country mallow)

Erect, branched shrubs or subshrubs upto 1.5 m tall. Stem is terete, green, densely tomentose with minute stellate and spreading simple hairs. Leaf blades are acute at apex, velvety to tomentose, often with simple hairs on nerves and soft tomentose, often with simple pilosa hairs, margins serrate to the base, basally 3-5 nerved, densely stellate tomentose beneath with simple hairs on nerves and soft tomentose above. Petiole is 3-60 mm long, pubescent with simple patent hairs. Stipules are filiform, 3-10 mm long. Flowers axillary, mostly solitary, occasionally arranged in a very short, axillary, almost leafless young branches or aggregated terminally into congested, paniculate or corymbiform inflorescences. Pedicel is 2-3 mm long in flower and 15 mm in fruits, articulated above the middle. Calyx is 5 mm

in diameter, 6-7 mm long, prominently 10 ribbed, densely tomentose externally by stellate hairs intermingled with simple hairs, inside on the segment sparsely hairy occasionally intermixed with simple hairs. Corolla is 10 mm in diameter, orange yellow or creamy white; petals, 1 × 0.7 cm, obliquely obovate, apex truncate or slightly emarginated, minutely hairy at base. Staminal column is 3 mm long glabrous or minutely hairy. Ovary is subglobose, pubescent with minute stellate hairs; styles 8-10; stigma capitate, yellow. Schizocarp is 6-7 mm in diameter, pubescent towards apex; mericarps 8-10, 3.5 × 2 mm, trigonous with acute angles, pale at maturity, prominently reticulate on the sides, especially towards base and transversely rugose on the back, apically 2-awned, awns far exceeding calyx (3-4 mm long) with simple retrose hairs. Seeds are brownish or black, glabrous or minutely hairy at hilum (Sivarajan and Pradeep, 1996).

2.1.3. *Sida alnifolia* L. (Arrowleaf Sida)

Woody herbs or sub shrubs upto 50cm, usually low and strongly branched. Branches are prostrate or ascending, terete, green or purplish-grey, stellate-tomentose to glabrescent. Leaf blades are 0.5-5 × 0.5-4 cm, leaves towards stem base always obovate with retuse or emarginated apex, rarely truncate; upper leaves obovate to elliptic-lanceolate with the rounded, subobtuse or acute apex, obtuse or rounded at base, margins irregularly serrate-dentate or crenate to the distal half, entire towards the proximal half, upper surface pubescent with short many rayed stellate hairs, lower surface densely grayish tomentose with short, many rayed stellate hairs. Petiole is 3-5 mm long, stellate-pubescent, shortly pulvinulate below the lamina. Stipules are 4.5 mm long, equal, linear to subulate, glabrasecent. Flowers are axillary, solitary, sometimes due to reduction of distal leaves in terminal clusters. Pedicels longer than the petioles, 3-4 mm long in flower, to 30 mm in fruits, glabrous, jointed at the middle. Calyx is 6-7 mm in diameter, 6 mm long, pubescent with minute stellate hairs, glabrous within, 5 lobed; the lobes 2 mm long, ovate to triangular. Corolla is

12 mm in diameter, orange-yellow; petals 7.8 × 6.5 cm, obliquely obovate, cuneate at apex, glabrous except for the minutely hairy base. Staminal column is 3 mm long, glabarous or minutely stellate-hairy, antheriferous at apex. Ovary is 1.5 mm diameter, depressed globose, glabrous; styles 7-10, white; stigmas capitate, creamy-yellow. Schizocarp is 4 mm long; mericarps 7-10, 2.5 -1.5 mm, included in the calyx, reticulate or rugose on the sides and back, with a pair of short stellately hairy mucro at apex, mucro obtuse, retuse or emarginate at apex. Seeds are 2 mm long, black, glabrous except for a puberulent hilum (Sivarajan and Pradeep, 1996).

2.1.4. Distribution and ecology

Sida acuta and Sida cordifolia are pantropical in distribution, occur nearly throughout the India at an altitude ranging from sea level to 1000 m. The plants grow along roadsides, wastelands, sandy sea coast, waste places and railway embankments and also as weed in upland cultivation. *Sida alnifolia* is restricted to the plains and hills of Southern peninsular India and occurs along roadsides and forest clearings as secondary growth in lateritic hill slopes and occasionally as a weed in upland cultivation (Sivarajan and Pradeep, 1996).

2.1.5. Medicinal uses

The drug is held in great repute by Ayurvedic physicians for the treatment of rheumatism and it forms the chief ingredient of several important preparations like ksirabala, dhanvantaram, balarishtam, rasnadi kasayam, asvagandhi leham etc. Root is the officinal part. It is reported to be cooling, sweet, demulcent, aphrodisiac and tonic. It produces strength, imparts beauty to the body and cures vatarakta, raktapitta, consumption, polyuria and ulcers. The drug is also useful in neurological disorders like hemiplegia, facial paralysis, sciatica, general debility, headache, opthalmia, dysuria, leucorrhea, tuberculosis, diabetes, fever and uterine disorders (Sharma, 1983).

Several pharmacological activities of various species of *Sida* have been reported. The roots of *Sida acuta* are extensively used as stomachic, diaphoretics and antipyretics, are cooling and astringent. Tonics are useful in treating nervous and urinary diseases and also disorders of the blood, bile and liver (Khare *et al*., 2002). The whole plant is used to treat snake bite of *Bothrops atrox* venom (Otero *et al*., 2000). *Sida acuta* has significant antiplasmodial activity due to its alkaloid cryptolepine (Karou *et al*., 2003; Banzouzi *et al*., 2004). Its aerial part is used in Central America to treat asthma, renal inflammation, cold, fever, headache, ulcers and worms (Caceres *et al*., 1987). *Sida cordifolia* roots are used to treat a variety of ailments including pulmonary tuberculosis, rheumatism, hematuria, urinary and heart diseases, Parkinson's disease and as a food supplement for fat loss. It is a tonic, astringent, emollient, aphrodisiac and is used in the treatment of leucorrhoea, gonorrhea and general debility (Khatoon *et al*., 2005). Decoction of the root bark is given in sciatica and rheumatism (Nair, 2004). *Sida alnifolia* is reputed as a remedy for curing neurological disorders, as an anti-rheumatic and anti-pyretic agent; also it is reported to possess anti-tumor, anti-HIV, hepatoprotective, abortifacient, antimicrobial and immunostimulant properties. The ethanolic extract of *Sida alnifolia* possesses hypoglycemic activity. It is a tonic, cures ulcer and biliousness and is useful in urinary infection, leprosy and skin infection. The roots are used as tonic, diaphoretic and useful in treatment of fever, debility, as demulcent in irritability of bladder (Khare *et al*., 2002).

2.1.6. Chemical constituents of *Sida* spp.

Due to pronounced therapeutic uses of various *Sida* species in medicine, various classes of chemical constituents are characterized from the genus *Sida*, include alkaloids, phytosterols, carbohydrates, flavanoids, fatty acids, amino acids, miscellaneous constituents like long chain hydrocarbons, alcohol, coumarins, phenolic acids etc. Presence of phytosterols, alkaloids and fatty acids may be

responsible for its medicinal properties. Due to presence of free amino acids and carbohydrates, the plants of this genus are being used as tonic. Elaboration of the quinoline alkaloid seems to be a characteristic feature of this genus. The favourable combination of sympathomimetic amines, choline, pseudoephedrine, betaphenethylamine, vascin, hipaphorine and related indole alkaloids and vasicinone of the different species would account for their major therapeutic uses in the Indian system of medicine (Shaman Australis Ethnobotanicals, 2002; Atul *et al*., 2007). The alkaloid content in the root was reported to be 0.1 per cent in *Sida rhombifolia* (Shaman Australis Ethnobotanicals, 2002). However, it was reported that the roots contained 450 ppm of alkaloids and leaves contain respectable amounts of nutrients, protein, carbohydrates, fiber, fat and ash.

2.1.7. Flowering and seed production potential

Callen *et al*. (1989) reported that *Sida* species as one of the main sources of pollen for *Apis mellifera* in Gabon and Ivory Coast. Sivarajan and Pradeep (1996) reported that *Sida acuta* flowers almost throughout the year. The flowers open between 9.15 and 9.30 in the morning on sunny days and wither by 11.00 am. In *Sida cordifolia*, peak flowering is from July to September. The flowers open at about 9.00 am and wither off by 2.00 pm. *Sida alnifolia* flowers from August to April in Kerala. The flowers open at about 9.00 am and wither by 2.00 pm. Parrotta (2001) reported that *Sida rhombifolia* flowers and fruits continuously, starting at 3 or 4 months of age in Puerto Rico. In Central India, plants flower from September to December and fruit from October to January.

Holm *et al*. (1997) and Calderon *et al*. (2000) reported that number of seeds per plant in *Sida rhombifolia* was 11,600 and 7,962 respectively. Gil *et al*. (2010) found that *Sida rhombifolia* to be most abundant and most frequent species in the pine forest. The pine forest showed a surface soil density of 2311 seeds m^{-2}.

2.2. DORMANCY

Dormancy is regarded as the temporary suspension of visible growth of any plant structure containing a meristem (Lang, 1987). Dormant seeds do not germinate when supplied with suitable temperature, adequate moisture and oxygen. The phenomenon of seed dormancy cannot be divorced from the processes of seed development and germination. Seed dormancy is initiated during seed development, the process being influenced by the parental and zygotic genotype (Simpson, 1990). The genotype will interact with the different environmental and physiological phenomena's which occur from anthesis to seed dispersal, producing varying degrees of dormancy (Jain, 1982; Nooden *et al*., 1985).

The final phase of seed development involves the loss of water and cessation of reserve synthesis, where after the seed enters a metabolically inactive state. The process of drying converts the seed to a dispersal structure, which will germinate if conditions are favourable. Thus seed desiccation achieves permanent changes in seed metabolism. The seed progresses from the developing stage to a propagule which is equipped for germination and establishment. In dormant seeds, the period between desiccation and germination is extended.

For germination to occur, a seed requires moisture, suitable temperature and in most cases an aerobic atmosphere. If one or more of these requirements are not met, germination will fail to take place and in this condition the seeds may be regarded as being in a state of imposed dormancy (Roberts, 1972).

2.2.1. Causes of dormancy

Causes of seed dormancy are many and varied. Impermeability of seed coat to water and gases, immaturity of the embryo, special requirement for temperature and light, presence of inhibitors and mechanical restriction to embryo growth are the

major reasons (Tran and Cavanagh, 1984). Primary dormancy was proposed for dormancies occurring due to pre-harvest or pre-dispersal changes in seeds and the secondary dormancy is the one induced following harvest or dispersal, by natural or artificial means (Khan and Karssen, 1980).

2.2.2. Coat-imposed dormancy

Although the causes of coat-imposed dormancy are well documented, in seeds of *Sida acuta, Sida rhombifolia, Sida spinosa, Urena lobata* and *Commelina benghalensis* (Egley, 1989; Seal and Gupta, 2000; Wang *et al*., 2009). It has become clear that seed coat dormancy is a more complex phenomenon than anticipated. Therefore, it is necessary to detail the sequence of events leading to dormancy in the seed, to identify the dormancy mechanism in the seed coat and finally to establish the precise location and nature of the barrier to germination. Developmental studies (anatomical and ultra structural) have proved to be vital in identifying the type of seed coat dormancy and in providing information as to how it is broken naturally. The seed coat has been shown to be a multifunctional organ which supplies nutrients to the embryo sac throughout the development (Murray, 1987) and is functional during drying of the seed (Hyde, 1954; Manning and Van Staden, 1985). The structural and chemical properties of the seed coat impose impermeability (Van Staden *et al*., 1989), regulate water entry once dormancy has been broken and reduce leakage from embryo during imbibition (Kelly and Van Staden, 1987).

2.2.3. Structural features responsible for seed impermeability

The impermeability of the seed coat to water and / gases and mechanical restriction of the embryo is achieved structurally and / chemically. Structurally, impermeability is imposed as the seed shrinks during maturation, the strips of thickenings in the upper part of each epidermal cell, become pressed together until they occlude the cell lumen entirely (Van Staden *et al*., 1989). Further loss of

moisture can be attributed to the action of the hilar mechanism in papilionoid legumes and the pleurogram in caesalpinoid and mimosoid legumes, while seeds lacking these mechanisms must lose water through the testa (Hyde, 1954). Compression of the palisade layer reinforces the impermeability to water (Corner, 1951; Graaff and Van Staden, 1983) while porous macrosclereids can be used as an indicator of permeability in *Aspalathus linearis* (Kelly and Van Staden, 1985), *Glycine max* (Harris, 1987) and *Lupinus augustifolius* (Serrato *et al*., 1989).

2.2.4. Chemical features responsible for impermeability of seed

Chemically, the deposition of hydrophobic substances in the palisade cells, such as callose in *Sesbania punicea* (Riggio *et al*., 1987), callose, lipid and suberin in *Melilotus alba* (Riggio *et al*., 1989) and the conversion of hydroxyphenolics to insoluble lignin polymers in *Sida spinosa* ensure that the seeds remain impermeable to water and / or gases (Egley *et al*., 1983).

2.2.5. Structural and chemical features responsible for impermeability of seed

Differences in the lignification of palisade cells in *Sida spinosa* (Egley and Paul, 1981), *Cuscuta pedicellata* and *Cuscuta campestris* (Lyshede, 1984) also produce permeable and impermeable seeds. In some cases the impermeable barrier include surface as well as palisade layers. For example, in *Rhynchosia minima*, impermeability is ensured by a waxy / lipoidal surface material, a hemicellulose / cellulose barrier, followed by the palisade cells containing cellulose fibrils, arabinans, phenolic compounds and tannins (Rangaswamy and Nandakumar, 1985). In *Halimium halimifolium* seeds, the exotesta exerts a physical effect in combination with the phenols in the coat to prevent dormancy (Pefia *et al*., 1988). However, there are not always structural and / chemical differences between impermeable and permeable seeds of the same species and the nature of the substances present in the testa differs from species to species. In *Cercis siliquastrum*, the impermeability is

due to a thin layer (the remains of the inner integument) of the copious endosperm (Riggio *et al*., 1985). In fact there may be a number of ways in which impermeability is achieved, the fundamental hypothesis remains, that the seeds are dormant due to the properties of the seed coat which prevent the entry of water and / oxygen.

2.2.6. The phenomenon of polymorphism

The occurrence of polymorphism in species which exhibit seed coat dormancy usually allows for a small percentage of seeds to be readily permeable. This may occur on the basis of seed colour, weight or size. Colour changes are associated with the onset of impermeability in *Ononis sicula* (Graaff and Van Staden, 1983). Long days permitted the developmental processes of the seed to overtake those of the fruit, mature impermeable seeds are then shed from the plant. Short days resulted in the developmental processes of the seed lagging behind those of the fruit, the pods dehisced and seeds were shed before testa maturation and seed impermeability. This may be the reason why a small percentage of *Sesbania punicea* seeds were permeable in a South African study (Graaff and Van Staden, 1983). In a similar European study only permeable seeds were found in *Sesbania punicea* (Riggio *et al*., 1987). In most cases, when polymorphism exists, anatomical differences can be identified between permeable and impermeable seeds (Kelly and Van Staden, 1985).

2.2.7. Mechanical constraints of coat-imposed dormancy

When the seeds of *Syringa* species imbibe and still do not germinate, the seed coat or endosperm may be preventing germination mechanically (Juntilla, 1973). The high resistance to radicle emergence may decrease when gibberellins are applied, or by incubating seeds at high levels of O_2 (Watkins and Cantliffe, 1983). Mechanical scarification of *Iris lorteti* seeds at the micropylar end allowed for radical protrusion and germination (Blumenthal *et al*., 1986). Non-imbibed (hard) *Iris lorteti* seeds required a pressure of 135 atmospheres to ‘force’ water into the seeds, ensuring

imbibition and germination, while for *Iris atropurpurea*, 75 atmospheres was sufficient (Blumenthal *et al*., 1986).

2.2.8. The role of inhibitory substances in coat-imposed dormancy

Soaking of impermeable seeds may result in substances leaching from the seed coat. If seeds germinate once, leaching is completed; an inhibitory substance may have been present. Very often inhibitors are present concurrently with other dormancy mechanisms. These substances need not be hormonal. *Sesbania punicea* seeds exude an orange substance upon imbibition, which could be an inhibitory substance (Graaff and Van Staden 1984).

Felix and Harr (1987) reported that polyamine content of 15 wild species and 15 crop species from 13 families including Malvaceae, before and after germination. A marked increase in polyamine content generally occurred in the cotyledons or endosperm on germination.

Newton and Egley (1977) found that both dormant and non-dormant *Sida spinosa* seeds contained water-soluble inhibitors. Williams and Hoagland (1982) reported the combination of coumarin with p hydroxybenzaldehyde to inhibit the germination of *Sesbania exaltata* and *Sida spinosa* to a greater extent than either compound alone.

Fischer *et al*. (1989) reported that seed germination of the dicotyledons and monocotyledons was both inhibited and promoted, depending on the compound and the specific species or cultivars, at concentration as low as 1 µM.

Colorado *et al*. (1994) reported that expression of the ABA-regulated clones was dependent on the presence of calcium ions, suggesting that calcium was involved in the response of the seeds to ABA.

Alhadi *et al*. (2012) suggested that amino acid reserves in dry seeds are major determinants for germination capacity and germination behaviour in the germination of pomegranate.

Hassan *et al*. (2013) reported that inhibitors present in the fruits of *Terminalia laxiflora* have inhibitory effect on the seed germination. Negi *et al*. (2014) reported that Nickel (Ni) when in excess, inhibits seed germination and reduces seedling growth in *Triticum aestivum*.

2.2.9. Removal of coat-imposed dormancy under natural conditions

Removal of the impermeable barrier is achieved by either mechanical or chemical scarification. Cracking of the testa during fires and exposure to solar radiation have all been mentioned as natural agents. As none of these treatments result in the immediate removal of dormancy, the duration and intensity will depend on the type of dormancy breaking agent involved.

Seed dormancy in *Leucospermum cordifolium* is imposed partly by the impermeability of the seed coat to oxygen. In the intact achene, the pericarp may also contribute to poor germination by impairing oxygen diffusion to the embryo (Van Staden and Brown, 1973). Ants disperse and bury achenes in which the edible pericarp (elaiosome) is intact (Slingsbury and Bond, 1985). The exotesta is decomposed by microbial action and is also ruptured by desiccation. If desiccation is followed by rain, the palisade (endotesta) ruptures as well, thus removing the mechanical constraint on the embryos. The second aspect of the dormancy involves an embryo requirement which is satisfied following exposure to alternating temperatures (Brits, 1986).

Seeds remaining in soil when subjected to repeated desiccation / hydration cycles cause the long-lived seeds of previous flowering seasons to become fully

scarified. In *Dichrostachys cinerea*, a mimosoid legume, seed burial for 71 weeks, five centimeter below the ground increased permeability from four per cent to 60.00 per cent. Microorganisms within the soil were degrading the seed coat with the appearance of fine cracks which were also present at the hilum. The testa topography of untreated seeds remained unaltered during a veld fire; seeds are exposed to temperatures ranging from 80° C to 300° C for a couple of seconds. The extreme range and duration of these temperatures resulted in disintegration of *Dichrostachys cinerea* seed exposed to the very high temperatures. The lower temperatures (80 and 100°C) usually resulted in imbibition (due to testa cracking) and germination. Therefore the intensity and duration of the fire will control the temperature reached at the soil surface and below, and thus would effect seed softening and the subsequent distribution of plants (Jones, 1963). In the legume seed, artificial treatments usually alter the macrosclereid layer, while natural agents and / or treatments alter the seed coat topography as well as the structure of the hilum, lens and / or micropyle (Van Staden *et al*., 1994). Once the seeds are permeable, germination was considerably improved by the exposure of seeds to light and this may have been another factor which increased permeability to 60.00 per cent after burial of seeds of *Dichrostachys cinerea* (Bell and Van Staden *et al*., 1993).

2.2.10. Occurrence of a natural site of permeability

The occurrence of a specific site of water entry in impermeable seeds is not restricted to Leguminosae. For example, seeds of *Sida* (Malvaceae) possess an impermeable area at the chalaza (Egley *et al*, 1986; Egley, 1989). *Beta vulgaris* a basal pore (Santos and Pereira, 1989) and *Ricinus communis* a caruncle (Lagoa and Pereira, 1987). The lens in the papilionoid legumes seed acts as the site of water entry, regulates the path of water movement (Kelly and Van Staden, 1987) and the rate of water entry (Manning and Van Staden, 1987).

2.2.11. Anatomy

Egley *et al*. (1983) reported that peroxidase is involved in the polymerization of soluble phenolics to insoluble lignin polymers during development of prickly *Sida* seeds, causing the formation of a water-impermeable barrier prior to seed dehydration. As dehydration proceeds, the chalazal area finally becomes impermeable resulting in the hard mature seeds of prickly *Sida*.

Egley *et al*. (1986) observed that subsequent expansion of the palisade cells causes the thin-walled subpalisade cells to break, resulting in separation of palisade from subpalisade cells and free passage of water through the exposed surface to the embryo, culminating in germination in *Sida spinosa.*

Paul (1981) reported that separation of the palisade and subpalisade layers in the chalazal area initiates imbibition of water by prickly *Sida spinosa* seeds.

Meryl and Moore (1959) found that water enters the cotton seed by an opening in the palisade layer at the chalazal end. In hard seed, this opening is made impervious by a chalazal cap and a seal between the cap and the palisade layer.

Nada *et al*. (1994) reported that okra seeds exhibit dormancy resulting from a hard, water resistant seed coat and chalazal plug, causing very slow water uptake.

Baskin and Baskin (2006) observed that seeds with physical dormancy have a water gap in the seed coat that opens in response to an environmental signal, thereby allowing water to enter.

2.2.12. Staggered dormancy

Rizk *et al*. (1969) reported that in Louisiana, seedlings of *Sida spinosa* and *Sida rhombifolia* under favourable conditions can emerge throughout the season

from a depth of down to 3.75 cm. Burial of seed in the field for 12 months did not reduce germinability.

Devillez and Capelle (1976) reported that *Alopercurus myosuroides* seeds exhibit staggered emergence.

Mott (1980) reported that *Sida acuta* is a serious weed species in the Northern territory and long-term dormant seed pool gives rise to new generation of seedlings. Species are dormant at seed-fall and require high alternating temperatures for germination and still retains 30.00 per cent hard seed after one dry season. Germination was spread over the first two months of the season and at the end of the wet season there could be a considerable amount of hard seeds.

Bhatia *et al*. (1990) reported that soil stored seeds of *Echinochloa crus-galli* under natural conditions had staggered germination from May to September. During one season 97.70 per cent of the seed reserves were exhausted and only 2.30 per cent were carried over to the second season. By the third season the soil was free of viable seeds.

Peirce (1990) reported that in *Carthamus lanatus* low rainfall and temperature in autumn were primarily responsible for the slow and staggered germination at the more southern site.

Cirujeda *et al*. (2008) reported that despite the fact that emergence will be staggered throughout several years and that there was a significant relationship between rainfall and emergence, so that dry years will cause a smaller emergence rate of the weed in *Papaver rhoeas.*

2.3. TREATMENTS FOR IMPROVING GERMINATION

2.3.1. Effect of seed scarification on germination

The simple observation or assumption of dormancy breaking through scarification without comphrensive anatomical investigation may often lead to false conclusion being drawn. A wide gap in the knowledge on the seed coat anatomy, effect of seed coat on imbition and different treatments aroused a renewed interest in many researchers. Some of the reviews on these topics are presented below.

2.3.1.1. Chemical scarification

Rizk *et al*. (1969) reported that *Sida rhombifolia* seeds required 4 to 6 weeks of after-ripening before appreciable germination was apparent and dormancy was overcome by treatment with water at 65º C or sulphuric acid. Datta and Sen (1981) reported that chemical treatment with Conc. H_2SO_4 produced better results in *Sida rhomboidea*. Lissy (2004) reported that treatment with sulphuric acid for varying periods showed significant results in *Sida rhombifolia*, giving 38.00 per cent of germination after 20 min of acid scarification. In *Sida retusa*, 53.00 per cent germination was exhibited after 20 min of acid scarification, whereas *Sida acuta* showed 56.00 per cent germination after 10 min scarification. Highest percentage of germination (83.00) was observed in *Sida cordifolia* after 20 min treatment. Chauhan and Johnson (2008) found that chemical scarification with Conc. H_2SO_4 released *Sida rhombifolia* seeds from dormancy and stimulated germination. Germination increased with increased duration of scarification with Conc. H_2SO_4 up to 120 min, giving 65.00 per cent germination compared with five per cent for nonscarified seeds. According to Packa *et al*. (2014), scarification for 30 minutes with 95.00 per cent sulphuric acid was most effective in breaking the physical dormancy of seeds of *Sida hermaphrodita* resulting in imbibition without impairing embryo viability.

Passam and Polyzou (1997) reported that acid scarification is essential for the successful germination of hard-seeded cultivars, such as Boyiatiou cultivar of okra.

Kader and Chacko (2000) reported that germination in *Thespesia populnea* can be enhanced from 2.30 per cent (untreated) to over 75.00 per cent either by nicking or by scarifying the seed coat for 20-60 min using 95 per cent Conc. H_2SO_4. Nicked and scarified seeds start germination from the fourth day and give a cumulative germination of 75.80 per cent and 85.00 per cent, respectively in two weeks. According to Gupta *et al*. (2004) scarification for 10-20 min was the most effective in seed dormancy breaking of *Thespesia populnea*. Vigour index was greatest (2180) for seeds treated with sulphuric acid for 20 min. Gagare and Mate (2009) obtained highest germination of 84.53 per cent when seeds of *Thespesia populnea* were scarified with Conc. H_2S0_4 for 30 min and germinated between paper at 30° C.

Solano *et al*. (1976) reported that *Anoda cristata* seed germination averaged 0.5 to 3.00 per cent for seeds buried in the field for up to 20 months under natural conditions. These same seeds averaged over 76.00 per cent germination after scarification with concentrated sulphuric acid, indicating hard seed coat involvement in seed dormancy.

Mechanical scarification and immersion in sulphuric acid reduced hard seededness in *Abutilon theophrasti* caused by seed coat impermeability to water (Horowitz and Taylorson, 1985; Cardina and Sparrow, 1997).

Michael *et al*. (2006) found that seeds of *Malva parviflora* exhibit physical dormancy developed after physiological maturity, once the seed moisture content declined below 20.00 per cent. Manual scarification or period of fluctuating summer was required to release dormancy. Germination of *Malva parviflora* (little mallow), was stimulated by seed scarification (Chauhan *et al*., 2006).

Seeds of *Hibiscus trionum* (Venice mallow) possessed physical dormancy that required immersion for 0.5 h in Conc. H_2SO_4 to break the seed coat without damaging the seed (Chachalis *et al*., 2008).

Wang *et al*. (2009) reported that cadillo seeds had significant levels of innate dormancy imposed by a hard seed coat and chemical scarification was the most effective technique for removing dormancy.

2.3.1.2. Mechanical scarification

Johnson *et al*. (1979) reported that seed dormancy of *Sesbania exaltata* was caused by impermeable seed coat. Acid scarification for 45.00 and 60.00 min and mechanical scarification for 20.00 and 30.00 seconds gave maximum germination.

Datta and Sen (1981) reported that mechanical scarification was more effective in breaking dormancy of *Ipomoea biloba, Neptunia oleracea* and *Tephrosia hamiltonii.*

Baskin and Baskin (1997) reported that dormancy in *Iliamna corei* was broken in a high percentage of seeds by mechanical scarification.

Gupta (2003) reported that germination of upto 80-95 per cent obtained with sand paper scarification for *Abelmoschus moschatus, Abrus precatorius, Cardiospermum halicacabum, Cassia* spp. and *Withania coagulans* and acid scarification for *Abrus precatorius, Argyreia nervosa, Bixa orellana, Helicteres isora* and *Indigofera tinctoria*.

Mohan (2005) reported that mechanical scarification, by way of seed rupturing in snake gourd recorded the highest germination per cent (93.00) and speed of germination (14.38), followed by treatment with 5N H_2SO_4, recording 89.00 per cent germination and speed of germination of 13.77.

Shooshtarian and Salehi (2010) reported that scarification with sand paper increased the germination percentage in *Alcea aucheria* Boiss.

Pallavi *et al*. (2014) reported that damaging the seed coat (nicking) enhanced germination percentage from 32.00 to 84.00 in *Abrus precatorius*.

Awan *et al*. (2014) reported that highest germination capacity (93.00 per cent) was achieved by mechanical scarification of previously stored seeds of *Urena lobata*, whereas the combination of mechanical and chemical seed scarifications provided maximum (99 per cent) seed germination.

2.3.1.3. Hot water scarification

Mechanical scarification and immersion in water at 60º C were the most efficient methods for *in vitro* seed germination of *Sida rhombifolia* (Pedroso *et al*., 2007).

Bhale *et al*. (1988) reported that complete removal of the hard seededness of F_1 hybrid H4 and its parent cultivars of cotton was obtained by exposure to water at 90º C for two minutes.

Hot water treatment was the most effective method for reducing the hard number of seeds in cotton, followed by pricking the seed coat, sulphuric acid and accelerated aging treatments (Patil and Andrews, 1985).

Gupta *et al*. (2001) reported that hot water treatment at 70º C for 10 min showed the highest reduction of hard seed occurrence (82.00 per cent) in *Abutilon indicum*.

Alberts and Mandel (2004) reported that hot water scarification is an effective treatment for breaking physical dormancy of *Callirhoe involucrata* seeds.

Kildisheva *et al*. (2011) reported that *Sphaeralcea munroana* seeds are physically dormant, possess a cap-like structure in the occlusion of the water gap, which inhibits imbibition and it can be artificially dislodged through boiling water scarification.

2.3.2. Effect of temperature and light on germination

Rizk *et al*. (1969) reported that *Sida* seeds (*Sida spinosa* and *Sida rhombifolia* required 4 to 6 weeks of after-ripening period before appreciable germination was apparent and dormancy was not broken by several light-dark regimes. *Sida spinosa* seed has an optimum temperature range for germination of 25^{o} C to 32^{o} C (Smith, 1977). Seed germination of *Sida rhombifolia* was tested under constant temperatures of 20º C, 25º C, 30º C and 35º C in light or darkness and also at 10º C and 15º C in the dark. *Sida rhombifolia* seeds germinated in higher rates at the constant temperature of 35º C or the alternating ones that included 35º C in the treatment (Rosa and Ferreira, 2001). In *Sida acuta* 64.00 per cent and in *Sida rhombifolia* 100 per cent germination was observed after seven min and 25 min of scarification respectively with Conc. H_2SO_4 and very low (less than ten per cent) germination was achieved under dark condition. According to Lissy (2004) germination of chemically scarified seeds was influenced by the interaction between temperature and light; germination was significantly lower in light / dark (3.00 per cent) than in the dark (59.00 per cent) indicating that germination of *Sida rhombifolia* is inhibited by light at suboptimal temperatures (Chauhan *et al*., 2006). Freshly harvested seeds of *Sida acuta* and *Sida rhombifolia* achieved 32.00 per cent and 62.00 per cent germination respectively, when subjected to alternate temperature treatments (80º C / 5º C).

Creel *et al*. (1968) reported that in *Cassia obtusifolia*, percentage of germination was high in the temperature range of 18º C to 36º C. No seeds germinated at 15º C and very few seeds germinated at 39º C.

Nada *et al*. (1994) reported that four-day treatment at 40º C was the most effective in increasing the water permeability of the seed and increasing germination in okra.

Singh and Singh (2009) reported that germination of Florida beggarweed (*Desmodium tortuosum*) was highest between 25º C to 40º C and of sicklepod (*Senna obtusifolia*) from 20º C to 40º C.

Wang *et al*. (2009) reported that in cadillo, seeds germinated from 15º C to 40º C, with an optimal temperature of 28º C.

2.3.3. Effect of salt stress on germination

Chauhan and Johnson (2008) reported that seeds of *Sida rhombifolia* may germinate in saline soils. A sigmoid response was observed in germination with increases in NaCl concentrations from zero to 200 mM. Germination per cent of chemically scarified seeds was greater than 55.00 upto the concentration of 50 mM NaCl; however, germination did not occur at 200 mM or greater concentration. As estimated from the fitted model, the concentration required for 50.00 per cent inhibition of maximum germination was 111 mM NaCl.

2.3.4. Effect of osmotic potential on germination

Chauhan and Johnson (2008) reported that *Sida rhombifolia* could germinate under moderate water stress conditions. Germination of *Sida rhombifolia* decreased from 64.00 to 25.00 per cent as osmotic potential decreased from 0 to -0.6 MPa and germination did not occur at -1.0 MPa. The osmotic potential required for 50.00 per cent inhibition of maximum germination was -0.49 MPa. In contrast to *Sida rhombifolia*, germination in *Malva parviflora* was completely inhibited at an osmotic potential of -0.6 MPa (Chauhan *et al.*, 2006).

Wang *et al*. (2009) reported that in cadillo seeds, water stress below -0.2 MPa reduced seed germination.

2.3.5. Effect of pH of buffered solution on germination

Chauhan and Johnson (2008) reported that germination of scarified seeds of *Sida rhombifolia* was not significantly affected by the tested range of pH solutions, and it varied from 60.00 to 65.00 per cent over the pH range of 05 to 09, indicating that *Sida rhombifolia* may germinate in many soil types used for growing most of the field crops in tropical countries.

Germination of prickly *Sida* was highest at pH of 09 and any increase or decrease in pH resulted in reduced germination (Singh and Singh, 2009).

Wang *et al*. (2009) reported that germination of cadillo seeds was unaffected by pH levels.

2.3.6. Effect of hydration treatments

When a seed is hydrated, physiological and biochemical changes begin to take place. A prolonged seed hydration particularly at low water potential profoundly increases the rapidity, synchrony and percentage of seeds that germinate.

Kidd and West (1918) demonstrated that short period of presoaking in water had a favorable effect on subsequent percentage germination and seedling growth.

According to Abdul-Baki and Anderson (1972), the differential germinability of hydrated and dehydrated seeds and control seeds have been related to dehydrogenase activity, membrane functions and lipid peroxidation.

Ma and Liu (1986) reported that forestry species with hard seed coat germinate better when they were previously soaked in water for 15 to 25 h.

Voll *et al*. (2003) reported that highest germination levels of 30.00 per cent was obtained for *Sida rhombifolia* seeds soaked in water for 24 h.

Mohan (2005) reported that seeds of snake gourd subjected to mechanical scarification imbibed water more rapidly after 24 h (88.62 per cent) and resulted in higher and early germination. On the other hand, per cent of mass increase was only 48.79 per cent and had lower germination in untreated seeds.

Puppala and Fowler (2003) found that soaking seeds of *Lesquerella fendleri* in water alone satisfied the light requirement under dark conditions suggesting that presoaking in water alone was sufficient to leach out or deactivate the light requiring dormancy factor. Soaking the seeds for four hours was just as effective as eight hours.

2.3.7. Effect of growth regulators

Prabhu *et al*. (2008) reported that treatment of seeds of one cultivated (*Abelmoschus esculentus*) and nine wild species of *Abelmoschus* seeds with 10 ppm or 50 ppm GA resulted in 100 per cent germination.

Pallavi *et al*. (2014) reported that seeds of *Abrus precatorius* soaked in 100 ppm of gibberllic acid for 24 h gave 78.00 per cent germination.

2.3.8. Effect of soaking in cow dung slurry.

Kundu *et al*. (1993) reported that cow's urine contain 2.50 per cent urea which is known to break dormancy and improve germination in rice.

Nene (2002) opined that cotton and other hard seeds were smeared with cow dung before sowing as in Kautilya's Arthashastra.

Elakkuvan and Manivannan (2010) reported 67.00 per cent germination of *Morinda citrifiolia* seeds soaked in cow dung slurry prepared with 100 g cow dung dissolved in 300 ml of water.

Mathad *et al*. (2013) tried treatment of sorghum seeds with dried cow dung powder and cow's urine before sowing. For one kg of seed, approximately 100 g cow dung powder and 250 ml cow urine were used for better germination.

2.3.9. Effect of seed burial depth on seedling emergence

Seed burial depth influenced seedling emergence of *Sida rhombifolia.* Though seedlings emerged from all burial depths ranging from 0 to 6 cm, fifty one per cent of the seedlings emerged from the seeds placed on the soil surface. Emergence was greater than 60.00 per cent at burial depths of 0.5 to 2 cm, but decreased thereafter. Seedlings did not emerge from the seed burial depth of 8 cm or greater. Decreased seedling emergence due to increased burial depth has been reported in several weed species (Chauhan *et al.*, 2006; Chauhan and Johnson, 2008), which could be linked to seed energy reserves. Larger seeds with greater carbohydrate reserves can emerge from greater depths of burial (Baskin and Baskin, 1998). On the other hand, small-seeded species such as *Sida rhombifolia* may have insufficient energy reserves to support hypocotyl elongation from deeper depths. Previous studies suggest that decreased germination at depth may be due to raised CO_2 derived from soil biological activity and slower gas diffusion, which is inversely correlated with burial depth (Benvenuti *et al*., 1994).

2.4. SEED STORAGE

Abdelmagid and Osman (1975) reported that the germination percentage of the cotton seeds decreased during 16 months of storage and results indicated that the deterioration in the viability of the seeds was associated with a decline in oil content.

Stewart and Duncan (1976) observed that seed lot of *Gossypium thurberi*, produced in 1931, had 64.00 per cent germination in 1968 and 39.00 per cent in 1974.

Thiagarajan and Krishnasamy (1985) reported that fumigated seeds stored in cloth bags showed higher germination percentage and vigour than when stored in polythene bags or paper-aluminium foil and polythene-laminated pouches. However, the latter containers were superior to cloth bags for non-fumigated seeds.

Hasanah (1987) compared the germination response in cotton seeds packed in plastic bags made of low, medium or high density polyethylene (0.85, 0.86 or 0.89 g/cm^3 respectively). Viability of seed was optimum using polyethylene bags of medium (85.00 µm) thickness.

Bhale *et al*. (1988) observed that cotton seeds stored for over 90 days showed an increase in the percentage of hard seeds but storage for over 180 days resulted in a reduced hard seed percentage. Near normal germination percentages were observed in seed stored for over 120 days.

Kausal and Patil (1989) reported that tin bins and cloth bags were better containers for maintaining viability and seed vigour of cotton. The acid-delinted seeds of cv. AKH-4 and SRT-1 can be safely stored for up to 28 and 33 months, respectively, under ambient conditions.

Bandopadhyay and Mahapatra (1990) found that seeds of *Hibiscus cannabinus* and *Hibiscus sabdariffa* stored in polythene bags and airtight glass bottles

under ambient conditions for 2-8 months were superior to those stored in cloth bags and paper packets in terms of percentage germination and seedling vigour. Treatment of seeds after storage for five months with low concentrations of iodine or chlorine vapour for 8-16 h increased viability and seedling vigour in accelerated aging tests and during subsequent storage under ambient conditions for two months; while treatment for 24 h showed some inhibitory effects.

Askari *et al*. (1995) reported that seeds of *Hibiscus sabdariffa* collected from the harvested crop showed a gradual decrease of seed viability for subsequently collected seeds. Seeds of *Hibiscus subdariffa* stored in glass jars or in controlled atmosphere chambers could be stored for four years without large increases in moisture content and without marked deterioration of viability (decrease of zero and five per cent in chambers and jars, respectively) and germination percentage (decrease of eight and 14 per cent in chambers and jars, respectively) (Ghosh and Das, 1997).

Doijode (1997) studied the germination behaviour of okra seeds of cv. Pusa Sawani. The polyethylene bags were effective in maintaining high viability at 5º C and could be used for seed storage, while aluminum foil pouches are suitable for the long term storage of seeds especially at sub-zero temperatures. Okra seed viability and germination reached its maximum after six months of storage in all the cultivars, thereafter, it declined gradually. Mean germination percentages were 82.80, 91.70, 78.40, 72.00 and 63.40 per cent at 0, 6, 12, 18 and 24 months of storage, respectively (Narwal *et al*., 1998). Suitability of pre-storage dry treatment with bleaching powder at 2 g/kg of seed in large sized okra seed was suggested for improved storability and field performance (Guha and Mandal, 2011). Seed treatments with red chilli powder and bleaching powder as well as mid-storage soaking-drying treatment may be suggested for the maintenance of germinability during storage and field performance of okra (Guha *et al*., 2012).

Galindez *et al*. (2010) reported that after four years of dry storage, a high proportion of *Collaea argentina* seeds were able to germinate, whereas *Abutilon pauciflorum* seeds continued to be dormant but were more sensitive to dormancy-breaking treatments.

Olasoji *et al*. (2012) reported that *Hibiscus cannabinus* seed deterioration during storage could be minimized by proper harvest timing. Seeds harvested five weeks after flowering and stored at 10º C showed the highest seed viability.

Kehinde *et al*. (2013) suggested that seed storage under ambient conditions should not exceed three months for best performance of amaranth seeds.

Materials and Methods

MATERIALS AND METHODS

The present investigation "Seed production potential, dormancy and seed storage behaviour in *Sida* spp." was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2012-2014.

The experimental site was located at an altitude of 22.50 m above M.S.L between $10^{o}32'$ N latitude. The location experiences a warm humid tropical climate. The soil of the experimental area comes under textural class of sandy clay loam and is acidic in reaction. The average monthly values of the meteorological parameters *viz*. rainfall, maximum and minimum temperatures and relative humidity were collected from the observatory attached to College of Horticulture and are presented in the Appendix-1.

The study consisted of the following four experiments

1. Assessment of seed production potential in *Sida* spp.
2. Characterization of dormancy behaviour in *Sida* spp.
3. Treatments for improving seed germination in *Sida alnifolia*
4. Seed storage studies in *Sida alnifolia*

The materials used and the methods adopted for the studies are briefly described below.

3.1. ASSESSMENT OF SEED PRODUCTION POTENTIAL IN *Sida* spp.

3.1.1. Seed collection and sowing

Seeds of three *Sida* species *viz*. *Sida acuta*, *Sida alnifolia* and *Sida cordifolia* (Plates 1-3) were collected from Vellanikkara campus of Kerala Agricultural

Young plant

Grown up plant

Flower

Fruits

Seeds

Plate 1. Habit of *Sida alnifolia* L. (Arrowleaf Sida)

Young plant

Grown up plant

Flower

Fruits

Seeds

Plate 2. Habit of *Sida acuta* Burm. (Common wireweed)

Young plant

Grown up plant

Flower

Fruits

Seeds

Plate 3. Habit of *Sida cordifolia* L. (Country mallow)

University. Seeds were pre-treated with Conc. H_2SO_4 for 20 min and sown on raised beds of 2 × 2 m at a spacing of 70 × 70 cm accommodating nine plants per bed. As growth advanced, thinning was done to maintain six plants per bed at three months.

The plants were raised as a rain-fed crop adopting uniform and minimum cultural operations. Application of FYM (5 kg bed^{-1}) as basal dose during bed preparation, two hand weedings at first and second month followed by earthing up and mulching with coirpith after second earthing up were done. The following observations were recorded from the five plants in three species.

3.1.2. Plant height

The height of the main stem was measured from collar region to tip of the stem in cm, during second, fourth and eighth month.

3.1.3. Primary branches per plant

Number of primary branches emerging from the main stem of the experimental plants was counted at second, fourth and eighth month.

3.1.4. Anthesis

The time of anthesis in the three species was recorded.

3.1.5. Days for first flowering

Number of days taken for first flowering in the three species was recorded from the date of sowing.

3.1.6. Days for first fruit harvest

Number of days taken for first plucking of matured fruits from the plants of three species was recorded from the date of sowing.

3.1.7. Number of fruits per plant

Number of fruits produced on the five observational plants, every week was recorded and the mean was computed to arrive at the fruits per plant.

3.1.8. Hundred fruit weight

Matured fruits were hand picked, at weekly interval and the total weight was recorded in grams.

3.1.9. Fruit yield per plant

The weight of fruits produced on the five observational plants, at weekly interval was recorded and the mean was computed to arrive at fruit yield per plant.

3.1.10. Number of seeds per fruit

Number of seeds produced from the single fruit was counted to arrive at the number of seeds produced per fruit.

3.1.11. Number of seeds per plant

Number of seeds obtained from the fruits produced on the five observational plants, at weekly interval was recorded and the mean was computed to arrive at the number of seeds produced per plant.

3.1.12. Hundred seed weight

Seeds extracted from the mature fruits were dried to a moisture content of eight per cent. Hundred seeds were selected and the weight was recorded in grams.

3.1.13. Seed yield per plant

Total weight of seeds collected from individual plants, was recorded in grams.

3.1.14. Seed yield per hectare

Seed yield per hectare was calculated from net seed yield per plant and expressed in kilograms.

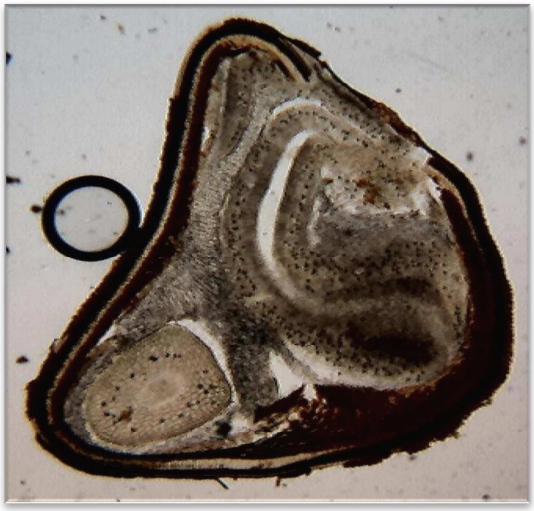
3.1.15. Seed rain

Seed rain was estimated by counting the number of schizocarps matured over the season / weeks in plants selected at random.

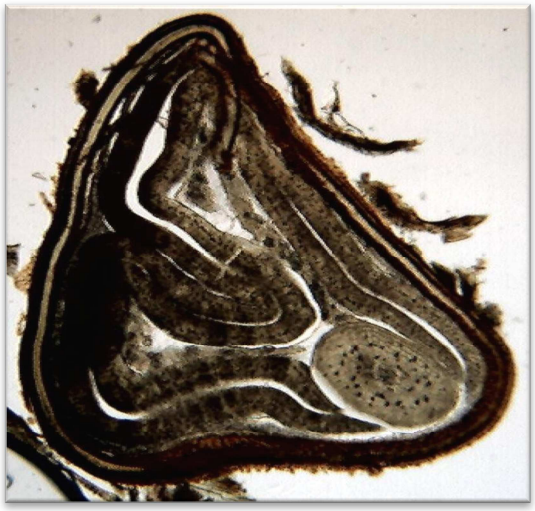
3.2. CHARACTERIZATION OF DORMANCY

3.2.1. Anatomical studies in *Sida* spp.

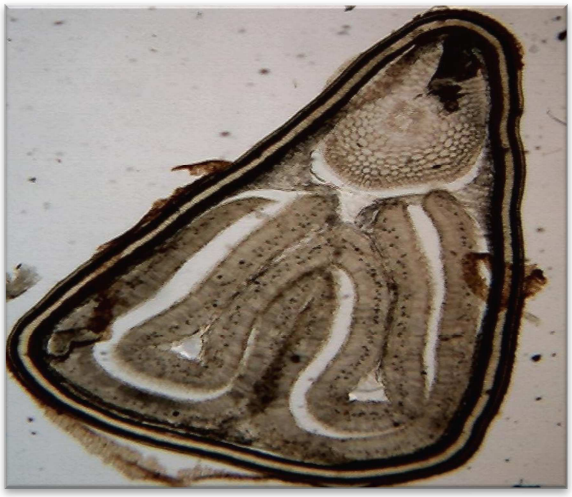
Anatomy of the seed coat was studied in three species (Plate 4 and 5). Due to small size, seeds were fixed in microtip, tightened from backside with tissue paper and sections were taken using a sliding microtome, Leica SM 2000 R at the Department of Wood Science, College of Forestry, Vellanikkara. The slides were made permanent using Dibutyl Phathalate Xylene (DPX) as a mounting medium and were examined under a digital microscope (LABOMED Digi 2 1500) attached to an image analyzer system using the software Digi pro, version 4.0. The seed coat



***Sida alnifolia* L.**



***Sida acuta* Burm.**



***Sida cordifolia* L.**

Plate 4. Longitudinal sections of seeds of *Sida* spp.

Embryo

Cut open seed exposing hard seed coat

Plate 5. Stereomicroscopic view of embryo and cut open seed of *Sida alnifolia*

thickness was noted by measuring thickness of the epicarp, mesocarp and endocarp and cuticle layer separately as well as total thickness of these layers. The seed perimeter, area and perimeter of the embryo were also recorded. Four measurements were taken from five sections of each seed and averages of four seeds were noted. The thickness and perimeter were expressed in µm and mm respectively, under 10 X magnification.

3.2.2. Physiological studies in *Sida* spp.

Fresh seeds of the three species, twenty five seeds in four replications were subjected to the following conditions and germination was recorded upto 14 days.

3.2.2.1. Exposure to varying temperature regimes

Seeds kept in petriplates were subjected to different temperature exposures *viz.* 30º C, 35º C and 40º C for 72 h (three days) in Biological Oxygen Demander (BOD). The exposed seeds were taken out and sown in sterilized petriplates after overnight water imbition and without imbibition. The seeds were kept under ambient conditions and moistened daily using hand sprayer.

3.2.2.2. Exposure to light and dark

Seeds sown in sterilized petriplates were exposed to eight hours of light and 16 h of darkness.

3.2.2.3. Exposure to stress

3.2.2.3.1. Hydration and dehydration

Seeds were subjected to hydration for 48 h followed by dehydration for 48 h and dried at room temperature to the initial weight of the seeds and sown in petriplates.

3.2.2.3.2. Hydration followed by storage

One lot of hydrated and dehydrated seeds was stored for four months and same procedure as in 3.2.2.3.1 was repeated and germination was recorded.

3.2.2.4. Germination of fresh and stored seeds of Sida spp.

Seeds of three *Sida* species both fresh and stored for one year under ambient condition, with and without pre-treatment with Conc. H_2S0_4 for 20 min, were sown in plastic trays containing sand and observed for 45 and 14 days respectively.

3.2.2.5. Germination of Sida spp. *under field conditions*

One month old seeds of three *Sida* species *viz. Sida acuta*, *Sida alnifolia* and *Sida cordifolia*, hundred seeds in four replications were sown in the pots filled with solarized potting media, lightly covering the seeds. Two sets were maintained. One set of pots was placed under open condition without watering and another set with regular watering. The observation on seed germination was recorded weekly upto one year.

3.2.3. Biochemical studies in *Sida* spp.

The content of total extractives, total alkaloid and total phenol in seeds of three *Sida* species were estimated (Plate 6). Seed leachates were extracted and the pH, EC, K, Na and Ca in the leachates were also estimated.

3.2.3.1. Total extractives content

Two grams of seed powder was reflexed with 125 ml of methanol and the extraction was continued till the solvent became colorless. The extract was transferred to a pre-weighed beaker and the solvent was evaporated and weight of the beaker along with the extract was noted and the recovery of extract was worked out and expressed in per cent (AOAC, 1980).

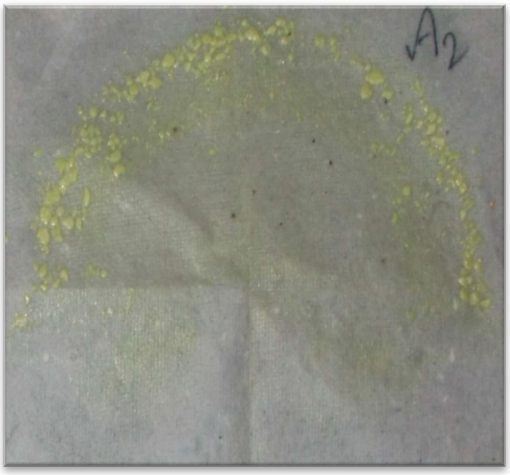
3.2.3.2. Total alkaloid content

Two grams of seed powder was weighed into a 250 ml beaker containg 80 ml of 10.00 per cent acetic acid in ethanol, kept covered and allowed to stand for four hours. Filtered the extract and concentrated on a water bath to bring down to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried, weighed and expressed as total alkaloid content in per cent (Harborne, 1973).

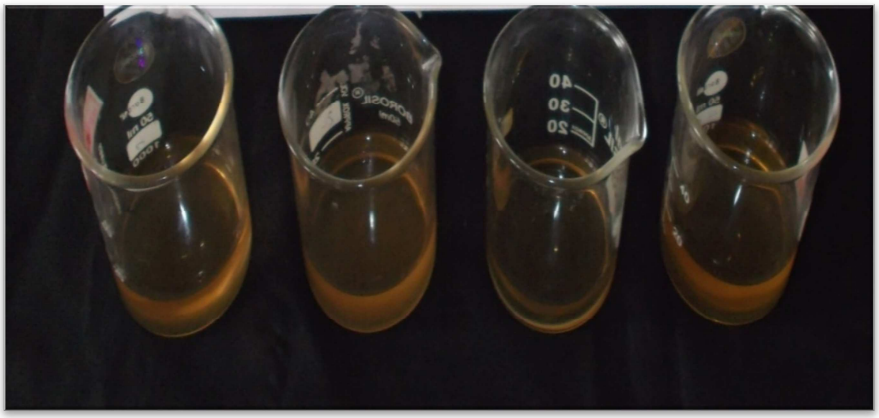
3.2.3.3. Total phenol content

One gram of seed powder was weighed and ground with a pestle and mortar. 10 ml of 80.00 per cent ethanol was added and centrifuged at 10,000 rpm for 20 min and re-extracted the residue with 05 ml of 80.00 per cent ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in a known volume of 05 ml distilled water. Pipetted out 0.2 ml in to test tubes. Made up the volume to 3 ml with distilled water. Added 0.5 ml of Folin-ciocalteau reagent. After three min, added 2 ml of 20 per cent Na_2CO_3 solution to each tube and mixed thoroughly. The tubes were placed in a boiling water bath for exactly one min, cooled and measured the absorbance at 650 nm against Folin cio-

Total phenol



Total alkaloid



Total extractives

Plate 6. Biochemical parameters

calteau reagent blank. Prepared a standard curve using different concentrations of pyrocatechol (Malick and Singh, 1980).

3.2.3.4. Extraction of seed leachates

Four replications of 25 seeds each were placed in beakers and washed in distilled water to remove all adhering dirt, soil or chemicals. The seeds were then soaked in 50 ml of distilled water for 24 h by occasionally stirring the contents. The beakers containing soaked seeds were covered to reduce evaporation and other probable contamination. The seed leachates were filtered and collected in 50 ml beaker. The pH, EC and contents of K, Na and Ca in the leachates were estimated.

3.2.3.4.1. pH analysis

The pH of the leachates solution was measured in a pH meter and recorded on a millivolt meter calibrated in pH units (Jackson, 1973).

3.2.3.4.2. Electrical conductivity

The electrical conductivity of the seed leachates was recorded in dS m^{-1} using EC meter (Jackson, 1973).

3.2.3.4.3. Potassium and sodium

The K and Na content of the leachates was measured using flame photometer and the values were expressed in ppm (Stanford and English, 1949).

3.2.3.4.4. Calcium

Calcium content of the leachates was estimated using Atomic Absorption Spectrophotometer (AAS) (Piper, 1996).

3.3. TREATMENTS FOR IMPROVING GERMINATION

Seeds of *Sida alnifolia* were collected from KAU campus during December, 2012 and January, 2013. Three months old seeds were sown in sterilized petriplates and kept under laboratory conditions, after subjecting the seeds to treatments indicated below (Plate 7). Seeds of treatments T_1 to T_{14} were subjected to overnight water soaking after the treatment. In the soaking treatments, constant seed water ratio was maintained. The petriplates were kept under observation for 45 days and germination percentage and speed of germination was noted.

Treatments for improving germination

T_1: Abrasion with hard sand paper
T_2: Conc. H_2SO_4 treatment for 05 min
T_3: Conc. H_2SO_4 treatment for 10 min
T_4: Conc. H_2SO_4 treatment for 20 min
T_5: Conc. H_2SO_4 treatment for 30 min
T_6: Boiling water treatment for 05 min
T_7: Hot water treatment at 90^oC for 05 min
T_8: Hot water treatment at 80^oC for 05 min
T_9: Hot water treatment at 70^oC for 05 min
T_{10}: Hot water treatment at 60^oC for 05 min
T_{11}: Hot water treatment at 50^oC for 05 min
T_{12}: Hot water treatment at 40^oC for 05 min
T_{13}: Treatment with boiling water for 05 min followed by freezing
T_{14}: Abrasion with hard sand paper followed by soaking in 1 per cent KNO_3 for 12 h
T_{15}: Soaking in water for 48 h
T_{16}: Making pin pricks followed by soaking in water for 48 h
T_{17}: Overnight soaking in cow dung slurry

T_{18}: Overnight water soaking

T_{19}: Control (untreated)

Design - CRD

Number of treatments - 19

Number of replications - 04

Seeds per replication - 25

3.3.1. Scarification treatments

The best four treatments from the above experiment were selected and compared with untreated control. Three months old seeds were subjected to the respective treatments and sown in the plastic trays containing sand and below mentioned parameters were recorded (Plate 8).

3.3.1.1. Germination percentage

The mean number of normal seedlings produced to the total number of seeds sown was expressed as germination percentage.

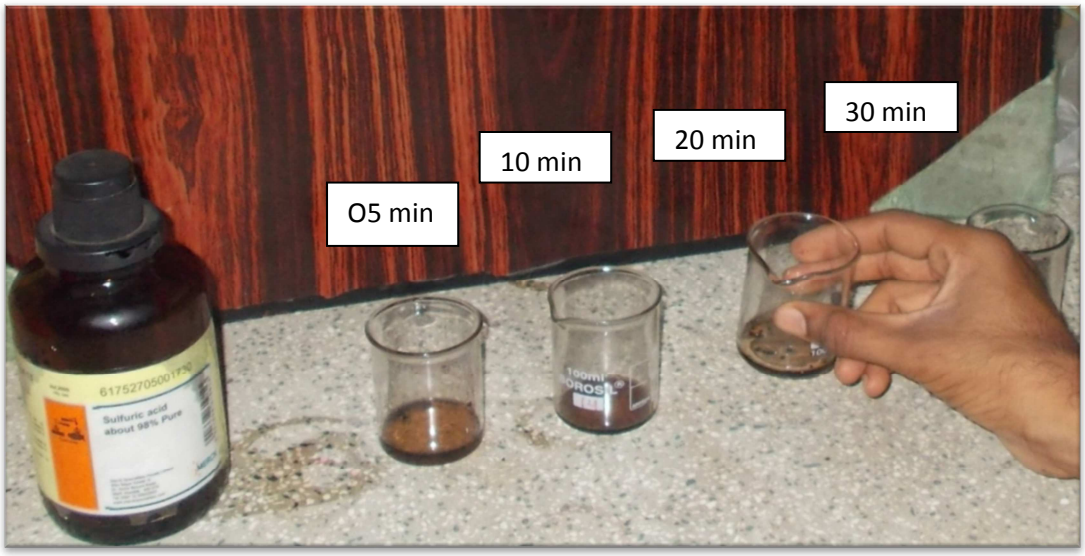
3.3.1.2. Speed of germination

From the samples sown for seed germination, number of seedlings emerged was recorded daily until the 14^{th} day of the emergence of first seedling. The condition wherein cotyledons slipping out of seed coat was taken as the criteria for emergence of normal seedling. From the mean germination percentage recorded on each counting date, speed of germination was calculated employing the following formula suggested by Maguire (1962).

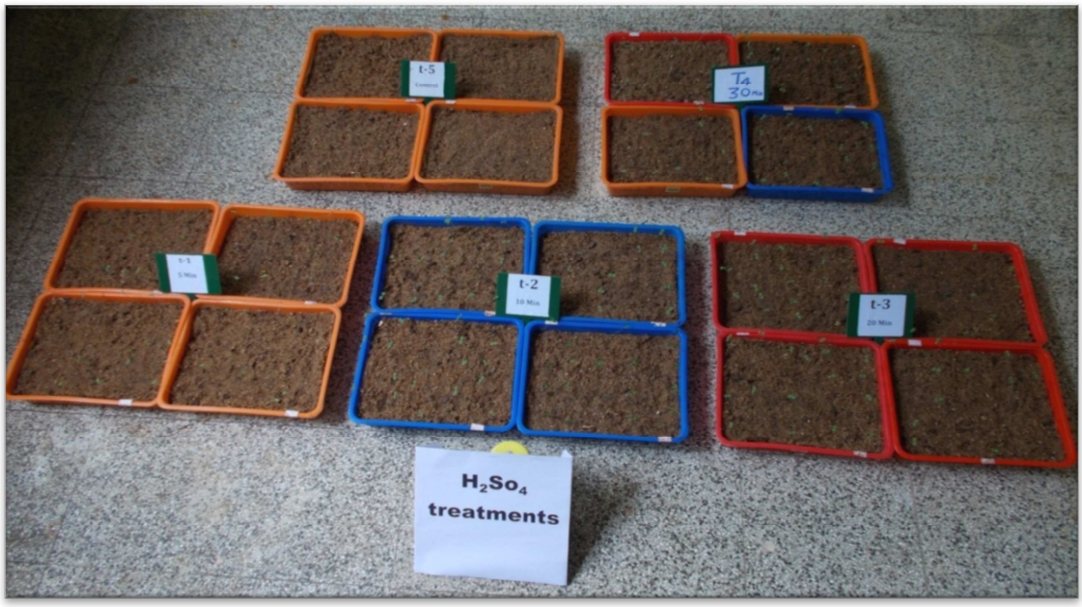
Nineteen pre-treatments

Untreated seeds **Treated seeds**

Plate 7. Seeds kept for germination after pre-treatment



Seed treatment with Conc. H_2SO_4



Germination of seeds after acid scarification

Plate 8. Acid scarification of seeds of *Sida alnifolia*

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots\ldots\ldots\ldots \frac{Xn - Xn\text{-}1}{Yn}$$

Where, Xn = Per cent germination on n^{th} day

Yn = Number of days from sowing to n^{th} count

3.3.1.3. Dormancy index

The speed of release of dormancy was estimated by counting number of seeds showing dormancy release on each day after sowing until the 14^{th} day. From this data, the dormancy index was calculated using the formula,

$$\text{Dormancy index} = \sum (Ni / Di)$$

Where, Ni= Number of seeds showing dormancy release on the i^{th} day

Di= Days after harvest

3.3.1.4. Intensity of dormancy

The number of non germinated seeds in the test at seven days after sowing (NGS_7) and 14 days after sowing (NGS_{14}) was noted, the mean number of non germinated seeds to the total number of seeds sown was expressed as intensity of dormancy in percentage (Swain *et al*., 2001).

3.3.1.5. Root length of the seedling

Ten normal seedlings were carefully uprooted at random from the test sample on the 14^{th} day; root length of individual seedlings was measured and expressed in cm.

3.3.1.6. Shoot length of the seedling

From the sample, after measuring root length, the length between collar region and tip of the leaf was measured in cm and the mean value was recorded as shoot length.

3.3.1.7. Total length of the seedling

The sum of shoot and root length value was recorded as total length of the seedling.

3.3.1.8. Fresh weight of seedling

Fresh weight of the uprooted seedlings was recorded and expressed in grams.

3.3.1.9. Dry weight of seedling

Ten normal seedlings were air dried initially for six hours and then in hot air oven maintained at 105^{o} C for 24 h dried seedlings were cooled for 45 min and the dry weight of a single seedling was calculated in grams.

3.3.1.10. Vigour index-I

Vigour index-I was computed adopting the formula suggested by Abdul-Baki and Anderson (1972) and expressed as whole number.

Vigour index-I = Per cent germination × Mean seedling root and shoot length.

3.3.1.11. Vigour index-II

Vigour index-II was computed, adopting the formula suggested by Bewley and Black (1994).

Vigour index-II = Percent germination × Seedling dry weight in g.

3.3.1.12. Water imbition rate

Mean weight of ten seeds was recorded as initial seed weight. Then the seeds were soaked in distilled water. After three hours, the seeds were taken out, dried between two Whatman No.1 filter paper and seed weight was recorded accurately. The seeds were kept back in the beaker containing water and the process was repeated after 6, 12 and 24 h. The amount of water imbition was determined as actual increase in seed weight and converted to percentage using the formula.

$$\text{Per cent water imbition} = [(W_i - W_d)/W_d] \times 100$$

Where, W_i and W_d are masses of imbibed and dry seeds respectively.

3.3.1.13. Electrical conductivity

The electrical conductivity of the seed leachates was measured in dS m^{-1} using EC meter (Jackson, 1973).

3.4. SEED STORAGE STUDIES

Moisture content of fresh seeds was brought down to four to five per cent and the seeds were stored for one year in self sealing polythene bags at ambient, refrigerated (6-7^{o} C) and freezer (0^{o} C) conditions (Plate 9). Samples were drawn at four months interval, sown in plastic trays containing sand, with and without pre-treatment with Conc. H_2SO_4 for 5, 10, 20 and 30 min. The same parameters as in 3.3.1 were recorded.

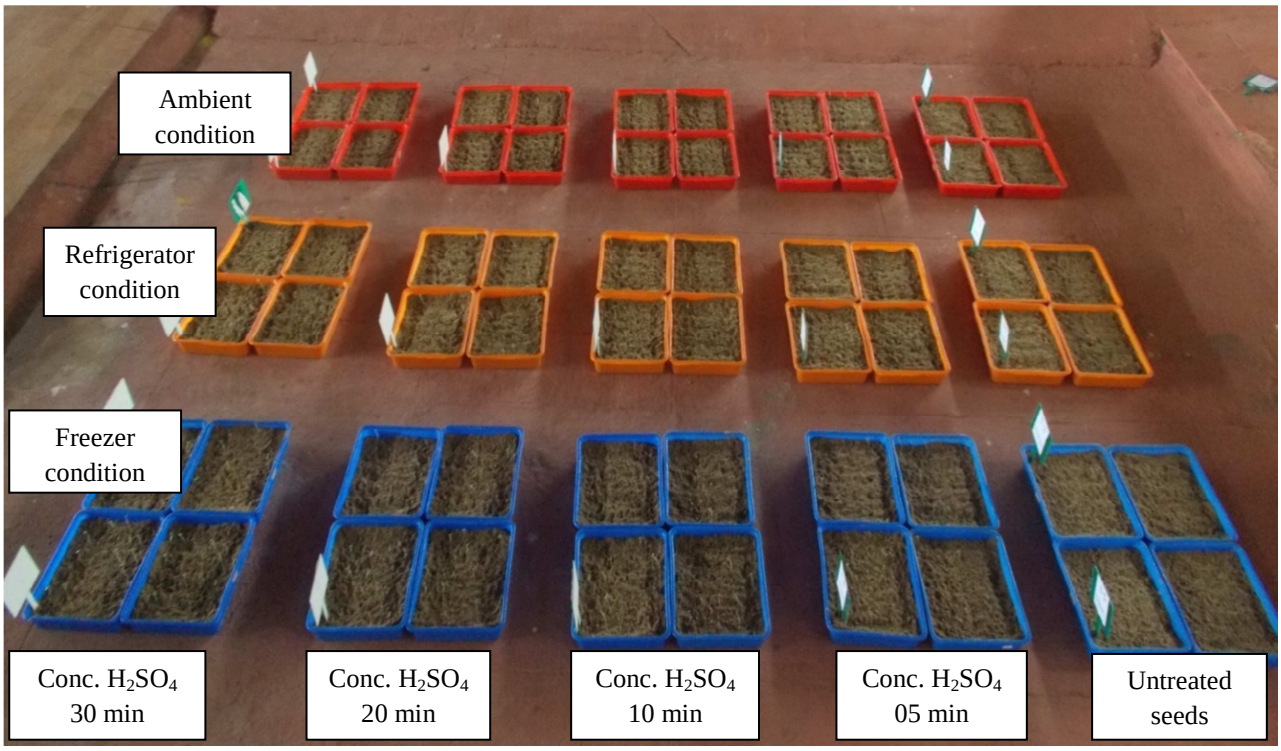


Plate 9. Seed germination under seed storage studies in *Sida alnifolia*

3.5. STASTICAL ANALYSIS

Analysis of variance was performed on the data collected from the experiments using Stastical Packages, MSTAT and multiple comparisons among the treatments were done using Duncan's Multiple Range Test (DMRT).

Results

RESULTS

The present investigations were carried out to assess the seed production potential and to characterize dormancy in *Sida* species *viz*. *Sida acuta*, *Sida alnifolia* and *Sida cordifolia*. Pre-treatments to improve the germination and seed storage behaviour in*Sida alnifolia* were also studiedThe experimental data collected were . tabulated, analyzed statistically and the results are presented in this chapter.

4.1. ASSESSMENT OF SEED PRODUCTION POTENTIAL

Plants of *Sida alnifolia*, *Sida acuta* and *Sida cordifolia* were raised in the field from seeds pre-treated with Conc. H_2SO_4 for 20 min. Growth at second, fourth and eighth month were recorded. Flowering, fruit and seed yield parameters of three species were also recorded and are presented in Tables 1, 2 and 3.

4.1.1. Plant height

The height of the plant differed greatly among the three species during second, fourth and eighth month, which corresponds to vegetative, flowering, seed setting and seed harvest stages respectively. *Sida acuta* recorded the maximum plant height at second month (26.77 cm) followed by *Sida alnifolia* (22.79 cm) and *Sida cordifolia* (17.20 cm). *Sida alnifolia* recorded the maximum plant height at fourth month (93.20 cm) followed by *Sida acuta* (83.00 cm) and *Sida cordifolia* (74.20 cm). The same trend was observed during eighth month also with *Sida alnifolia* recording the maximum plant height (163.00 cm).

4.1.2. Number of Primary branches

Number of primary branches varied greatly among the three species. *Sida alnifolia* recorded highest number of branches at second month (12.50), followed by *Sida acuta* (12.40) and the least was for *Sida cordifolia* (9.20). Similar trend was

seen during the fourth and eight months. The difference in number of primary branches was significant for all the stages except for the second month where *Sida acuta* and *Sida alnifolia* were on par.

4.1.3. Anthesis

Anthesis in the species started at 7:30 am in *Sida acuta*, 8:00 am in *Sida alnifolia* and *Sida cordifolia* was the last for flower opening (8:15 am).

4.1.4. Days for flowering

There was significant difference among the species with respect to days for flowering from the date of sowing. Blooming was noticed in October for *Sida acuta* (120 days) and *Sida cordifolia* (131 days) whereas *Sida alnifolia* bloomed during November (142 days).

4.1.5. Days for first fruit harvest

Significant difference was observed among the species with respect to days for the first fruit harvest from the date of sowing. Harvesting of fruits started early in *Sida acuta* during October (137 days) followed by *Sida cordifolia* (149 days) and *Sida alnifolia* (160 days) during November. Harvesting was stopped during the fortnight of January (186 days).

4.1.6. Number of fruits per plant

There was significant difference among the species with respect to number of fruits per plant. *Sida acuta* registered highest fruits per plant (3825.80), followed by *Sida alnifolia* (1919.97) and lowest for *Sida cordifolia* (1459.94).

4.1.7. Hundred fruit weight

Hundred fruit weight differed significantly among the species. *Sida cordifolia* (5.27 g) had significantly bigger fruits, whereas fruit weights were on par in *Sida acuta* (2.40 g) and *Sida alnifolia* (2.20 g).

4.1.8. Fruit yield per plant

There was significant difference among the species with respect to fruit yield per plant. Among the three species, highest fruit yield was obtained from *Sida acuta* (91.86 g) followed by *Sida cordifolia* (76.96 g) and the least in *Sida alnifolia* (42.26 g).

4.1.9. Number of seeds per fruit

Among the species with respect to number of seeds per fruit. *Sida cordifolia* produced nine seeds per fruit, whereas *Sida alnifolia* and *Sida cordifolia* produced six seeds each.

4.1.10. Number of seeds per plant

There was significant difference among the species with respect to number of seeds produced per plant. *Sida acuta* registered more seeds per plant (23005.78), whereas *Sida alnifolia* (11520.53) and *Sida cordifolia* (10043.84) were on par with respect to seed production potential.

4.1.11. Hundred seed weight

There was significant difference among the species with respect to hundred seed weight (Plate 10). Seeds of *Sida cordifolia* (0.48 g) were bolder followed by *Sida acuta* (0.33 g) and *Sida alnifolia* (0.30 g).

***Sida alnifolia* L.**

***Sida acuta* Burm.**

***Sida cordifolia* L.**

Plate 10. Seed size in *Sida* spp.

4.1.12. Seed yield per plant

There was a significant difference among the species with respect to seed yield per plant. Since fruit yield is directly related to seed yield, the same trend as in fruit yield per plant was seen for seed yield per plant also with *Sida acuta* giving highest seed yield.

4.1.13. Seed yield per hectare

There was a significant difference among the species with respect to seed yield per hectare. *Sida acuta* (1562.65 kg) out yielded *Sida cordifolia* (1255.51 kg) and *Sida alnifolia* (713.87 kg).

4.1.14. Seed rain

Sida acuta started bearing early followed by *Sida cordifolia* and *Sida alnifolia*. The maximum number of weekly harvest of fruits (10) was made from *Sida acuta* followed by *Sida cordifolia* (09) and *Sida alnifolia* (06), yielding 76.57 g, 61.52 g and 34.98 g of seeds per plant respectively. Though there was potential of a few more weeks of harvest but, exposure of plants to hot summer (February) and lack of rain, plants started wilting (natural silencing) and seed development was arrested leading to no more further seed harvests.

4.2. CHARACTERIZATION OF DORMANCY

4.2.1. Physical characterization of dormancy

4.2.1.1. Seed coat thickness of three Sida spp.

Seed coat thicknesses of the three species in case of fresh and seeds stored for one year and scarified seeds of *Sida alnifolia* were studied (Table 4). Fresh seeds of *Sida cordifolia* recorded the maximum thickness of endoderm (46.16 µm), mesoderm

Table 1. Vegetative characters of *Sida* spp. at different stages of growth

Characters	Month	*Sida alnifolia*	*Sida acuta*	*Sida cordifolia*
Plant height (cm)	02	22.79^{b}	26.77^{a}	17.20^{c}
	04	93.20^{a}	83.00^{ab}	74.20^{b}
	08	163.00^{a}	105.90^{b}	97.80^{b}
No. of primary branches	02	12.50^{a}	12.40^{a}	9.20^{b}
	04	45.70^{a}	30.90^{b}	24.70^{c}
	08	71.90^{a}	38.00^{b}	31.50^{c}

Values having common superscript are not significantly different from each other.

Table 2. Flowering and fruiting characters of *Sida* spp.

Characters	*Sida alnifolia*	*Sida acuta*	*Sida cordifolia*
Anthesis (am)	$8:00^{b}$	$7:30^{a}$	$8:15^{b}$
Days for first flowering	142^{c}	120^{a}	131^{b}
Days for the first fruit harvest	160^{c}	137^{a}	149^{b}
Number of fruits per plant	1919.97^{b}	3825.8^{a}	1459.94^{c}
Hundred fruit weight (g)	2.20^{b}	2.40^{b}	5.27^{a}
Fruit yield per plant (g)	42.26^{c}	91.86^{a}	76.96^{b}
Number of seeds per fruit	06	06	09
Number of seeds per plant	11520.53^{b}	23005.78^{a}	10043.84^{b}
Hundred seed weight (g)	0.30^{b}	0.33^{b}	0.48^{a}
Seed yield per plant (g)	34.98^{c}	76.57^{a}	61.52^{b}
Seed yield (kg ha^{-1})	713.87^{c}	1562.65^{a}	1255.51^{b}

Values having common superscript are not significantly different from each other.

Table 3. Number of seed rain and seed yield in *Sida* spp.

No. of seed rains	*Sida alnifolia*			*Sida acuta*			*Sida cordifolia*		
	Fruit yield (g)	**Seed yield (g)**	**No. of seeds**	**Fruit yield (g)**	**Seed yield (g)**	**No. of seeds**	**Fruit yield (g)**	**Seed yield (g)**	**No. of seeds**
1	-	-	-	2.34	1.95	586.76	-	-	-
2	-	-	-	3.85	3.21	966.17	1.60	1.28	209.81
3	-	-	-	5.97	4.96	1489.82	5.64	4.51	736.03
4	-	-	-	8.32	6.94	2084.10	9.02	7.21	1177.66
5	3.75	3.11	1016.58	11.97	9.98	2998.69	11.74	9.39	1532.23
6	7.58	6.28	2052.92	19.95	16.64	4997.40	14.91	11.92	1946.06
7	10.77	8.92	2916.09	16.36	13.64	4097.84	12.15	9.72	1586.00
8	8.66	7.17	2346.17	10.85	9.05	2718.20	10.36	8.28	1352.14
9	7.02	5.81	1900.22	8.06	6.72	2019.74	6.73	5.38	878.54
10	4.46	3.69	1207.55	4.18	3.48	1047.06	4.79	3.83	625.37
Total	42.26	34.98	11520.53	91.86	76.57	23005.78	76.96	61.52	10043.84

(47.86 µm), periderm (40.22 µm) and total thickness (151.61 µm) and maximum perimeter (8.41 mm) followed by *Sida acuta* and *Sida alnifolia*. *Sida alnifolia* showed maximum cuticle thickness (18.64 µm) followed by *Sida acuta* (18.43 µm) and *Sida cordifolia* (17.36 µm). In case of stored seeds also, seed coat thickness followed the same trend with *Sida cordifolia* having highest thickness of various layers except for cuticle thickness, whereas *Sida acuta* recorded highest cuticle thickness. Upon storage for one year, substantial reduction in thickness of various layers was observed, irrespective of the species, except for cuticle thickness which showed an increasing trend. Scarified seeds of *Sida alnifolia* showed reduction in all layers of the seed coat with maximum reduction in cuticle thickness.

4.2.1.2. Embryo area and perimeter of three Sida spp.

Embryo area and perimeter of fresh and stored seeds followed reverse trend as in the seed coat thickness and perimeter, with *Sida alnifolia* having the highest values followed by *Sida cordifolia* and *Sida acuta* (Plate 11). Upon storage for one year, the embryo area and perimeter increased slightly, irrespective of the species (Table 5).

4.2.2. Physiological characterization of dormancy

4.2.2.1. Exposure to different temperatures

Imbibed and non-imbibed fresh seeds of the three species were exposed to different temperatures, 30^{o} C, 35^{o} C and 40^{o} C for 72 h. One per cent germination was recorded in imbibed seeds of *Sida cordifolia* at 40^{o} C whereas *Sida acuta* and *Sida alnifolia* failed to germinate. Non imbibed seeds of three species did not germinate at different temperatures (Table 6).

Table 4. Seed coat thickness of fresh, stored and sulphuric acid treated seeds in *Sida* spp.

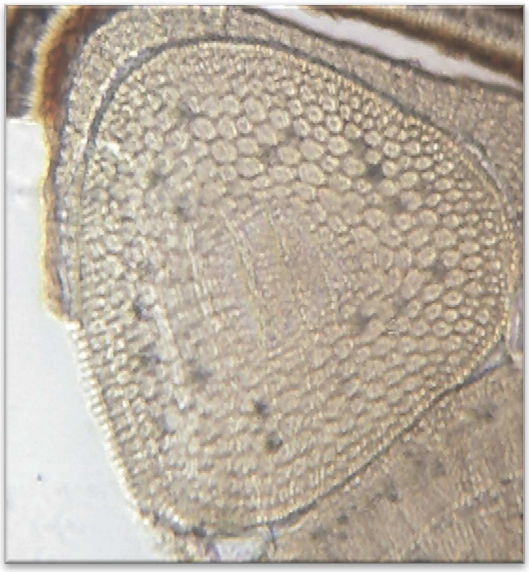
Treatments	Species	Endoderm (µm)	Mesoderm (µm)	Periderm (µm)	Cuticle (µm)	Total thickness (µm)	Perimeter (mm)
Fresh Seeds	*Sida alnifolia*	41.30[a]	34.33[b]	32.55[b]	18.64[a]	126.82[c]	7.35[b]
	Sida acuta	44.99[a]	38.44[b]	33.61[b]	18.43[a]	135.47[b]	7.64[b]
	Sida cordifolia	46.16[b]	47.86[a]	40.22[a]	17.36[a]	151.60[a]	8.41[a]
Stored seeds (1 year)	*Sida alnifolia*	34.18[b]	33.16[b]	30.42[a]	20.02[a]	117.78[b] (7.12)	7.17[b] (2.44)
	Sida acuta	39.63[a]	37.04[a]	30.77[a]	20.13[a]	127.57[a] (5.83)	7.33b (4.05)
	Sida cordifolia	43.40[a]	44.87[a]	37.04[a]	19.65[a]	144.96[a] (4.37)	8.01[a] (4.75)
Sulphuric acid treated seeds	*Sida alnifolia*	39.75	33.23	33.06	9.215 (50.56)	115.25 (9.12)	6.95 (5.44)

Values having common superscript are not significantly different from each other.
Values in brackets indicate per cent reduction

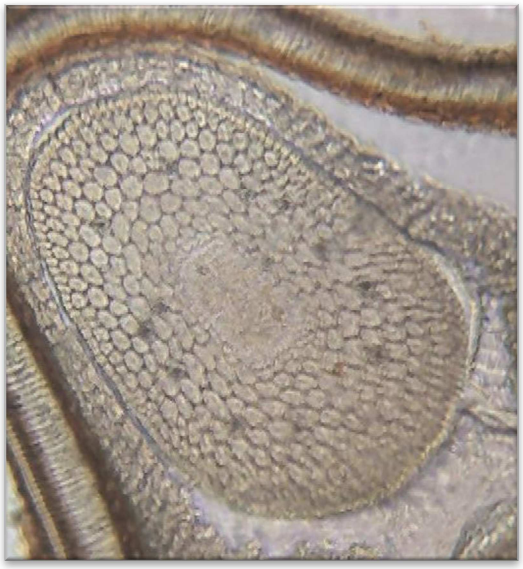
Table 5. Embryo area and perimeter of fresh and stored seeds of *Sida* spp.

Treatments	Species	Embryo area (mm)	Embryo perimeter (mm)
Fresh seeds	*Sida acuta*	0.21[b]	1.60[b]
	Sida alnifolia	0.25[a]	1.83[b]
	Sida cordifolia	0.21[b]	1.68[a]
Stored seeds (1 year)	*Sida acuta*	0.23[b]	1.68[b]
	Sida alnifolia	0.26[a]	1.86[ab]
	Sida cordifolia	0.24[b]	1.73[a]

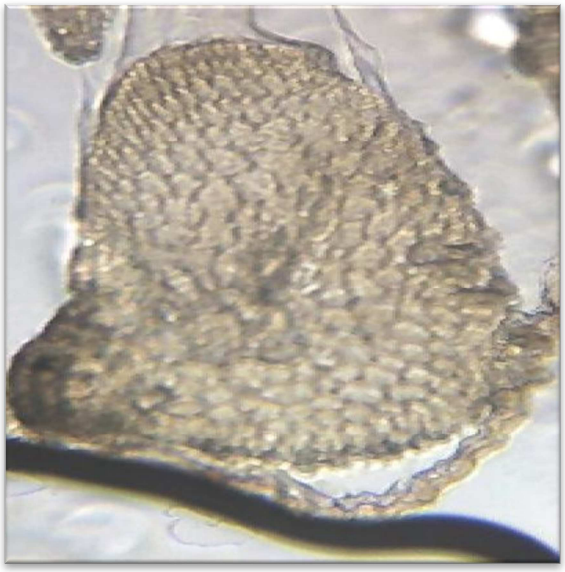
Values having common superscript are not significantly different from each other.



***Sida alnifolia* L.**



***Sida acuta* Burm.**



***Sida cordifolia* L.**

Plate 11. Longitudinal sections of embryo of *Sida* spp.

4.2.2.2. Light and dark exposure

Fresh seeds sown in sterilized petriplates exposed to eight hours of light and sixteen hours of darkness showed no germination response in the three species.

4.2.2.3. Exposure to stress

4.2.2.3.1. Hydration and dehydration treatments

Hydration and dehydration treatments tried in freshly harvested seeds, showed response only in the case of *Sida cordifolia*, giving one per cent germination (Table 6).

4.2.2.3.2. Hydration and dehydration treatments followed by storage

Hydrated and dehydrated seeds were stored for four months and the same seeds when hydrated and dehydrated before sowing, showed two per cent germination in *Sida cordifolia*, one per cent in *Sida acuta* and no germination at all in *Sida alnifolia*.

4.2.2.4. Germination of fresh and stored seeds

4.2.2.4.1. Germination, seedling growth and dormancy behaviour of fresh and stored seeds of Sida spp.

Irrespective of the species, seeds whether fresh or stored, failed to germinate without treatment. In the case of fresh seeds, even with Conc. H_2SO_4 scarification for 20 min, maximum germination per cent recorded was only two per cent for *Sida cordifolia* and one per cent each for *Sida alnifolia* and *Sida acuta* witnessing low speed of germination also. The growth parameters of seedlings were also low leading to low vigour index in the case of fresh seeds. There was no appreciable reduction in intensity of dormancy of fresh seeds which were scarified. Among the species,

Table 6. Effect of temperature, light and hydration treatments on fresh seeds germination in *Sida* spp.

Physiological conditions		Germination per cent		
		Sida alnifolia	***Sida acuta***	***Sida cordifolia***
Exposure to different temperatures (imbibed)	30^{o} C	0	0	0
	35^{o} C	0	0	0
	40^{o} C	0	0	1
Exposure to different temperatures (Non-Imbibed	30^{o} C	0	0	0
	35^{o} C	0	0	0
	40^{o} C	0	0	0
Light and dark exposure (8 h light, 16 h dark)		0	0	0
Hydration and dehydration treatments		0	0	1
Hydration and dehydration treatments (4 MAS)		0	1	2

MAS- months after storage

treating fresh seeds of *Sida cordifolia* gave slightly better performance with respect to germination (2.0 per cent), seedling growth and reduction of intensity of dormancy (98.00 per cent) (Table 7).

After storage for one year, seeds of *Sida cordifolia* failed to germinate even with acid scarification whereas *Sida acuta* and *Sida alnifolia* gave 48.00 and 33.40 % germination respectively. *Sida acuta* showed better performance with respect to germination per cent (48.00) and speed of germination (16.46), resulting in reduction of intensity of dormancy whereas growth of seedling was better for *Sida alnifolia*. The germination per cent, speed of germination, growth parameters of seedling and vigour index were improved upon storage of seeds for one year, whereas intensity of dormancy got reduced (Table 7).

4.2.2.4.2. Seed leachates of Sida spp. stored under ambient condition

Seed leachates of three *Sida* species stored for one year under ambient condition were analyzed for the content of minerals, pH and EC (Table 8). Among the three species, *Sida alnifolia* recorded significantly highest calcium (2.65 ppm) and sodium (3.20 ppm) contents followed by *Sida cordifolia* and *Sida acuta*. Significantly highest potassium (2.16 ppm) and EC (0.0031 dS m^{-1}) values were recorded for *Sida acuta* followed by *Sida alnifolia* and lowest for *Sida cordifolia*. pH values were on par for the three species but *Sida cordifolia* (6.20) recorded the highest value.

4.2.2.5. ***Germination of Sida*** **spp.** ***under field conditions***

Seeds of three *Sida* species (two months after collection) were sown in pots during February, 2013 and maintained under both rain-fed and irrigated condition for one year (January, 2014) (Plate 12 and 13). The data pertaining to germination per cent under two conditions of sowing, irrigated and rain-fed, are presented in Table 9.

Table 7. Effect of fresh and stored seeds of *Sida* spp. on germination, seedling growth and dormancy behaviour

Parameters	*Sida alnifolia*		*Sida acuta*		*Sida cordifolia*	
	Fresh seeds	Stored seeds	Fresh seeds	Stored seeds	Fresh seeds	Stored seeds
Germination per cent	1.00	33.33	1.00	48.00	2.00	0
Speed of germination	0.25	11.18	0.11	16.46	0.071	0
Seedling shoot length (cm)	1.62	6.26	1.60	4.90	2.05	0
Seedling root length (cm)	0.70	1.44	0.65	1.00	0.52	0
Seedling total length (cm)	2.32	7.70	2.25	5.90	2.57	0
Fresh weight of seedling (g)	0.0072	0.027	0.0070	0.020	0.0087	0
Dry weight of seedling (g)	0.0007	0.0028	0.0007	0.0021	0.0009	0
Vigour index 1	2.32	256.64	2.25	283.20	5.14	0
Vigour index 2	0.0007	0.093	0.0007	0.10	0.0018	0
Dormancy index	0.062	0.02	0.027	0.05	0.017	0
ID NGS 7 (%)	99	70.66	100	52.00	100	100
ID NGS 14 (%)	99	66.66	99	52.00	100	100
ID NGS 30 (%)	99	-	99	-	98	-
ID NGS 45 (%)	99	-	99	-	98	-

4.2.2.5.1. Sida alnifolia L.

In *Sida alnifolia*, germination started from February in irrigated condition and germination was continuous upto October, except for July. The highest germination per cent (4.25) was recorded after four months of sowing. The overall germination per cent in *Sida alnifolia* (14.50) recorded was higher than the *Sida acuta* (7.20 per cent) and *Sida cordifolia* (11.75 per cent). Germination of seeds under rain-fed condition started two days after receipt of the rain and germination was completed within seven days, giving a germination percentage of 67.50.

4.2.2.5.2. Sida acuta Burm.

In *Sida acuta*, seeds kept under irrigated condition started germination from the month of sowing itself and it was continuous upto September except during the month of June with the highest germination per cent (1.75) at second month after sowing. Overall germination per cent under irrigated condition was 7.20. With respect to pots kept under rain-fed condition, germination was spontaneous on receipt of rain during June, giving 74.00 per cent germination. Germination was completed within five days after the rain.

4.2.2.5.3. Sida cordifolia L.

In *Sida cordifolia*, emergence of seedlings started from February under irrigated condition. The germination was continuous upto November. Maximum germination per cent (1.75) was recorded during second and ninth month, with over all germination per cent of 11.75. With respect to rain-fed condition, germination started soon after receipt of rain and germination was completed within 10 days, with an overall germination percentage of 42.50, which was less than the other two species.

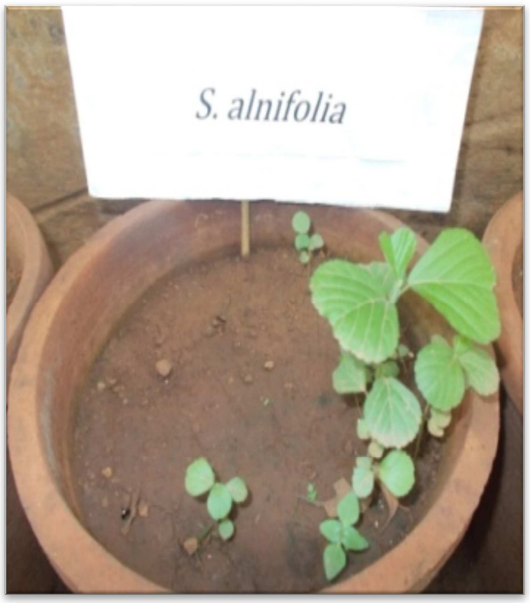
Table 8. Effect of storage on the seed leachates of *Sida* spp. under ambient conditions

Parameters	*Sida alnifolia*	*Sida acuta*	*Sida cordifolia*
Ca (ppm)	2.65^{a}	1.33^{b}	1.42^{b}
Na (ppm)	3.20^{a}	2.46^{b}	2.23^{b}
K (ppm)	1.96^{b}	2.16^{a}	1.78^{b}
pH	5.94^{a}	6.19^{a}	6.20^{a}
EC (dS m^{-1})	27.01^{b}	31.33^{a}	15.76^{c}

Values having common superscript are not significantly different from each other.

Table 9. Germination percentage of *Sida* spp. under irrigated and rain-fed conditions

Germination Percentage						
	Sida alnifolia		***Sida acuta***		***Sida cordifolia***	
Month	**Irrigated**	**Rain-fed**	**Irrigated**	**Rain-fed**	**Irrigated**	**Rain-fed**
February	2.50	0	1.25	0	1.25	0
March	1.50	0	1.75	0	1.75	0
April	3.00	0	1.50	0	1.00	0
May	4.25	0	0.75	0	0.50	0
June	2.00	67.50	0	74.00	1.50	42.50
July	0	0	0.75	0	1.50	0
August	0.50	0	0.75	0	1.50	0
September	0.25	0	0.50	0	0.25	0
October	0.50	0	0	0	1.75	0
November	0	0	0	0	0.75	0
December	0	0	0	0	0	0
January	0	0	0	0	0	0
Total	14.50	67.50	7.25	74.00	11.75	42.50



***Sida alnifolia* L.**

***Sida acuta* Burm.**

***Sida cordifolia* L.**

Plate 12. Seed germination of *Sida* spp. under irrigated system

***Sida alnifolia* L.**

***Sida acuta* Burm.**

***Sida cordifolia* L.**

Plate 13. Seed germination of *Sida* spp. under rain-fed system

4.2.4. Biochemical parameters

Biochemical parameters such as total extractives, total alkaloid and total phenol content of fresh seeds of three species and seeds of *Sida alnifolia* stored for one year are presented in Table 10.

Biochemical parameters of fresh seeds significantly differed among the three species. *Sida acuta* recorded maximum total extractives (8.87 per cent) and total alkaloid (1.85 per cent) followed by *Sida alnifolia* (8.12 per cent and 1.56 per cent) and *Sida cordifolia* (7.87 per cent and 1.45 per cent). Highest phenol content was recorded for seeds of *Sida alnifolia* (5.85 per cent) followed by *Sida acuta* (4.75 per cent) and *Sida cordifolia* (4.40 per cent). Stored seeds of *Sida alnifolia* showed significant reduction in the biochemical components with maximum reduction in alkaloid content (42.30 per cent) followed by phenol content (38.46 per cent) and total extractives (26.71 per cent).

4.3. PRE-TREATMENTS FOR IMPROVING GERMINATION

Eighteen pre-treatments comprising of mechanical scarification, chemical scarification, scarification followed by osmoticum treatment, hydration and boiling followed by freezing treatments were tried to soften the seed coat and these were compared with untreated control. Four months old seeds of *Sida alnifolia*, subjected to pre-treatments, were sown in sterilized petriplates with blotter paper and emergence of seedlings was noted up to 45 days (Plate 14). Effect of treatments on germination per cent and speed of germination in *Sida alnifolia* are presented in Table 11.

4.3.1. Germination per cent

Among the eighteen pre-treatments applied for softening the seed coat, chemical scarification treatments using Conc. H_2SO_4 were highly promising with respect to early emergence and maximum germination per cent.

Table 10. Biochemical parameters of seeds of three *Sida* spp.

Parameters	*Sida acuta*	*Sida cordifolia*	*Sida alnifolia*	
			Fresh seeds	**Stored seeds**
Total extractives (%)	8.87[a]	7.87[b]	8.12[b]	6.50[c] (26.71)
Total alkaloid (%)	1.85[a]	1.45[b]	1.56[b]	0.90[c] (42.30)
Total Phenol (%)	4.75[b]	4.40[b]	5.85[a]	3.60[c] (38.46)

Values having common superscript are not significantly different from each other.
Values in brackets indicate per cent reduction.

Germination of seeds

Seedlings

Plate 14. Germinated seeds and seedlings

Among the chemical scarification treatments, treatment with Conc. H_2SO_4 for a longer duration of 30 min was proved as the best and outstanding giving 94.00 per cent germination. Germination percentage was on par for treatments of 10 and 20 min duration with Conc. H_2SO_4 with 78.00 and 72.00 per cent respectively. Treatment for five minutes was ranked low (54.00 per cent) among the chemical scarification treatments. Mechanical scarification with sand paper has given 26.00 per cent germination, but scarification followed by osmoticum treatment (one per cent KNO_3) has given only six per cent germination and did not satisfy the minimum mandatory germination requirement of 60.00 per cent. The rest of the treatments including untreated seeds failed to soften the seed coat and give appreciable germination per cent (0-6).

4.3.2. Speed of germination

Among the chemical scarification treatments using Conc. H_2SO_4, treatment for highest duration of 30 min has proved to be the best and significantly superior to other treatments giving maximum speed of germination of 89.96. This was followed by acid treatment for 10 min and 20 min with a speed of germination of 72.33 and 69.33 respectively, which were on par. Speed of germination was minimum for five minutes (36.08). Sand paper rubbing was slightly better than other treatments with a speed of germination of 10.20, while all other treatments were significantly inferior, untreated seeds failed to germinate.

The best four treatments from the above experiment (scarification with Conc. H_2SO_4 for 05 min, 10 min, 20 min and 30 min) along with untreated control was employed using three months old seeds to study their influence on seedling growth, vigour and dormancy factors. The seeds were sown in plastic trays containing sand and observed for 14 days. The results are presented in Tables 12 and 13.

Table 11. Effect of pre-treatments on seed germination in *Sida alnifolia*

Treatments	Germination per cent	Speed of germination
T_1	26.00[c] (5.05)	10.20[d] (3.21)
T_2	54.00[b] (7.37)	36.08[c] (6.03)
T_3	78.00[ab] (8.84)	72.33[b] (8.51)
T_4	72.00[ab] (8.48)	69.33[b] (8.32)
T_5	94.00[a] (9.72)	89.96[a] (9.51)
T_6	0.00[e] (0.70)	0.00[e] (0.70)
T_7	0.00[e] (0.70)	0.00[e] (0.70)
T_8	2.00[de] (1.41)	0.14[e] (0.79)
T_9	0.00[e] (0.70)	0.00[e] (0.70)
T_{10}	0.00[e] (0.70)	0.00[e] (0.70)
T_{11}	0.00[e] (0.70)	0.00[e] (0.70)
T_{12}	6.00[d] (2.12)	0.22[e] (0.84)
T_{13}	2.00[de] (1.41)	0.06[e] (0.75)
T_{14}	6.00[d] (2.51)	0.74[e] (1.07)
T_{15}	0.00[e] (0.70)	0.00[e] (0.70)
T_{16}	2.00[de] (1.41)	0.14[e] (0.79)
T_{17}	2.00[de] (1.41)	0.15[e] (0.80)
T_{18}	0.00[e] (0.70)	0.00[e] (0.70)
T_{19}	0.00[e] (0.70)	0.00[e] (0.70)

T_1: Rubbing with hard sand paper
T_2: Conc. H_2SO_4 treatment for 05 min
T_3: Conc. H_2SO_4 treatment for 10 min
T_4: Conc. H_2SO_4 treatment for 20 min
T_5: Conc. H_2SO_4 treatment for 30 min
T_6: Boiling water treatment for 05 min
T_7: Hot water treatment at 90^o C for 05 min
T_8: Hot water treatment at 80^o C for 05 min
T_9: Hot water treatment at 70^o C for 05 min
T_{10}: Hot water treatment at 60^o C for 05 min
T_{11}: Hot water treatment at 50^o C for 05 min
T_{12}: Hot water treatment at 40^o C for 05 min
T_{13}: Treatment with boiling water for 05 min followed by freezing
T_{14}: Rubbing with hard sand paper followed by soaking in 01 % KNO_3 for 12 h
T_{15}: Soaking in water for 48 h
T_{16}: Making pin pricks followed by soaking in water for 48 h
T_{17}: Overnight soaking in cow dung slurry
T_{18}: Overnight water soaking
T_{19}: Absolute control (untreated)

4.3.2.1. Germination

Germination per cent and speed of germination of seedlings showed significant difference between treated and untreated seeds(Table 12Within the .(.conC chemical scarification treatmets usingH ,$_4SO_2$the maximum germination per cent)72 per cent) was realized for seeds treated for30 minfollowed by 10 min (60 per cent), 20 min (54per cent), 05 min (30per cent) and the lowest for the untreated seeds (01per cent). Similarly, speed of germination was highest for 30 min treated seeds (19.33) followed by 20 min (18.12), 10 min (17.18) and five minutes (6.77) and the lowest for untreated seeds (0.14).

4.3.2.2. Dormancy index

Dormancy index showed significant difference between treated and untreated edsse(Table 12Within the scarification treatmets .(, though maximum dormancy .conealized for seeds treated with Cindex was rH for $_4SO_2$30 min(0.047) it was on par with10 min and20 min(0.042), but differed significantly from treatment for five minutes (0.02). Minimum dormancy index was recorded for untreated seeds (0.001).

4.3.2.3. Intensity of dormancy

Intensity of dormancy at seven days and 14 days showed significant difference between treated and untreated seeds(Table 12n Within the scarificatio .(treatments, minimumintensity of dormancy was recorded for 30 min treatment (28.00 per cent) which was on par with 20 min (46.00 per cent) and 10 min treatment (40.00 per cent), but differed significantly from five minutes treatment. Untreated control recorded constant and maximum intensity of dormancy (99.00 per cent).

4.3.2.4. Seedling growth

Significant difference was noted in seedling growth between treated and untreated seeds. All the scarification treatments were on par and significantly

superior to untreated control with respect to seedling shoot length, root length and total length(Table13.(

4.3.2.5. Fresh weight and dry weight of seedling

Fresh weight of seedlings showed significant difference between treated and untreated seeds(Table 13Within the scarification treatmets .(, fresh weight of seedling did not differ significantly but maximum fresh weight was realized for seeds .cond with CtreateH for $_4SO_2$30 min and 20min)0.028 g). The seedling dryweight the scarification treatments and maximum dry weight differed significantly among .conC was realized for seeds treated with$H_4SO_2$for 20min)0.0031 (followed by 30 min (0.0030 g), 20 min (0.028 g), 05 min (0.025 g) and the minimum for the untreated (0.0005g) seeds.

4.3.2.6. Vigour index-1 and vigour index-II

Vigour index of seedlings showed significant difference between treated and untreated seeds(Table 13Within the scarification treatmets .(, the maximumvigour index-1 and vigour index-2 .conC were realized for seeds treated withH_4SO_2 for30 minfollowed by 10 min, 20 min, 05 min and minimum for the untreated seeds. Vigour index-I differed signifcantly between scarification treatements whereas vigour index- II was on par for30 min, 20min and 10min treatment with .conCH_4SO_2

4.3.2.7. Rate of water imbibition

The rate of water imbition in seeds of *Sida alnifolia* which are freshly harvested and stored for one year and treated with Conc. H_2SO_4 for different durations of 30 min, 20 min, 10 min and five minutes was compared with untreated control (Table 14). The rate of water imbition was faster for the fresh seeds than stored seeds. Irrespective of treatments, the maximum mass increase was noticed within three hours of soaking for the scarification treatments, but untreated seeds absorbed maximum water after six hours of soaking. After 24 h of soaking there was

notable difference in the water imbibition between treated and untreated seeds, with untreated seeds recording lower values.

Irrespective of age of seeds, with increase in duration of scarification, rate of imbition was also higher but the rate of increase was more pronounced in fresh seeds than in stored seeds. However, increase in water imbition rate after at six hours, 12 h and 24 h was low for the fresh seeds than the stored seeds and after the 24 h of soaking in water, per cent of mass increase of fresh and stored seeds were near similar.

4.3.2.8. Electrical conductivity of seed leachates

Untreated seeds recorded highest electrical conductivity for both the fresh seeds (0.045 dS m^{-1}) and stored seeds (0.049 dS m^{-1}). There was linear decrease in the EC values with increase in duration of scarification and treatment for five minutes recorded higher EC values (Table 15).

4.4. SEED STORAGE STUDIES

Seeds of *Sida alnifolia* were stored for one year in polythene bags under three conditions *viz*. ambient, refrigerated and freezed conditions. Samples drawn at four months interval were sown with and without pre-treatments. The individual effect and combination effect of pre-treatments, storage conditions and intervals of storage on germination, seedling growth and dormancy behaviour are presented in Tables 16 -17 and 18-29 respectively.

4.4.1. Germination and dormancy behaviour

When the effect of treatments alone was considered, pre-treatment with Conc. H_2SO_4 for 30 min recorded significantly highest germination per cent (51.55) and speed of germination (17.20) while all other pre-treatments were on par and significantly superior to untreated seeds (Table 16). This treatment has also given

Table 12. Effect of acid scarification treatments on germination and dormancy behaviour

Treatments	Germination per cent	Speed of germination	Dormancy index	Intensity of dormancy at 7 days (%)	Intensity of dormancy at 14 days (%)
T1	30.00^{bc}	6.77^{b}	0.024^{b}	70.00^{b}	70.00^{b}
T2	60.00^{ab}	17.18^{a}	0.042^{a}	51.00^{c}	40.00^{c}
T3	54.00^{abc}	18.12^{a}	0.041^{a}	51.00^{c}	46.00^{c}
T4	72.00^{a}	19.33^{a}	0.047^{a}	48.00^{c}	28.00^{c}
T5	1.00^{d}	0.14^{c}	0.001^{c}	99.00^{a}	99.00^{a}

Values having common superscript are not significantly different from each other.

Table 13. Effect of acid scarification treatments on growth and vigour of seedlings

Treatments	Shoot length (cm)	Root length (cm)	Total length (cm)	Fresh weight (g)	Dry weight (g)	Vigour index-I	Vigour index-II
T1	6.75^{a}	1.09^{a}	7.84^{a}	0.024^{a}	0.0025^{b}	235.20^{d}	0.075^{b}
T2	7.24^{a}	1.37^{a}	8.62^{a}	0.026^{a}	0.0028^{b}	517.20^{b}	0.168^{a}
T3	7.15^{a}	1.25^{a}	8.40^{a}	0.028^{a}	0.0031^{a}	453.30^{c}	0.167^{a}
T4	7.19^{a}	1.13^{a}	8.32^{a}	0.028^{a}	0.0030^{ab}	590.90^{a}	0.216^{a}
T5	1.02^{b}	0.25^{b}	1.27^{b}	0.004^{b}	0.0005^{c}	1.27^{e}	0.0005^{c}

Values having common superscript are not significantly different from each other.

T_1: Conc. H_2SO_4 treatment for 05 min.
T_2: Conc. H_2SO_4 treatment for 10 min.
T_3: Conc. H_2SO_4 treatment for 20 min.
T_4: Conc. H_2SO_4 treatment for 30 min.
T_5: Control/untreated seeds

Table 14. Effect of acid scarification treatments on water imbibition per cent

Treatments	Seeds	After 3 hours	After 06 hours	After 12 hours	After 24 hours
Conc. H_2SO_4 05 min	Fresh	36.25	53.64	61.14	68.04
	Stored	24.10	32.26	48.46	64.01
Conc. H_2SO_4 10 min	Fresh	44.02	55.1	60.1	65.1
	Stored	24.55	36.55	56.61	64.22
Conc. H_2SO_4 20 min	Fresh	60.4	65.44	67.25	72.25
	Stored	28.56	48.63	56.22	68.66
Conc. H_2SO_4 30 min	Fresh	60.45	65.48	67.25	70.44
	Stored	28.54	40.11	52.77	64.78
Control	Fresh	8.98	40.11	42.5	45
	Stored	8.11	20.11	36.22	44.52

Table 15. Effect of acid scarification treatments on electrical conductivity

Treatments	Electrical conductivity (dS m^{-1})	
	Fresh seeds	Stored seeds
05 min	0.034	0.043
10 min	0.032	0.034
20 min	0.030	0.034
30 min	0.026	0.030
Control	0.045	0.049

high dormancy index (0.05) and minimum intensity of dormancy at seven days (54.77 per cent) and 14 days after sowing (47.61 per cent).

Comparing the storage conditions, it was observed that storage in refrigerator and freezer were on par with respect to germination per cent and speed of germination and it was significantly superior to storage under ambient condition (Table 16). Dormancy index was significantly high for refrigerated storage (0.043) followed by freezed storage (0.036). Intensity of dormancy at seven days and 14 days were significantly lower for storage under cooler conditions whereas ambient storage recorded higher intensity of dormancy (69.20 per cent).

With advancement in duration of storage, the germination per cent, speed of germination and dormancy index showed a significantly linear increase, having highest values at one year whereas the intensity of dormancy at seven days (49.84) and fourteen days after sowing (47.13) showed a reverse trend with minimum values at one year (Table 16).

4.4.2. Seedling growth and vigour

Treatment difference was noted for growth parameters. Maximum shoot length, root length, total length of seedling, vigour index-1 and vigour index-2 were observed for seeds treated with Conc. H_2SO_4 for 30 min and the differences were significant, except for root length. The fresh weight and dry weight of seedling did not differ markedly among the treatments with Conc. H_2SO_4, but there was marked difference when compared to untreated seeds for dry weight. The dry weight was significantly more for treated seeds. Untreated seeds recorded significantly lower values for all the above parameters (Table 17).

As regards to storage conditions, freezed storage recorded significantly higher shoot length and total length of seedling while the root length was on par to seeds stored under refrigerated condition (Table 17). Fresh weight and dry weight of

seedling differed significantly, cooler atmosphere were better and on par. Refrigerated storage proved to be the best with respect to vigour index-I (260.36) but for vigour index-II, freezed storage was the best (0.081).

As storage period advanced, shoot length, root length, total length of seedling, fresh and dry weight of seedling, vigour index-I and vigour index-II showed linear increase and the values differed significantly among storage intervals (Table 17).

Combination effect of treatments, storage conditions and intervals of storage were significant for the above parameters except for the germination per cent, speed of germination and fresh weight, vigour index-I and intensity of dormancy at 14 days after sowing. Overall, treatment for higher duration of 30 min with Conc. H_2SO_4, storage under freezed condition and storage duration of minimum eight months were needed for obtaining minimum mandatory requirement of 60.00 per cent germination. Sufficiently high germination percentage in *Sida alnifolia* (Table 18-29).

4.4.3 Water imbition percentage

The seeds stored in cooler conditions had higher per cent water imbition rate at all intervals of soaking and the mass of seeds was constantly increasing from three to 24 h of soaking. Maximum imbibition of water was observed within three hours of soaking irrespective of storage conditions and thereafter, rate of water imbition decreased gradually. Comparing the storage intervals, there was a progressive decrease in the rate of water imbibition with advancement in storage period and the same trend was evident at different periods of soaking in water (Table 30).

4.4.4. Electrical conductivity and mineral content of seed leachates

Electrical conductivity of seed leachates showed pronounced difference among different storage conditions, highest values being recorded for seeds stored under freezed condition (0.041 dS m^{-1}) followed by the refrigerated condition (0.036

dS m^{-1}) and the least under ambient condition (0.030 dS m^{-1}). Intervals of storage also showed significant variation in EC values, with the highest EC being recorded for seeds stored upto one year (0.042 dS m^{-1}) (Table 31).

Seed leachates of *Sida alnifolia* stored for one year under different conditions were analyzed for mineral elements such as calcium, sodium, potassium, pH and electrical conductivity. Significant variations depending on storage conditions were noted for all the parameters except for sodium content. Among the three storage conditions, highest pH, EC and contents of minerals were noted for the seeds stored in freezed condition followed by refrigerator and the least was in ambient condition (Table 32).

Table 16. Effect of pre-treatments, conditions and intervals of storage on germination and dormancy behaviour in *Sida alnifolia*

	Germination per cent	Speed of germination	Dormancy index	Intensity of dormancy @ 7 days (%)	Intensity of dormancy @ 14 days (%)
T_1	44.33[b] (6.21)	14.63[b] (3.59)	0.038[d] (0.73)	63.22 (7.85)	55.66[b] (7.30)
T_2	44.33[b] (6.31)	13.35[b] (3.48)	0.041[c] (0.73)	60.73[bc] (7.56)	55.66[b] (7.30)
T_3	41.44[b] (6.08)	13.25[b] (3.45)	0.045[b] (0.73)	59.44[bc] (7.56)	54.55[b] (7.21)
T_4	51.55[a] (6.89)	17.20[a] (4.01)	0.050[a] (0.74)	54.77[c] (7.21)	47.61[c] (6.59)
T_5	3.33[c] (1.56)	0.819[c] (1.03)	0.003[e] (0.70)	97.33[a] (9.88)	96.66[a] (9.85)
C_1	30.80[b] (4.71)	9.91[b] (2.76)	0.027[c] (0.72)	71.27[a] (8.25)	69.20[a] (8.11)
C_2	41.00[a] (5.84)	12.87[a] (3.28)	0.043[a] (0.73)	66.56[ab] (8.01)	59.00[b] (7.48)
C_3	39.20[a] (5.68)	12.86[a] (3.30)	0.036[b] (0.73)	63.46[b] (7.78)	60.30[b] (7.51)
I_1	14.80[c] (3.33)	4.65 (1.99)[c]	0.023[c] (0.72)	87.56[a] (9.35)	85.20[a] (9.21)
I_2	42.33[b] (6.09)	11.45[b] (3.19)	0.036[b] (0.73)	63.90[b] (7.87)	56.16[b] (7.30)
I_3	52.86 (6.80)a	19.45[a] (4.15)	0.047[a] (0.73)	49.84[c] (6.82)	47.13[c] (6.59)

Values having common superscript are not significantly different from each other.
Values in parenthesis are transformed values

T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 months)
I_2: Second interval (8 months)
I_3: Third interval (12 months)

Table 17. Effect of pre-treatments, conditions and intervals of storage on seedling growth and vigour in *Sida alnifolia*

	Shoot length (cm)	Root length (cm)	Total length (cm)	Fresh weight (g)	Dry weight (g)	Vigour index-1	Vigour index-2
T_1	3.90^{b} (2.03)	1.73^{a} (1.42)	5.63^{b} (2.41)	0.017^{b} (0.719)	0.002^{a} (0.708)	269.71^{b} (15.12)	0.088^{c} (0.76)
T_2	4.25^{ab} (2.14)	2.05^{a} (1.52)	6.27^{ab} (2.57)	0.019^{a} (0.720)	0.002^{a} (0.708)	274.68^{b} (15.66)	0.089^{b} (0.76)
T_3	4.04^{ab} (2.08)	2.21^{a} (1.56)	6.27^{ab} (2.57)	0.019^{a} (0.720)	0.002^{a} (0.708)	259.71^{b} (15.3)	0.095^{d} (0.76)
T_4	4.31^{a} (2.16)	2.18^{a} (1.56)	6.57^{a} (2.65)	0.019^{a} (0.721)	0.002^{a} (0.708)	337.22^{a} (17.50)	0.107^{a} (0.77)
T_5	1.49^{c} (1.23)	0.88^{b} (1.05)	2.38^{c} (1.42)	0.014^{b} (0.807)	0.001^{b} (0.708)	20.80^{c} (3.16)	0.006^{e} (0.71)
C_1	3.15^{c} (1.77)	1.39^{b} (1.27)	4.59^{c} (2.099)	0.015^{c} (0.717)	0.002^{a} (0.708)	188.13^{b} (11.16)	0.065^{b} (0.75)
C_2	3.55^{b} (1.929)	2.01^{a} (1.48)	5.55^{b} (2.36)	0.022^{b} (0.719)	0.002^{a} (0.708)	260.36^{a} (14.52)	0.080^{a} (0.76)
C_3	4.10^{a} (2.094)	2.03^{a} (1.51)	6.13^{a} (2.52)	0.029^{a} (0.776)	0.002^{a} (0.708)	248.78^{a} (14.20)	0.081^{a} (0.76)
I_1	2.96^{c} (1.49)	1.02^{a} (1.20)	3.98^{b} (2.27)	0.015^{b} (0.716)	0.001^{b} (0.708)	58.90^{c} (3.37)	0.020^{c} (0.72)
I_2	3.94^{b} (2.04)	0.90^{b} (1.17)	4.84^{b} (2.24)	0.029^{a} (0.776)	0.002^{a} (0.708)	241.26^{b} (14.15)	0.090^{b} (0.76)
I_3	4.90^{a} (2.26)	1.06^{a} (1.23)	5.96^{a} (2.47)	0.031^{a} (0.721)	0.002^{a} (0.709)	351.79^{a} (17.36)	0.116^{a} (0.78)

Values having common superscript are not significantly different from each other
Values in parenthesis are transformed values

T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 MAS)
I_2: Second interval (8 MAS)
I_3: Third interval (12 MAS)

Table 18. Combinatorial effect of pre-treatments, conditions and intervals of storage on germination per cent

T	Condition-I			Condition-II			Condition-III		
	I_1	I_2	I_3	I_1	I_2	I_3	I_1	I_2	I_3
T1	1 (1.06)	49 (6.97)	50 (7.0)	45 (6.66)	46 (6.73)	77 (8.79)	9 (2.96)	62 (7.89)	60 (7.75)
T2	7 (2.47)	48 (6.72)	55 (7.41)	38 (6.07)	61 (7.79)	64 (8.01)	09 (3.02)	57 (7.57)	60 (7.72)
T3	6 (2.51)	43 (6.25)	53 (7.23)	24 (4.79)	42 (6.36)	57 (7.53)	15 (3.83)	58 (7.61)	75 (8.63)
T4	20 (4.11)	54 (7.27)	73 (8.53)	28 (5.27)	56 (7.49)	72 (8.44)	16 (3.89)	64 (7.98)	81 (9.02)
T5	0 (0.70)	0 (0.70)	3 (1.61)	0 (0.70)	1 (1.06	4 (1.96)	0 (0.70)	9 (3.02)	13 (3.60)

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Table 19. Combinatorial effect of pre-treatments, conditions and intervals of storage on speed of germination

T	Condition-I			Condition-II			Condition-III		
	I_1	I_2	I_3	I_1	I_2	I_3	I_1	I_2	I_3
T1	0.37 (0.88)	12.45 (3.56)	20.76 (4.59)	13.37 (3.69)	8.65 (2.98)	33.01 (5.78)	2.84 (1.77)	17.51 (4.23)	22.82 (4.89)
T2	4.02 (1.81)	10.84 (3.22)	18.42 (4.30)	10.61 (3.15)	13.13 (3.66)	23.45 (4.87)	2.54 (1.72)	14.97 (3.92)	22.17 (4.70)
T3	1.8 (1.50)	12.56 (3.36)	15.01 (3.86)	6.20 (2.50)	11.52 (3.35)	22.54 (4.78)	5.45 (2.32)	15.67 (4.01)	28.49 (5.36)
T4	8.47 (2.73)	18.29 (4.29)	25.38 (5.01)	7.04 (2.70)	13.85 (3.76)	27.19 (5.24)	6.02 (2.48)	20.16 (4.49)	28.34 (5.36)
T5	0 (0.70)	0 (0.70)	0.28 (0.86)	0.95 (1.16)	0.25 (0.83)	0 (0.70)	0 (0.70)	1.90 (1.53)	3.98 (2.08)

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T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 months)
I_2: Second interval (8 months)
I_3: Third interval (12 months)

Table 20. Combinatorial effect of pre-treatments, conditions and intervals of storage on dormancy index

T	Condition-I			Condition-II			Condition-III		
	I_1	l_2	I_3	I_1	l_2	I_3	I_1	l_2	I_3
T1	0.002^{wx} (0.708)	0.044^{l} (0.738)	0.033^{p} (0.730)	0.052^{i} (0.743)	0.036^{o} (0.732)	0.052^{ij} (0.743)	0.018^{s} (0.72)	0.062^{e} (0.750)	0.039^{n} (0.734)
T2	0.014^{t} (0.717)	0.041^{mn} (0.735)	0.036^{o} (0.732)	0.071^{b} (0.756)	0.052^{ij} (0.743)	0.043^{lm} (0.737)	0.018^{s} (0.720)	0.059^{f} (0.748)	0.04^{n} (0.735)
T3	0.012^{t} (0.715)	0.039^{n} (0.734)	0.033^{p} (0.730)	0.028^{r} (0.726)	0.118^{a} (0.784)	0.041^{mn} (0.735)	0.028^{r} (0.727)	0.056^{gh} (0.746)	0.05^{gk} (0.742)
T4	0.04^{n} (0.734)	0.057^{fg} (0.746)	0.049^{k} (0.741)	0.032^{pq} (0.729)	0.069^{c} (0.754)	0.051^{ij} (0.742)	0.03^{q} (0.728)	0.064^{d} (0.751)	0.055^{h} (0.745)
T5	0^{x} (0.707)	0^{x} (0.707)	0.003^{v} (0.709)	0^{x} (0.707)	0.006^{v} (0.711)	0^{x} (0.707)	0^{x} (0.707)	0.009^{u} (0.713)	0.009^{u} (0.713)

Values having common superscript are not significantly different from each other.

Table 21. Combinatorial effect of pre-treatments, conditions and intervals of storage on intensity of dormancy at seven days

T	Condition-I			Condition-II			Condition-III		
	I_1	l_2	I_3	I_1	l_2	I_3	I_1	l_2	I_3
T1	99.00^{a} (9.97)	53.00^{hijkl} (7.22)	54.00^{ghijkl} (7.36)	$59.00^{fghijkl}$ (7.66)	$69.00^{bcdefghi}$ (8.31)	23.00^{p} (4.78)	92.00^{ab} (9.615)	42.00^{jklm}_{n} (6.47)	44.00^{jklmn} (6.62)
T2	95^{ab} (9.76)	$63^{efghijk}$ (7.73)	47^{ijkkmn} (6.85)	$74^{abcdefgh}$ (8.60)	$63^{efghijk}$ (7.93)	40^{klmno} (6.34)	91^{abc} (9.56)	51^{hijll} (7.16)	45^{jklmn} (6.71)
T3	95^{ab} (9.77)	$64^{defghijk}$ (7.93)	25.63^{p} (4.70)	83^{abcdef} (9.12)	$72^{abcdefghi}$ (8.41)	47^{ijklmn} (6.85)	86^{abcde} (9.28)	$45j^{klmn}$ (6.71)	29^{nop} (5.29)
T4	81^{abcdef} (8.95)	$44.5j^{klmn}$ (6.48)	49.00^{jklm} (6.95)	$77.5^{abcdefg}$ (8.82)	$64^{cdefghij}$ (8.01)	31^{mnop} (5.42)	84^{abcdef} (9.17)	38^{lmnop} (6.13)	24^{op} (4.19)
T5	100^{a} (10.02)	100^{a} (10.02)	99^{a} (9.97)	97^{ab} (9.87)	97^{a} (9.97)	100^{a} (10.02)	100^{a} (10.02)	91^{abc} (9.56)	90^{abcd} (9.51)

Values having common superscript are not significantly different from each other.

T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 months)
I_2: Second interval (8 months)
I_3: Third interval (12 months)

Table 22. Combinatorial effect of pre-treatments, conditions and intervals of storage on intensity of dormancy at 14 days

T	Condition-I			Condition-II			Condition-III		
	I_1	l_2	I_3	I_1	l_2	I_3	I_1	l_2	I_3
T1	99 (9.975)	51 (7.098)	50 (7.091)	55 (7.395)	54 (7.318)	23 (4.784)	91 (9.562)	38 (6.182)	40 (6.308)
T2	93 (9.666)	52 (6.937)	45 (6.688)	62 (7.853)	39 (6.159)	36 (6.011)	91 (9.564)	43 (6.581)	40 (6.275)
T3	94 (9.721)	57 (7.4)	47 (6.789)	76 (8.724)	58 (7.501)	43 (6.514)	85 (9.237)	42 (6.452)	25 (4.484)
T4	80 (8.901)	46 (6.58)	27 (4.958)	72 (8.5)	44 (6.648)	28 (4.994)	84 (9.176)	28.5 (5.168)	19 (4.385)
T5	100 (10.025)	100 (10.025)	97 (9.873)	96 (9.822)	99 (9.975)	100 (10.025)	100 (10.025)	91 (9.564)	87 (9.350)

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Table 23. Combinatorial effect of pre-treatments, conditions and intervals of storage on shoot length of the seedling

T	Condition-			Condition-II			Condition-III		
	I_1	l_2	I_3	I_1	l_2	I_3	I_1	l_2	I_3
T1	0.07^{m} (0.75)	3.79^{defgh} (2.03)	5.68^{abc} (2.46)	2.70^{fghij} (1.79)	4.73^{abcde} (2.28)	5.14^{abcd} (2.37)	3.30^{efghi} (1.93)	4.48^{abcde} (2.23)	5.50^{abc} (2.44)
T2	2.12^{jk} (1.54)	3.79^{cdefg} (2.07)	5.68^{ab} (2.48)	2.65^{fghij} (1.77)	4.76^{abcde} (2.29)	5.40^{abcd} (2.43)	3.70^{defgh} (2.02)	4.62^{abcde} (2.26)	5.52^{abcd} (2.45)
T3	1.97^{jk} (1.50)	4.1^{bcdef} (2.14)	5.84^{ab} (2.51)	1.86^{jk} (1.52)	5.12^{abcd} (2.37)	5.36^{abcd} (2.42)	2.27^{hijk} (1.65)	4.52^{abcde} (2.24)	5.35^{abcd} (2.42)
T4	2.02^{ijk} (1.57)	4.34^{abcde} (2.20)	6.32^{a} (2.61)	2.67^{fghij} (1.77)	5.16^{abcd} (2.37)	5.23^{abcd} (2.39)	2.59^{ghij} (1.74)	4.67^{abcde} (2.27)	5.81^{ab} (2.51)
T5	0^{m} (0.70)	0^{m} (0.70)	1.80^{kl} (1.36)	1.57^{kl} (1.36)	0.95^{lm} (1.04)	0^{m} (0.70)	0^{m} (0.70)	4.22^{bcde} (2.17)	4.93^{m} (2.33)

Values having common superscript are not significantly different from each other.

T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 months)
I_2: Second interval (8 months)
I_3: Third interval (12 months)

Table 24. Combinatorial effect of pre-treatments, conditions and intervals of storage on root length of the seedling

T	Condition-I			Condition-II			Condition-III		
	I_1	I_2	I_3	I_1	I_2	I_3	I_1	I_2	I_3
T1	1.17 ^{fgh} (1.1)	0.93 ^{efgh} (1.19)	1.06 ^{efg} (1.23)	3.92 ^{abc} (2.09)	0.92 ^{efgh} (1.19)	1.08 ^{efg} (1.25)	3.92 ^{abc} (2.07)	1.29 ^{def} (1.33)	1.26 ^{defg} (1.32)
T2	3.87 ^{abc} (1.95)	0.84 ^{efgh} (1.16)	1.14 ^{efg} (1.28)	4.10 ^{ab} (2.13)	1.08 ^{efg} (1.25)	0.99 ^{efg} (1.22)	4.10 ^{abc} (2.10)	1.30 ^{def} (1.34)	1.01 ^{efg} (1.22)
T3	3.02 ^{bcd} (1.77)	0.90 ^{efgh} (1.17)	1.21 ^{defg} (1.30)	5.17 ^{a} (2.38)	1.08 ^{efg} (1.25)	1.04 ^{efg} (1.24)	5.31 ^{a} (2.40)	1.09 ^{efg} (1.26)	1.06 ^{efg} (1.24)
T4	3.83 ^{abc} (2.02)	1.01 ^{efg} (1.22)	1.34 ^{def} (1.35)	4.33 ^{ab} (2.19)	0.94 ^{efgh} (1.20)	1.09 ^{efg} (1.26)	5.00 ^{a} (2.33)	1.10 ^{efg} (1.26)	0.98 ^{efg} (1.21)
T5	0 ^{h} (0.70)	0 ^{h} (0.70)	0.537 ^{fgh} (0.97)	4.175 ^{abc} (2.02)	0.2 ^{gh} (0.81)	0 ^{h} (0.70)	0 ^{h} (0.70)	0.88 ^{fgh} (1.16)	2.20 ^{cde} (1.64)

Table 25. Combinatorial effect of pre-treatments, conditions and intervals of storage on total length of the seedling

T	Condition-I			Condition-II			Condition-III		
	I_1	I_2	I_3	I_1	I_2	I_3	I_1	I_2	I_3
T1	1.25 ^{cd} (1.11)	4.56 ^{ab} (2.25)	6.65 ^{ab} (2.67)	6.62 ^{ab} (2.66)	5.65 ^{ab} (2.47)	6.22 ^{ab} (2.59)	7.20 ^{ab} (2.77)	5.77 ^{ab} (2.50)	6.76 ^{ab} (2.69)
T2	6 ^{ab} (2.36)	4.65 ^{ab} (2.27)	6.82 ^{ab} (2.70)	6.61 ^{ab} (2.66)	5.87 ^{ab} (2.52)	6.4 ^{ab} (2.62)	7.69 ^{a} (2.86)	5.92 ^{ab} (2.53)	6.53 ^{ab} (2.65)
T3	5.12 ^{ab} (2.20)	5.02 ^{ab} (2.34)	7.05 ^{ab} (2.74)	7.04 ^{ab} (2.74)	6.19 ^{ab} (2.58)	6.40 ^{ab} (2.62)	7.61 ^{ab} (2.84)	5.62 ^{ab} (2.47)	6.41 ^{ab} (2.62)
T4	6.45 ^{ab} (2.62)	5.36 ^{ab} (2.42)	7.66 ^{ab} (2.85)	6.98 ^{ab} (2.72)	6.10 ^{ab} (2.56)	6.33 ^{ab} (2.61)	7.61 ^{ab} (2.84)	5.82 ^{ab} (2.51)	6.79 ^{ab} (2.70)
T5	0 ^{d} (0.70)	0 ^{d} (0.70)	2.33 ^{c} (1.49)	5.75 ^{ab} (2.31)	1.15 ^{cd} (1.09)	0 ^{d} (0.70)	0 ^{d} (0.70)	5.10 ^{ab} (2.36)	7.13 ^{ab} (2.76)

Values having common superscript are not significantly different from each other.

T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 months)
I_2: Second interval (8 months)
I_3: Third interval (12 months)

Table 26. Combinatorial effect of pre-treatments, conditions and intervals of storage on fresh weight of the seedling

T	Condition-I			Condition-II			Condition-III		
	I_1	l_2	I_3	I_1	l_2	I_3	I_1	l_2	I_3
T1	0.002 (0.709)	0.019 (0.72)	0.022 (0.723)	0.016 (0.719)	0.02 (0.721)	0.019 (0.721)	0.017 (0.719)	0.019 (0.721)	0.022 (0.722)
T2	0.011 (0.715)	0.021 (0.722)	0.021 (0.722)	0.017 (0.719)	0.02 (0.721)	0.021 (0.722)	0.016 (0.718)	0.019 (0.721)	0.021 (0.722)
T3	0.012 (0.715)	0.020 (0.721)	0.022 (0.723)	0.017 (0.719)	0.02 (0.721)	0.023 (0.723)	0.016 (0.718)	0.020 (0.721)	0.021 (0.722)
T4	0.018 (0.72)	0.02 (0.721)	0.023 (0.723)	0.016 (0.718)	0.020 (0.721)	0.021 (0.722)	0.017 (0.719)	0.019 (0.721)	0.021 (0.722)
T5	0 (0.707)	0 (0.707)	0.009 (0.713)	0.016 (0.718)	0.03 (0.709)	0 (0.707)	0 (0.707)	0.02 (1.569)	0.024 (0.724)

NS

Table 27. Combinatorial effect of pre-treatments, conditions and intervals of storage on dry weight of the seedling

T	Condition-I			Condition-II			Condition-III		
	I_1	l_2	I_3	I_1	l_2	I_3	I_1	l_2	I_3
T1	0 [a] (0.707)	0.002 [a] (0.709)	0.002 [a] (0.709)	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.708)	0.002 [a] (0.708)	0.002 [a] (0.708)	0.002 [a] (0.709)
T2	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)	0.001 [a] (0.708)	0.002 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)
T3	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)
T4	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)	0.001 [a] (0.708)	0.002 [a] (0.709)	0.002 [a] (0.709)
T5	0.00 [a] (0.707)	0.00 [a] (0.707)	0.001 [a] (0.708)	0.001 [a] (0.708)	0.00 [a] (0.707)	0.00 [a] (0.707)	0.00 [a] (0.707)	0.002 [a] (0.708)	0.002 [a] (0.709)

Values having common superscript are not significantly different from each other.

T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 months)
I_2: Second interval (8 months)
I_3: Third interval (12 months)

Table 28. Combinatorial effect of pre-treatments, conditions and intervals of storage on vigour index-1

T	Condition-I			Condition-II			Condition-III		
	I_1	I_2	I_3	I_1	I_2	I_3	I_1	I_2	I_3
T1	5 (1.66)	224.83 (14.85)	333.92 (18.20)	301.2 (17.09)	261.56 (15.95)	478.28 (21.84)	60.49 (7.57)	355.96 (18.86)	406.22 (20.08)
T2	57.6 (6.55)	214.34 (14.27)	373.46 (19.25)	259.34 (15.63)	360.46 (18.84)	409.62 (20.21)	69.19 (8.18)	337.26 (18.35)	390.88 (19.65)
T3	27.8 (4.68)	213.51 (13.87)	371.34 (19.10)	177.26 (12.73)	260.16 (15.73)	364.14 (18.97)	115.18 (10.42)	330 (18.02)	478.02 (21.76)
T4	134.23 (10.26)	292.68 (16.83)	559.56 (23.54)	203.54 (13.94)	341.28 (18.44)	454.62 (21.18)	123.08 (10.61)	375.23 (19.24)	550.75 (23.46)
T5	0 (0.70)	0 (0.70)	13.7 (2.97)	29.4 (4.86)	4.6 (1.61)	0 (0.70)	0 (0.70)	47.09 (6.71)	92.42 (9.45)

NS

Table 29. Combinatorial effect of pre-treatments, conditions and intervals of storage on vigour index-2

T	Condition-I			Condition-II			Condition-III		
	I_1	I_2	I_3	I_1	I_2	I_3	I_1	I_2	I_3
T1	0.001^{y} (0.70)	0.104^{k} (0.77)	0.114^{i} (0.78)	0.0601^{n} (0.74)	0.098^{l} (0.77)	0.150^{d} (0.80)	0.015^{v} (0.71)	0.121^{h} (0.78)	0.131^{f} (0.79)
T2	0.011^{w} (0.71)	0.1^{l} (0.74)	0.122^{h} (0.78)	0.050^{o} (0.74)	0.120^{h} (0.78)	0.139^{e} (0.79)	0.013^{v} (0.71)	0.119^{h} (0.78)	0.129^{g} (0.79)
T3	0.005^{x} (0.71)	0.091^{m} (0.76)	0.110^{j} (0.78)	0.034^{q} (0.73)	0.092^{m} (0.76)	0.132^{f} (0.79)	0.024^{t} (0.72)	0.120^{h} (0.78)	0.160^{c} (0.81)
T4	0.028^{s} (0.72)	0.120^{h} (0.78)	0.170^{b} (0.81)	0.038^{p} (0.73)	0.120^{h} (0.78)	0.158^{c} (0.81)	0.018^{u} (0.72)	0.133^{f} (0.79)	0.182^{a} (0.82)
T5	0^{y} (0.70)	0^{y} (0.70)	0.006^{x} (0.71)	0.006^{x} (0.71)	0.001^{y} (0.70)	0^{y} (0.70)	0^{y} (0.70)	0.014^{v} (0.71)	0.031^{r} (0.72)

Values having common superscript are not significantly different from each other.

T_1: Conc. H_2SO_4 treatment for 05 min
T_2: Conc. H_2SO_4 treatment for 10 min
T_3: Conc. H_2SO_4 treatment for 20 min
T_4: Conc. H_2SO_4 treatment for 30 min
T_5: Control (untreated seeds)

C_1: Ambient condition
C_2: Refrigerated condition
C_3: Freezer condition

I_1: First interval (4 months)
I_2: Second interval (8 months)
I_3: Third interval (12 months)

Table 30. Effect of conditions and intervals of storage on water imbibition of *Sida alnifolia*

Water imbibition (per cent)					
Treatments		**After 3 hour**	**After 06 hour**	**After 12 hour**	**After 24 hour**
Conditions	Ambient	22.06	29.76	32.89	36.51
	Refrigerator	33.74	38.91	41.69	45.03
	Freezer	41.62	45.69	48.06	51.40
Intervals	First	41.07	45.32	48.60	52.11
	Second	36.02	41.61	43.87	46.95
	Third	20.32	27.42	30.17	33.88

Table 31. Effect of conditions and intervals of storage on electrical conductivity of seed leachates

Electrical conductivity (dS m^{-1})		
Treatments	**Fresh seeds**	**Stored seeds**
Conditions	Ambient	0.030
	Refrigerator	0.036
	Freezer	0.041
Intervals	First	0.030
	Second	0.035
	Third	0.042

Table 32. Effect of storage conditions on content of minerals, EC and pH of seed leachates of *Sida alnifolia* after one year

Parameters	Storage conditions		
	Ambient	**Refrigerator**	**Freezer**
Ca (ppm)	1.44^{c}	1.67^{b}	2.60^{a}
Na (ppm)	2.90	3.09	3.99
K (ppm)	2.00^{c}	2.66^{b}	3.93^{a}
pH	5.65^{b}	5.83^{b}	6.16^{a}
EC (dS m^{-1})	0.036^{c}	0.043^{b}	0.048^{a}

Values having common superscript are not significantly different from each other.

Discussion

DISCUSSION

Sida species, a panaceae for rheumatism and neurological complaints in indigenous medicine is gaining more importance as a phyto pharmaceutical due to the presence of indoquinoline alkaloids with pharmacological effects such as anti microbial, anti hyperglycemic and cytotoxic effects. Rarity in the population of *Sida* species is observed presently, owing to over exploitation, destructive harvesting for collection of roots and habitat destruction. Specific information on seed production potential, dormancy and germination behaviour and response to storage are needed to plan and execute conservation strategies and domestication programmes, which are rather limited.

5.1. ASSESSMENT OF SEED PRODUCTION POTENTIAL IN *Sida* spp.

Sida alnifolia was found to have a vigorous growth as compared to *Sida acuta* and *Sida cordifolia* as indicated by more height (Fig. 1) and number of primary branches (Fig. 2). But the seed production potential was highest for *Sida acuta* as evidenced by early flowering and fruiting, extended seed rain (Fig. 4), fruit yield and seed yield (Fig. 3). *Sida cordifolia* was intermediate in flowering and fruiting whereas *Sida alnifolia* flowered last. In contrast, *Sida cordifolia* had largest fruits and seeds and more seeds (09) per fruit whereas only six seeds in fruits of *Sida alnifolia* and *Sida acuta*. The least sized fruits and seeds were in *Sida alnifolia*.

The flowering observations of this study is in concurrence with Sivarajan and Pradeep (1996) wherein *Sida acuta* has an early and extended flowering and *Sida alnifolia* is relatively late to flower (Table 2).

Seed yield per plant (seed number) observed in the study (10043.84 to 23005.78) relates to the seed yield reported by Holm *et al*. (1997) and Calderon *et al*. (2000) in *Sida rhombifolia* (7962 to11, 600).

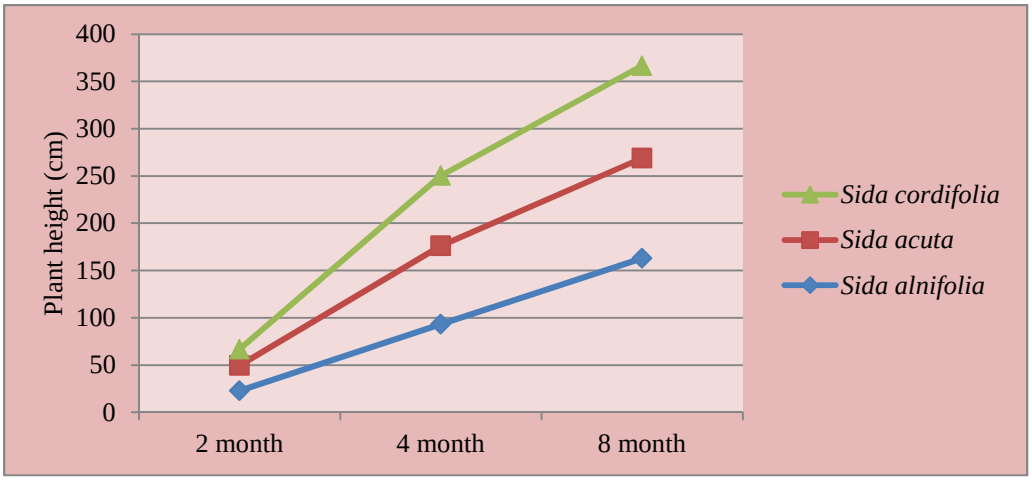


Fig. 1 Plant height in *Sida* spp.

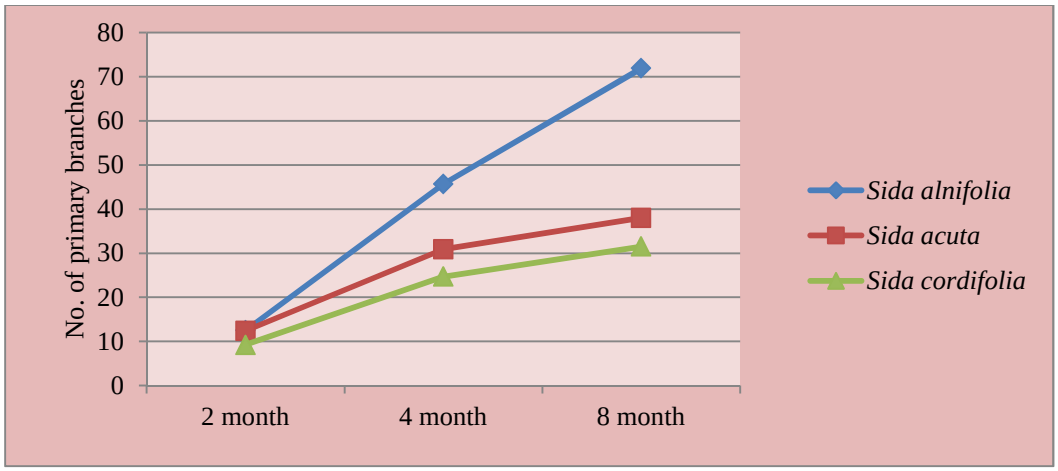


Fig. 2 Number of primary branches in *Sida* spp.

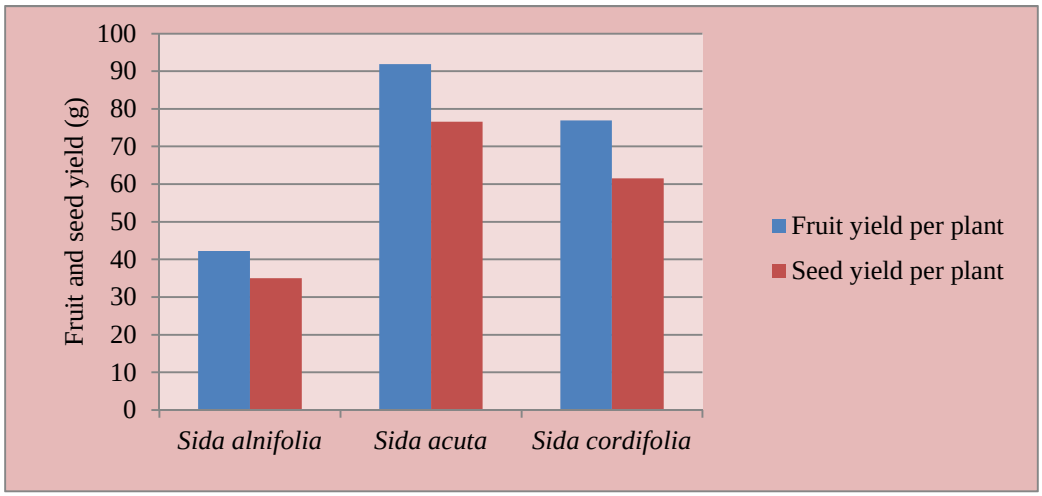


Fig. 3 Fruit and seed yield in *Sida* spp.

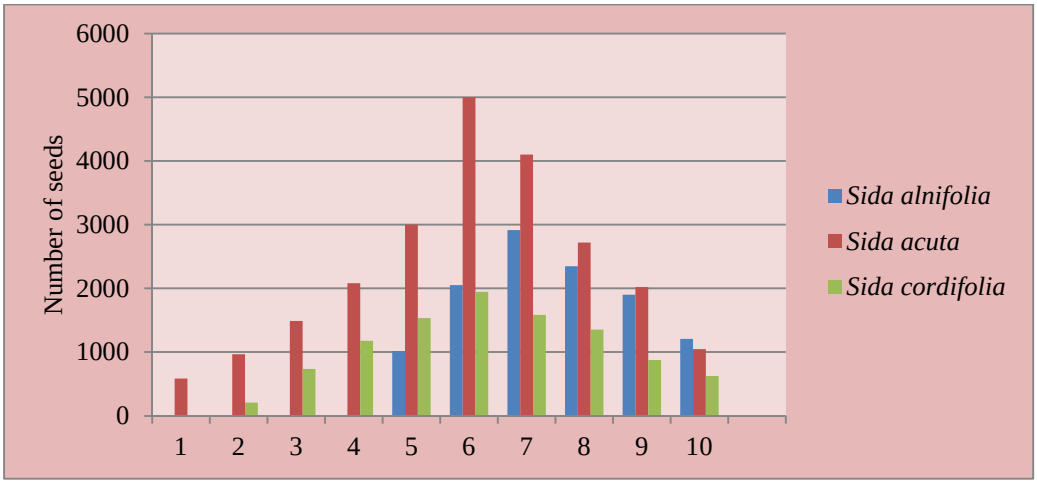


Fig. 4 Seed rain in *Sida* spp.

Anthesis in the three species occurred between 7.30 am to 8.15 am with *Sida acuta* having early anthesis. Slight variation in time of anthesis among *Sida* species has been already reported by Sivarajan and Pradeep (1996). An early anthesis observed in the study may be due to climate changes and variations in the locations of the study.

5.2. CHARACTERIZATION OF DORMANCY

Dormancy in the three *Sida* species was studied from different perspective *viz.* physical, physiological and biochemical. Dormancy behaviour of fresh seeds and those stored for one year under ambient condition and those sown under field conditions was also studied.

5.2.1. Physical factors inducing dormancy in *Sida* spp.

Seed coat thicknesses of the three species in case of fresh seeds (Table 4), seeds stored for one year and scarified seeds of *Sida alnifolia* were studied (Fig. 5). The thickness of different layers of seed coat (periderm, mesoderm and endoderm) and perimeter of seed, except cuticle was highest for *Sida cordifolia* followed by *Sida acuta* whereas cuticle thickness was highest for *Sida alnifolia* followed by *Sida acuta* (Fig. 6). This is in agreement with the report of Rao and Dave (2006) that seeds of *Sida rhombifolia* possesses thick and corrugated cuticle, which act as an impermeable layer restricting germination. Conversion of hydroxyphenolics to insoluble lignin polymers forming a water impermeable barrier at the chalazal area resulting in hard mature seeds of prickly *Sida* has been suggested by Egley *et al*. (1983).

Apparent reduction in thickness of different layers of seed coat and the impervious cuticle upon storage and treatment with Conc. H_2SO_4 was observed. Scarification of seeds brought out a drastic reduction in thickness of different layers especially cuticle wherein 50.56 per cent reduction was noted. Thus it can be

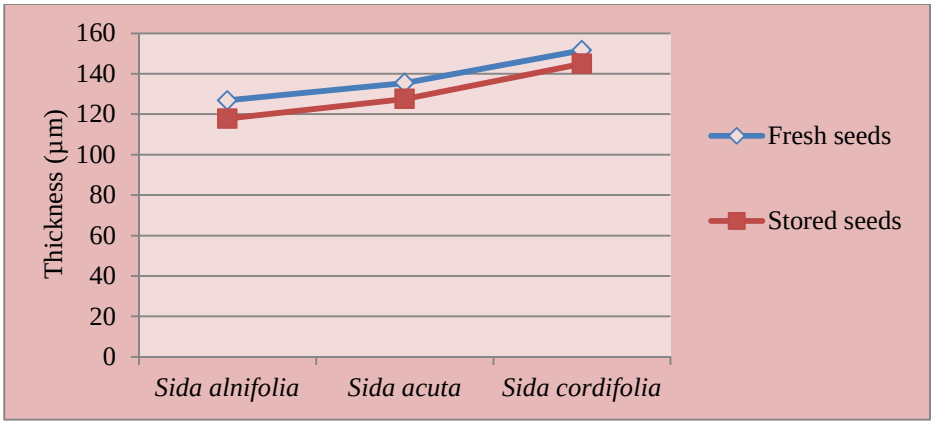


Fig. 5 Total seed coat thickness of *Sida* spp. upon storage

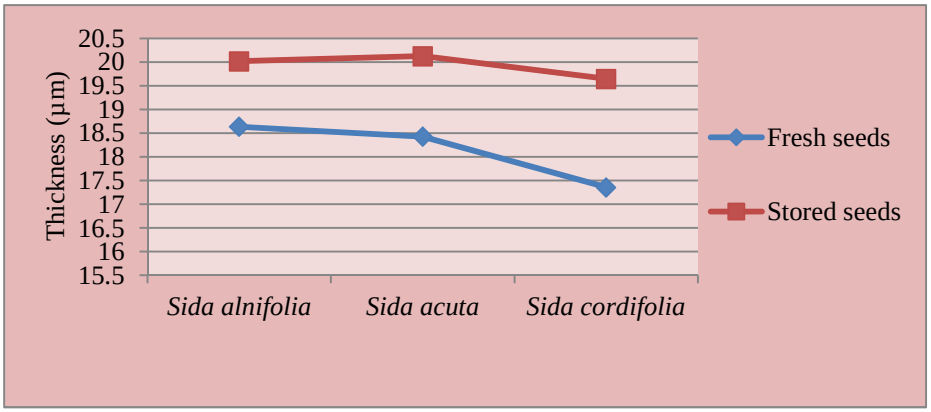


Fig. 6 Cuticle thickness upon storage in *Sida* spp.

concluded that fresh seeds have higher seed coat thickness and this acts as a mechanical barrier for water imbibition and reduction in cuticle layer after acid scarification might have reduced the impermeable layer (Plates 15 and 16). Physical dormancy due to hard seed coat in *Sida species* has been reported by Egley (1989) and Seal and Gupta (2000).

Embryo area and perimeter of seeds increased slightly during storage, compared to fresh seeds (Fig. 7 and 8), with *Sida alnifolia* recording maximum values and least in S*ida cordifolia* (Table 5). Similarly Rizk *et al*. (1969) stated that *Sida rhombifolia* seed required four to six weeks of after-ripening of embryo, before appreciable germination was apparent. The increase in the size of the embryo observed in the study can be attributed to after ripening / maturation of embryo during storage.

5.2.2. Physiological factors inducing dormancy in *Sida* spp.

Under physiological studies, freshly harvested seeds of three *Sida* species were sown in sterilized petriplates and observed for 14 days, to record germination per cent (Table 6). Seeds with and without imbibition were exposed to different temperatures (30º C, 35º C and 40º C), light and dark conditions (08 h and 16 h) and hydration and dehydration treatments and sowing of same seeds was done, repeating the hydration and dehydration treatments. Germination was very poor irrespective of the species and treatments recording maximum of two per cent.

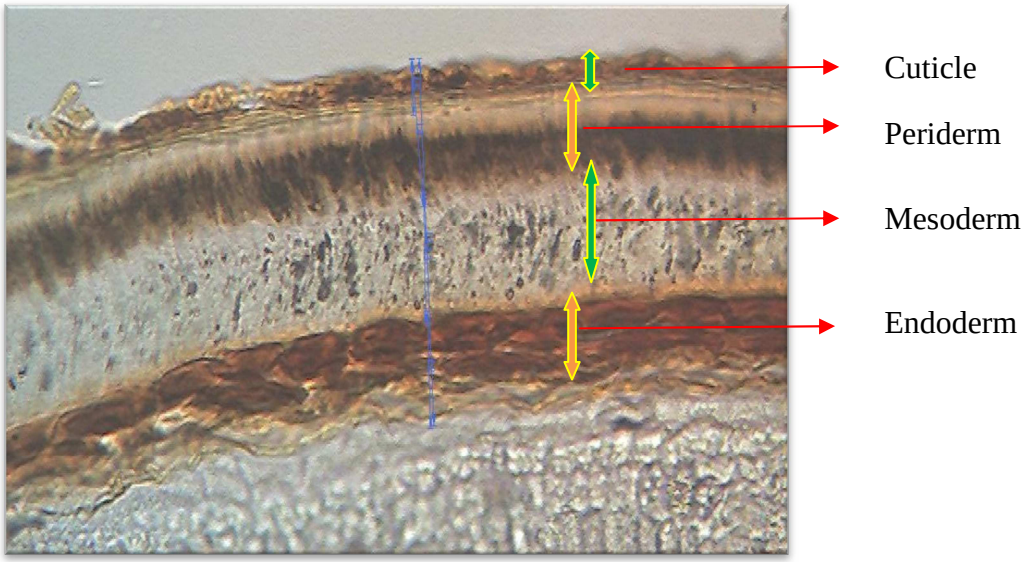
Since fresh seeds are used for the study, the findings of the study are in agreement with Rizk *et al*. (1969) who found that *Sida* seeds required four to six weeks of after-ripening before appreciable germination was apparent and dormancy was not broken by several light-dark regimes.

Untreated seeds

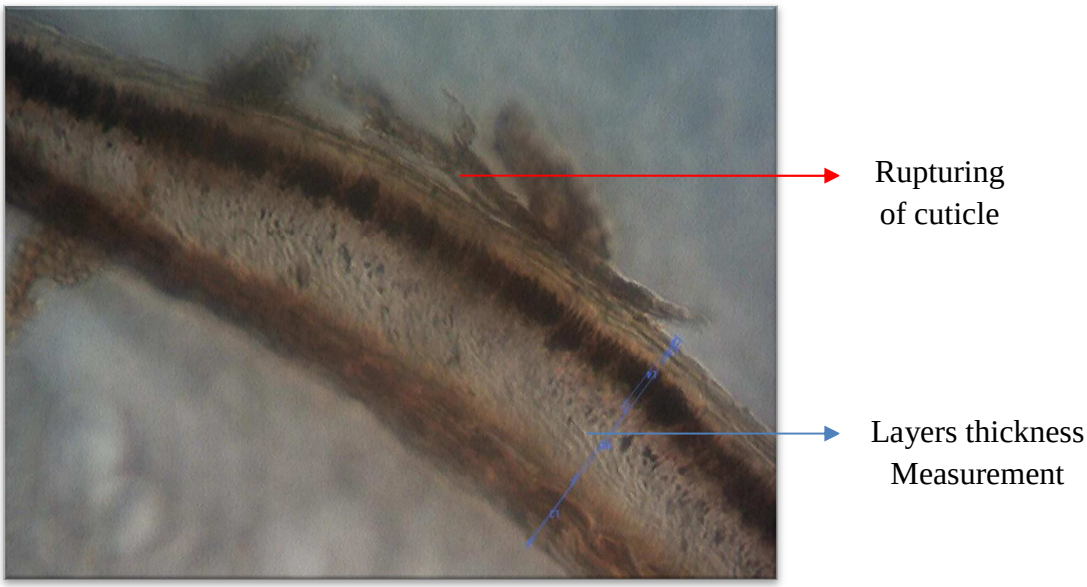
Seeds treated with Conc. H_2SO_4

Seed coat removed after scarification

Plate 15. Effect of acid scarification on seeds



Untreated seeds



Treated seeds

Plate 16. Seed coat thickness of sulphuric acid treated and untreated seeds

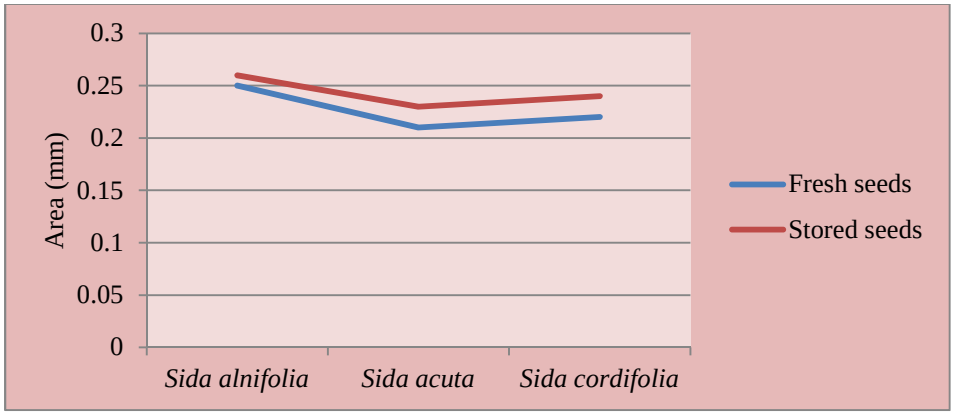


Fig. 7 Embryo area upon storage in *Sida* spp.

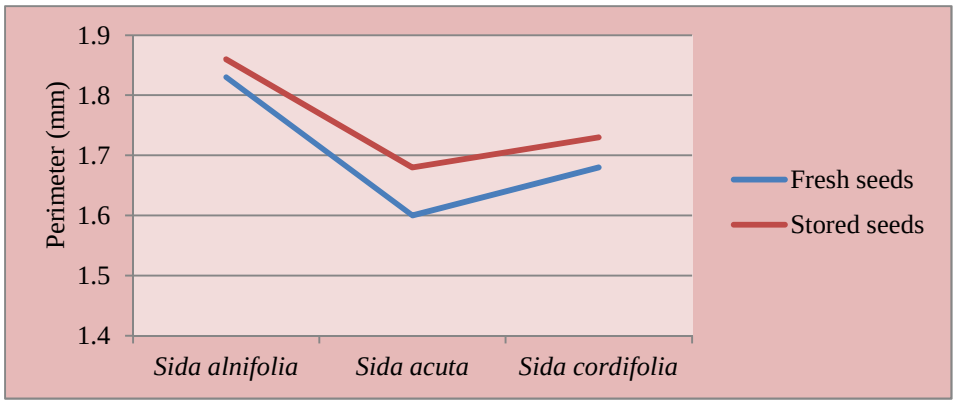


Fig. 8 Embryo perimeter upon storage in *Sida* spp.

The reduction in the biochemicals such as total extractives, content of total alkaloid and total phenol and increased in minerals and electrolytes (Ca, K and Na and EC) upon storage can be attributed to inter conversions or utilization of the metabolites taking place during after ripening maturation of embryo. Resulting in better germination. Presence of inhibitors and mechanical restriction to embryo growth.

Chauhan and Johnson (2008) also found that germination at higher temperatures (30º C / 20º C to 35º C / 25º C) was not influenced by light conditions. The non response of hydration and dehydration treatments may be due to the fact that these treatments may not be sufficient to soften the hard seed coat. In general, requirement of after ripening period of embryo and no response of seeds to temperature and light conditions may be reasons for very poor germination of fresh seeds observed in the above experiments.

5.2.2.1. Germination of fresh and stored seeds

Germination studies in both fresh and stored seeds, sown in protrays containg sand and observed for germination and growth of seedlings revealed that irrespective of the species, seeds whether fresh or stored failed to germinate without pre-treatment with Conc. H_2SO_4 for 20 min (Table 7). In the case of fresh seeds, germination was extremely low with *Sida cordifolia* recording maximum of two per cent.

After storage for one year, seeds of *Sida cordifolia* failed to germinate even with acid scarification whereas *Sida acuta* and *Sida alnifolia* gave 48.00 and 33.40 per cent germination respectively. The germination per cent, speed of germination, growth parameters of seedling and vigour index was improved with considerable reduction in intensity of dormancy upon storage of seeds for one year.

As reported by Lissy (2004) difference in embryo viability between *Sida* species may be a reason for the loss of viability and no germination in *Sida cordifolia* observed after one year in this study. Also it is probable that all the seeds produced by a plant may not have a well formed embryo to resume growth or the embryo inside the seed has a definite life span under the given environmental conditions, which may determine the spread of the species within the ecosystem with respect to time (Misra, 1968).

5.2.2.2. Germination of Sida spp. *under field conditions*

Seeds of *Sida acuta*, *Sida alnifolia* and *Sida cordifolia*, collected during December 2012 were sown during February 2013 (Table 9). Pots were kept under irrigated and rain-fed systems. In irrigated condition, germination started from February itself and germination was continuous upto 6-9 months recording less than 15 per cent germination in all the three species with highest germination in *Sida alnifolia* (14.50 per cent). The occurrence of polymorphism in species which exhibit seed coat dormancy has been reported and allows for a small percentage of seeds to be readily permeable (Graaff and Van Staden, 1983).

Under rain-fed system, pots were left in open condition for natural weathering. In all the three species, germination started spontaneously on receipt of rain during June and completed within ten days, recording germination of 42.50 per cent in *Sida cordifolia* and maximum of 74.00 per cent in *Sida acuta*. This may be because, the seeds are dormant at seed fall and require high alternating temperature for germination as reported by Mott (1980). The spontaneous germination observed in the study is in agreement with the report of Rizk *et al*. (1969) that alternating temperature in soil seed banks of *Sida* results in softening of the thick hard impermeable seed coat permitting imbibition of water and exchange of gases. It is also probable that in nature, exotesta is decomposed by microbial action or ruptured

by desiccation due to fluctuating temperatures (Jones, 1963; Van Staden *et al*., 1994). Desiccation followed by rain induces the rupture of palisade (endotesta) layer, thus removing the mechanical constraint on the embryos.

In conformity with the findings of this study that per cent of hard seeds 26.00-57.50 are left after the wet season in three *Sida* species. The occurrence of 30.00 per cent hard seeds after the wet season in *Sida acuta* has been reported by Mott (1980). In seeds with physical dormancy opening of a water gap in the seed coat in response to an environmental signal, thereby allowing water to enter has been suggested by Baskin and Baskin (2006). Differences in the lignification of palisade cells produce permeable and impermeable seeds as reported by Egley and Paul (1981) in *Sida spinosa* holds true in this study also.

5.2.3. Biochemical

Estimates of the contents of total extractives, total alkaloid and total phenol in the seed and contents of major salts, EC and pH of seed leachates of three species were made (Fig. 9 and 10). It was observed that the species differed significantly in the contents of total extractives and total alkaloid content, with *Sida acuta* having higher total extractives (8.87 per cent) and total alkaloid content (1.85 per cent) followed by *Sida cordifolia* and *Sida alnifolia* which were on par. *Sida alnifolia* recorded maximum content of phenols (5.85 per cent), followed by *Sida acuta* (4.75 per cent) and *Sida cordifolia* (4.40 per cent), which were on par. Stored seeds of *Sida alnifolia* registered significant reduction in total extractives (26.71 per cent), total alkaloid (42.30 per cent) and total phenol (38.46 per cent) with maximum reduction in total alkaloid, compared to fresh seeds of *Sida alnifolia*. In general, upon storage, the biochemical parameters reduced significantly. Indicating a probable change taking place during after ripening maturation of embryo.

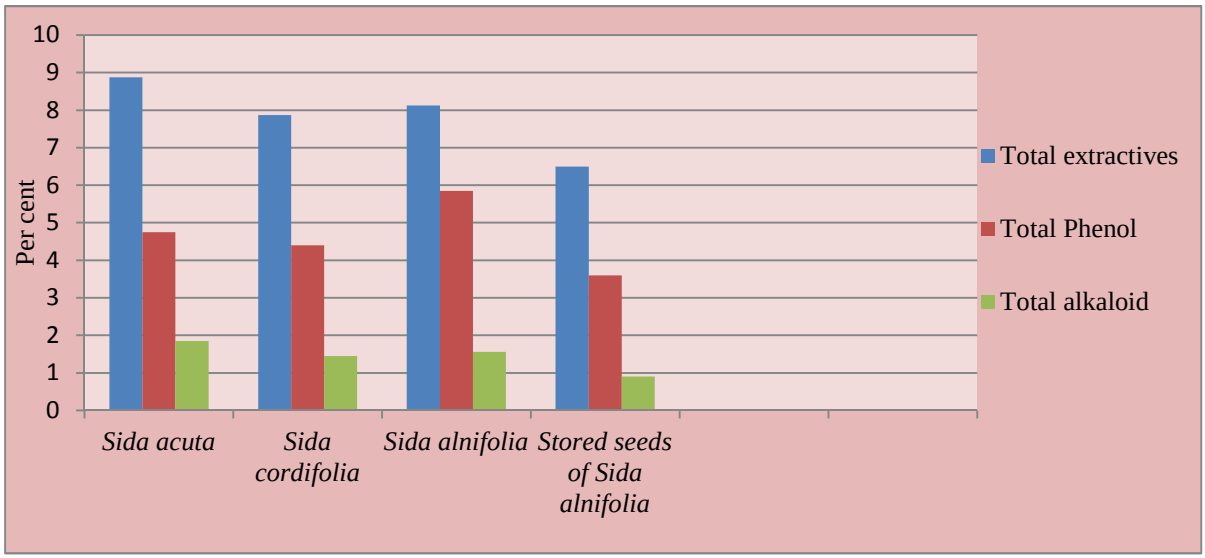


Fig. 9 Biochemical parameters of seeds of *Sida* spp.

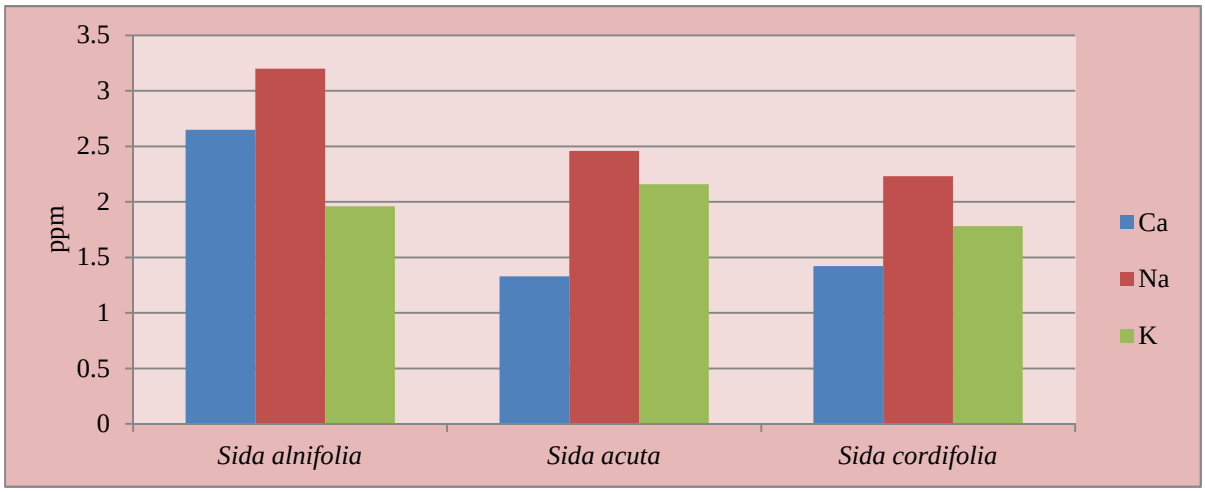


Fig. 10 Effect of storage on the mineral content of seeds of *Sida* spp. under ambient conditions

Newton and Egley (1977) found that both dormant and non-dormant *Sida spinosa* seeds contained water-soluble inhibitors. Williams and Hoagland (1982) reported the combination of coumarin with p hydroxybenzaldehyde to inhibit the germination of *Sesbania exaltata* and *Sida spinosa* to a greater extent than either compound alone. Felix and Harr (1987) reported that marked increase in polyamine content generally occurred in the cotyledons or endosperm on germination of many species. Fischer *et al*. (1989) reported that seed germination of the dicotyledons and monocotyledons was both inhibited and promoted, depending on the compound and the specific species or cultivars, at concentration as low as 1 µM.

5.3. TREATMENTS FOR IMPROVING GERMINATION

Studies on characterization of dormancy revealed that freshly harvested seeds of the three *Sida* species failed to germinate and that seed coat imposed impermeability and requirement of after ripening maturation of embryos are primary reasons restricting germination.

Eighteen pre-treatments comprising scarification (mechanical scarification, chemical scarification), scarification followed osmoticum, boiling water treatments, boiling followed by freezing, hydration (water soaking) and soaking in cow dung slurry, were tried for softening the seed coat and improving germination. This was compared with untreated control.

Germination per cent and speed of germination varied significantly among the various pre-treatments (Fig. 11). Pre-treatment of seeds with Conc. H_2SO_4 was significantly superior to rest of the treatments. Increase in the duration of scarification showed gradual increase in germination percentage and speed of germination and maximum values were seen for 30 min treatment with Conc. H_2SO_4.

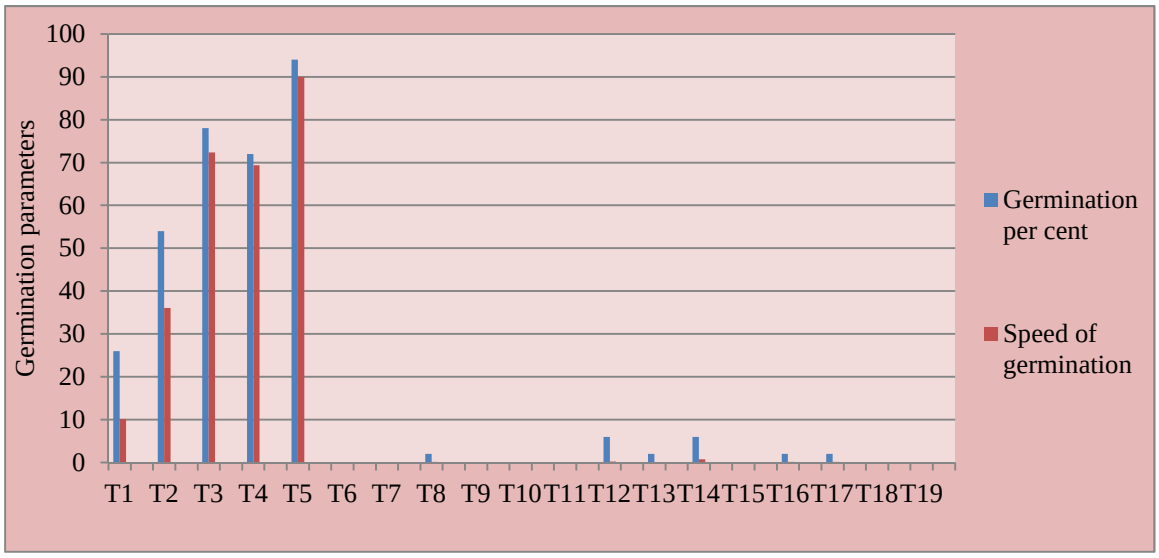


Fig. 11 Effect of pre-treatments on seed germination in *Sida alnifolia*

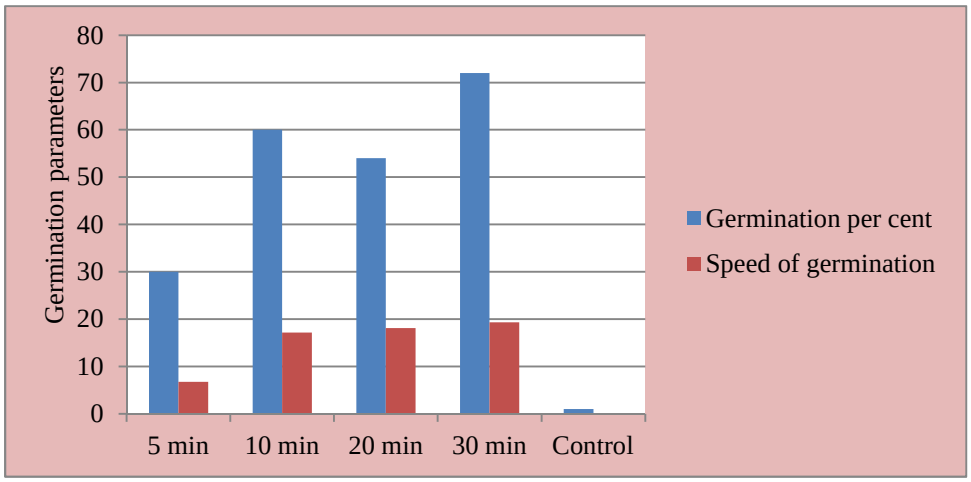


Fig. 12 Effect of scarification treatments on germination and speed of germination

Similar to the present findings, Packa *et al*. (2014) also found that scarification for 30 minutes with 95.00 per cent sulphuric acid was most effective in breaking the physical dormancy of seeds of *Sida hermaphrodita*, resulting in imbibition without impairing embryo viability. Lissy (2004) reported beneficial effect of acid scarification in four *Sida* species with the optimum duration to be 10-20 min as well as variation in germination per cent, with the highest in *Sida cordifolia* and lowest in *Sida rhombifolia*. The favourable influence of Conc. H_2SO_4 in improving germination was strongly supported by Chauhan and Johnson (2008) whom suggested that seeds of *Sida* treated with sulphuric acid for 120 min resulted in 65.00 per cent germination compared with five per cent, for non-scarified seeds and predicting the response to scarification indicates that a hard seed coat is the primary mechanism restricting germination.

Mechanical scarification with sand registered 26.00 per cent germination, less than 60.00 per cent which is minimum mandatory germination requirement. In contrast, beneficial effect of mechanical scarification was reported by Pedroso (2007) in *Sida rhombifolia* and Shooshtharian and Salehi (2010) in *Alcea aucheria*; probably the agents used and extent of scarification may be different.

Sand paper rubbing followed by soaking in one per cent KNO_3 for 12 h was not very effective giving only six per cent germination. Oxidants like KNO_3 help to break dormancy in seeds of many species (Roberts and Smith 1997). As per the findings of Bewley and Black (1994), KNO_3 enhances germination by enhancing the O_2 level. However, in the case of *Sida*, dormancy may not be related to oxygen availability, since soaking seeds for 12 h in KNO_3 could not improve germination percentage.

Germination percentage for the hot water boiling and soaking treatments, ranged from zero to six per cent. Hot water boiling followed by freezing, overnight

soaking in cow dung slurry and making pin pricks followed by soaking in water for 48 h recorded only two per cent germination. Soaking in water for 48 h, overnight water soaking and untreated seeds failed completely to soften the seed coat and none of the seeds germinated.

Among the eighteen pre-treatments tried to soften the seed coat, scarification treatments with Conc. H_2SO_4 were the best. The effect of best four treatments of acid scarification with Conc. H_2SO_4, treatment for 05 min, 10 min, 20 min and 30 min compared with untreated control on growth and vigour of seedlings was studied.

Effect of scarification using Conc. H_2SO_4 for different durations on the growth and vigour of seedlings indicated that acid scarification treatments of all durations were significantly superior compared to untreated control seeds (Fig. 12). Scarification for higher duration of 30 min recorded highest germination percentage, speed of germination, dormancy index, seedling growth and vigour parameters and lowest intensity of dormancy (28.00 per cent), which is inversely related to germination. Scarification for 10 min and 20 min were on par but superior than scarification for the duration of five minutes. Untreated seeds recorded highest intensity of dormancy (99.00 per cent), exhibiting only one per cent germination (Table 12 and 13).

The rate of water imbition was faster for the fresh seeds than stored seeds (Table 14). Irrespective of treatments, the maximum mass increase was noticed within three hours of soaking for the scarification treatments, but untreated seeds absorbed maximum water after six hours of soaking. Irrespective of age of seeds, with increase in duration of scarification, rate of imbition was also higher but the rate of increase was more pronounced in fresh seeds than in stored seeds. However, increase in water imbition rate after at six hours, 12 h and 24 h was low for the fresh

seeds than the stored seeds and after the 24 hours of soaking in water, per cent of mass increase of fresh and stored seeds were near similar.

Therefore, it is presumed that the difference in germination percentage between acid scarification treatments, mechanical and hot water treatments and other treatments might be due to differences in the rates of seed coat softening and water imbibition as achieved by the pre-treatment techniques.

5.4. SEED STORAGE STUDIES

In this study, the main focus was on the impact of storage conditions and intervals of storage on storability of seeds (Tables 18-29). Freshly harvested seeds of *Sida alnifolia* whose moisture content was brought down to 4-5 per cent were stored for one year, in self sealing polythene bags at ambient, refrigerated (6-7^{o} C) and freezed (0^{o} C) conditions. Seed quality parameters like germination per cent, speed of germination, intensity of dormancy, dormancy index, vigour of seedlings, water imbibition and electrical conductivity were recorded at four months interval for one year. The effect of pretreatment with acid scarification on seedling quality parameters at different durations (05, 10, 20 and 30 min) consequent to storage of seeds was also studied.

Acid scarification treatments improved germination and scarification for higher duration of 30 min recorded highest germination per cent of 51.55 per cent and least for untreated seeds (3.33 per cent). Similarly, storage of seeds under cooler conditions had a beneficial effect on these parameters compared to seeds stored under ambient condition. There was no germination for untreated seeds stored at ambient condition even after storage for one year, whereas germination per cent was nine and 13.00 per cent for the seeds under refrigerator and freezer conditions. Respectively Minimum mandatory germination requirement of 60.00 per cent was achieved for seeds stored under freezer after eight months of storage and reached 81.00 per cent

after one year of storage. During the seed storage period of one year, germination per cent showed an increasing trend the increase was linear from 14.80 per cent germination at four months interval to 42.33 per cent from at eight months storage and 52.86 per cent at one year storage.

This finding is consistent with the results obtained by Bhale *et al*. (1988) who observed that cotton seeds stored for over 90 days showed an increase in the percentage of hard seeds but storage for over 180 days resulted in a reduced hard seed percentage. Near normal germination percentages were observed in seeds stored for over 120 days. Similarly, Narwal *et al*. (1998) also found that seed viability and germination reached its maximum after six months of storage in all the cultivars of okra, thereafter, it declined gradually.

In the case of speed of germination, highest speed of germination was recorded for the seeds invigorated with higher duration of Conc. H_2SO_4 for 30 minutes (17.20) and least for untreated seeds (0.81). With respect to storage conditions, cooler conditions (freezer-12.86 and refrigerator-12.87) were on par but significantly superior to storage under ambient condition (9.91). Speed of germination increased substantially with the intervals of storage, with 4.65 in first interval, 11.45 in second interval and a maximum of 19.45 during third interval of storage.

Other seed quality parameters like growth of seedling (shoot length, root length, total length, fresh weight and dry weight), vigour index-I, vigour index-II and dormancy index, showed gradual increase for higher durations of acid scarification and 30 min treatment, recorded maximum values. Seed storage under cooler conditions was significantly superior over seeds stored in ambient condition. Growth and vigour exhibited increasing trend and seeds stored for one year recorded maximum values.

In the case of intensity of dormancy, untreated seeds exhibited highest values (96.66 per cent) where as lowest values were recorded for acid scarification for 30 min. Among the storage conditions, seeds stored in refrigerator (59.00 per cent) and freezer (60.30 per cent) stored recorded significantly lower intensity of dormancy and were on par, where as seeds stored under ambient condition showed higher intensity of dormancy (69.20 per cent). Similarly, seeds stored for longer intervals witnessed very low intensity of dormancy (47.13 per cent) unlike the four months old seeds (85.20 per cent). Similar findings were reported by Doijode (1997) who observed that highest seed viability and extended storage in okra for ten years was achieved for the seeds stored under low temperatures and under sub-zero temperatures.

There was an explicit increase in leakage of seed leachates with increase in the duration of storage. In conformity with the present finding, Seal and Gupta (2000) also observed elevated leakage of electrolytes in aged seeds than freshly harvested seeds of *Sida acuta* and *Sida rhombifolia*. The observed increase in EC values of seed leachates upon storage could be due to membrane aberration of the seeds, as observed by Berjak and Villers (1972) in maize. Yadav *et al*. (1987) also related the increased EC values with weakening of cell membrane integrity in case of sal seeds and they opined that soluble leakage would increase germinability of seeds. When the storage conditions are compared, seeds stored in freezer recorded maximum content of minerals and pH.

When a comparison of seeds stored under different conditions was made, it was observed cooler conditions exhibited the higher concentration of salts, pH and electrical conductivity of the seed leachates than the seeds stored under ambient condition and this may be a reason for increased germination for the seeds stored under cooler conditions.

With advance in storage period there was gradual decrease in per cent imbibition of water, perhaps due to the thickening of seed coat as indicated by the anatomical studies (Fig. 13). Similar findings of decreased water imbibition over period of storage was reported by Packa *et al*. (2014) in *Sida hermaphrodita* seeds,

Seeds stored under cooler conditions imbibed water quickly and highest water imbibition was noted for the seeds stored in freezer (Fig. 14). Seeds stored in ambient condition showed a slow and low water imbibition. In consequence with the present findings, Tubic *et al*. (2005) found that amount of water absorbed by sunflower seed in a 24 h period decreased with age. The rate of decrease was higher both in seeds stored in ambient conditions than in those stored at 4°C and 80-85 per cent relative humidity. Mota *et al*. (2012) observed the decreased germination percentage in seeds of *Jatropha curcas*, when the time of water imbibition was increased.

The better response of seeds to low temperature storage especially is attributed to increased water imbibition, electrical conductivity (Fig. 15) and content of minerals (Fig. 16).

As reported by Tran and Cavanagh (1984), the observed causes of seed dormancy in *Sida* are hard seed coat with an impervious cuticle, impermeability of seed coat to water and gases, immaturity of the embryo, presence of inhibitors and mechanical restriction to embryo growth.

The present investigation could thus establish that seeds of *Sida* species exhibit coat imposed dormancy and require two months of after ripening period. Fresh seeds and seeds stored for one year under ambient condition failed to germinate without pre-treatment. Acid scarification has brought out significant reduction in the seed coat thickness, caused abrasions and improved water imbibition. Pre-treatment of seeds with Conc. H_2SO_4 for 30 min is suggested to bring down dormancy and

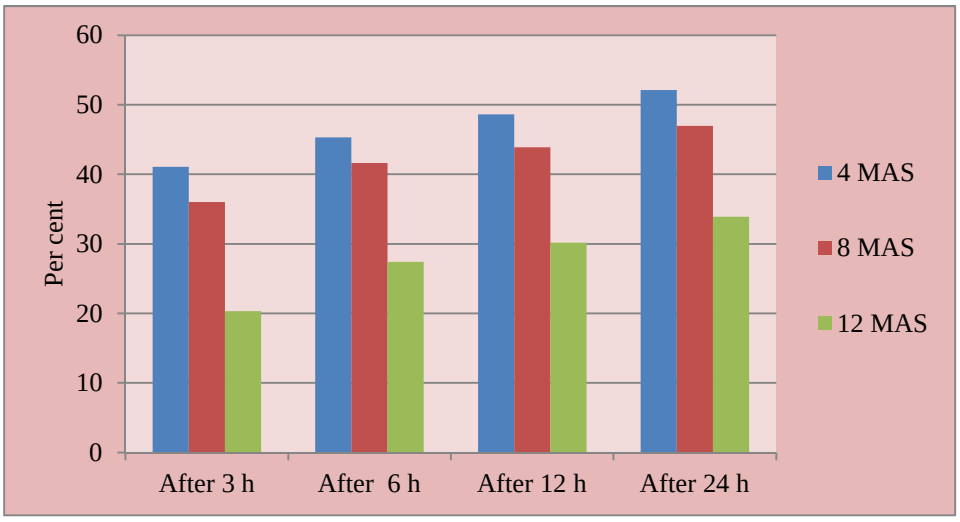


Fig. 13 Effect of storage intervals on water imbibition per cent in *Sida alnifolia*

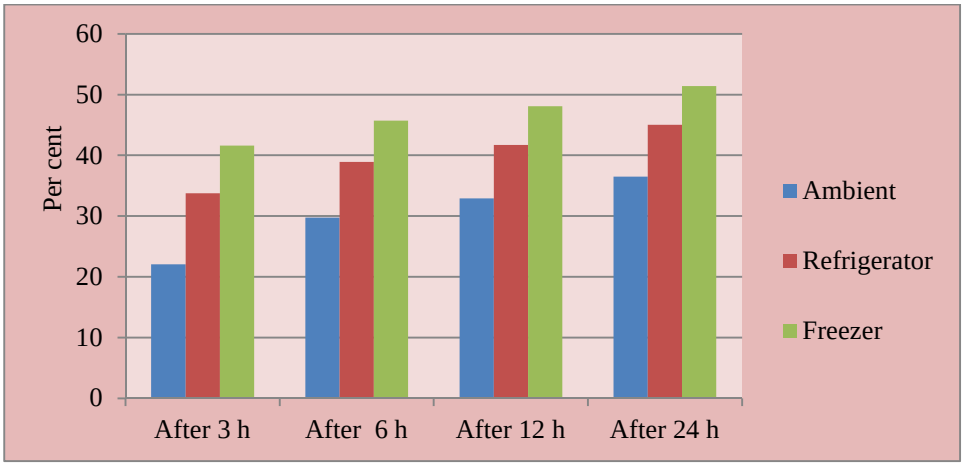


Fig. 14 Effect of storage conditions on water imbibition per cent in *Sida alnifolia*

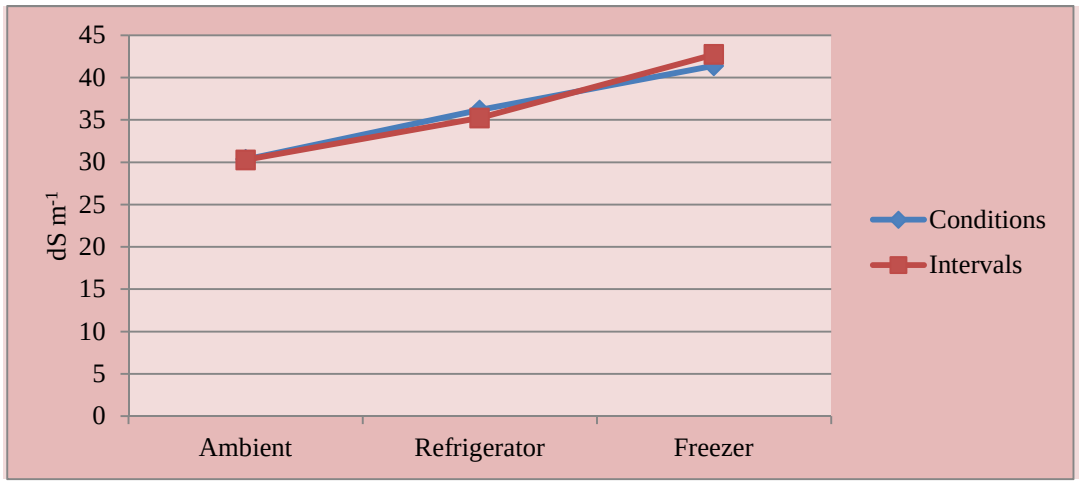


Fig. 15 Effect of storage intervals and conditions on electrical conductivity of seed leachates in *Sida alnifolia*

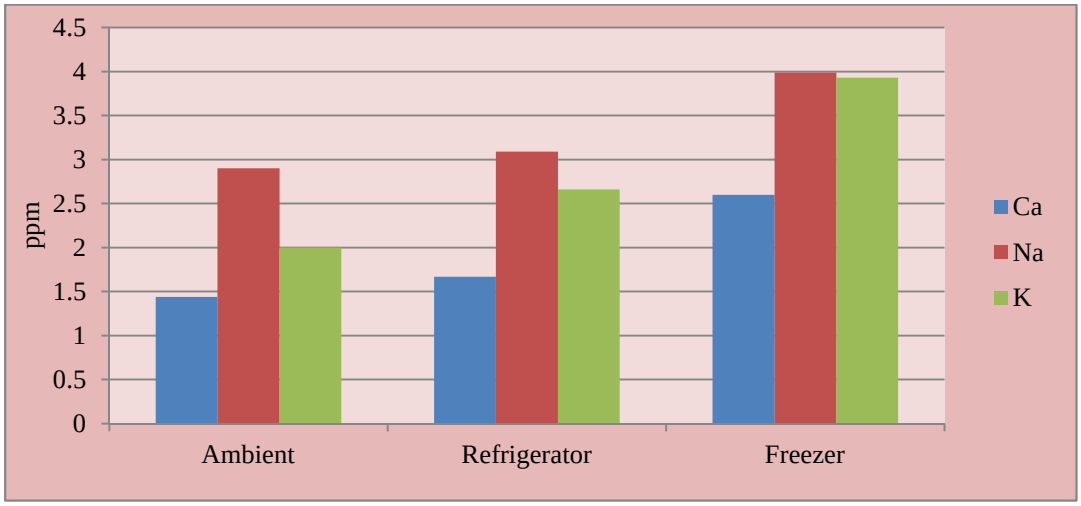


Fig. 16 Effect of storage conditions on content of minerals in seed leachates of *Sida alnifolia*

improving germination without impairing embryo viability and vigour of seedlings. Subjecting the seeds to natural weathering is a simple way of taking care of dormancy and improving germination. The seeds can be effectively stored under cooler conditions in refrigerator / freezer for one year or longer, which also lowered dormancy and improved germination and vigour of seedlings. Storage of seeds for eight months under freezed condition and scarification with Conc. H_2SO_4 for 30 min is found necessary to get minimum mandatory germination of 60.00 per cent.

Future line of work suggested:

1. Response of seeds to long term storage
2. Role of inhibitors in dormancy behaviour
3. Characterization of after ripening of embryo

Summary

SUMMARY

The present investigation on "Seed production potential, dormancy and seed storage behaviour in *Sida* spp." was undertaken at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2012-2014. The study aimed to assess seed production potential and characterize dormancy in three *Sida* species such as *Sida acuta*, *Sida alnifolia* and *Sida cordifolia* and to standardize ideal pre-treatment for improving germination and to study seed storage behaviour in *Sida alnifolia*.

- Significant difference in vegetative growth parameters was observed in the three species and *Sida alnifolia* was most vigorous, recording highest plant height and number of primary branches. Flowering and fruiting in *Sida* species was staggered and started four months after planting. *Sida acuta* was early to flower (120 days) with more weeks of seed rain (10) and seed yield (1562.65 kg ha^{-1}) or seed production potential. *Sida alnifolia* was late to flower (142 days) with less weeks of seed rain (06) and witnessed lowest, seed yield (713.87 kg ha^{-1}). Highest hundred fruit weight (5.27 g), hundred seed weight (0.48 g) and number of seeds per fruit (09) recorded high in *Sida cordifolia*.

- Physical characterization of dormancy by analysis of seed coat thickness and embryo size of three species revealed that hard thick seed coat comprising endoderm; mesoderm, periderm and presence of thick corrugated impermeable cuticle were major limiting factors restricting the exchange of water and gases. *Sida cordifolia* registered highest seed coat thickness and least in *Sida alnifolia*. Upon storage, the total thickness of the coat and individual layers was slightly reduced but embryo area, perimeter and cuticle

thickness increased slightly especially, *Sida alnifolia* has maximum embryo area, perimeter and cuticle thickness.

- Physiological conditions like exposure of seeds to different temperatures *viz*. 30º C, 35º C and 40º C sown with and without imbibition, light and dark exposure for eight and sixteen hours and exposure to stress by hydration and dehydration for 48 h and resowing of same seeds after four months repeating hydration and dehydration, failed to supplement the embryo requirements or make alterations in the seed coat, which were found essential to improve germination.

- Germination studies in both fresh and stored seeds of three *Sida* species, revealed that irrespective of the species, whether fresh or stored failed to germinate without pre-treatment even after 45 days and 14 days of observation respectively. Fresh seeds pre-treated with Conc. H_2SO_4 for 20 min recorded maximum of two per cent germination in *Sida cordifolia* and only one per cent each in *Sida alnifolia* and *Sida acuta* whereas stored seeds *Sida acuta* and *Sida alnifolia* gave 48.00 and 33.40 per cent germination respectively. *Sida acuta* showed better performance with respect to germination per cent (48.00) and speed of germination (16.46), reduced intensity of dormancy (52.00) whereas growth of seedling was better for *Sida alnifolia*. The germination per cent, speed of germination, growth parameters of seedling and vigour index were improved upon storage of seeds for one year, whereas intensity of dormancy got reduced.

- Germination studies (two months old) of *Sida* species in field under rain-fed and irrigated conditions indicated that seeds exposed to natural weathering showed a spontaneous response on receipt of rain during June and the

germination completed in ten days with the highest germination (74.50 per cent) being recorded in *Sida acuta* followed by *Sida alnifolia* (67.50 per cent) and lowest in *Sida cordifolia* (42.50 per cent). Under irrigated conditions, germination started from the month of sowing in February itself and was continuous upto ten months (November) giving germination per cent of 7.25-14.50.

- Biochemical analysis of seeds revealed that *Sida* species differed in the content of total extractives, total phenol and total alkaloid and *Sida acuta* recorded higher contents of total extractives and total alkaloid whereas total content of phenol was highest in *Sida alnifolia*. Seeds of *Sida alnifolia* stored for one year recorded significant reduction in the biochemical constituents, with highest reduction in the total alkaloids, indicating a probable change taking place during after ripening maturation of embryo.

- Among the eighteen pre-treatments tried for the improving germination, chemical scarification using Conc. H_2SO_4 was significantly superior and recorded higher germination per cent and speed of germination. Abrasion with sand paper was found the next better treatment. Untreated seeds failed to germinate. As indicated by anatomical studies of seed coat, scarification has brought out significant reduction in the seed coat thickness especially cuticle and disruption of the seed coat, imparting germination. Scarification of seeds with Conc. H_2SO_4 for higher duration of 30 min was selected as the best treatment considering the improvement in germination per cent, speed of germination, growth and vigour of seedling and significant reduction in the intensity of dormancy.

- Storage of seeds for one year revealed that improvement of germination and vigour of seedling and reduction in dormancy factors was at a positive side

compared to fresh seeds. The overall germination per cent was 14.80 at four months after storage which reached 52.86 per cent after 12 months. There was also significant increase in growth and vigour of seedlings with advancement in storage period. Storage under cooler atmosphere either refrigerator or freezer, was found to improve the germination and vigour of seedling compared to ambient storage.

- Seeds stored under cooler conditions recorded higher values for content of minerals such as calcium, sodium and potassium, pH and electrical conductivity than seeds stored under ambient condition. The significant increase in germination and vigour of seedling during storage especially under cooler conditions can possibly be attributed to either one or combination of the factors like after ripening of the embryo, reduced per cent of hard seeds, reduction in biochemical constituents and increase in minerals content and increased leakage of seed leachates due to membrane aberration.

- The present investigation could thus establish that seeds of *Sida* species exhibit coat imposed dormancy and require two months of after ripening period. Fresh seeds and seeds stored for one year under ambient condition failed to germinate without pre-treatment. Acid scarification has brought out reduction in the seed coat thickness, caused abrasions and improved water imbibition. Pre-treatment of seeds with Conc. H_2SO_4 for 30 min is suggested to bring down dormancy without impairing embryo viability and vigour of seedlings. Subjecting the seeds to natural weathering is a simple way of taking care of dormancy and improving germination. The seeds can be effectively stored under cooler conditions in refrigerator / freezer for one year or longer, which also lowered dormancy and improved germination and vigour of seedlings. Storage of seeds for eight months under freezed

condition and scarification with Conc. H_2SO_4 for 30 min is found necessary to get minimum mandatory germination of 60 per cent.

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* Original not seen

Appendices

Appendix I. Meteorological data during the crop growing period

Month (2013-14)	Mean Max. Temp (° C)	Mean Min. Temp (° C)	Mean RH (%)	Rainfall (mm)
June	28.50	22.70	90.00	1031.80
July	28.40	22.70	91.00	932.30
August	29.90	22.90	84.00	305.90
September	30.00	22.20	85.00	344.10
October	30.80	22.60	83.00	309.80
November	32.60	23.90	73.00	82.00
December	31.90	22.30	61.00	0.50
January	32.90	23.00	59.00	00
February	34.70	22.90	56.00	00
Total	-	-	-	3006.40
Average	**31.07**	**22.80**	**75.77**	**334.04**

SEED PRODUCTION POTENTIAL, DORMANCY AND SEED STORAGE BEHAVIOUR IN *Sida* spp.

by
VEERESH NETEKAL

(2012-12-120)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the

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DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF HORTICULTURE
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ABSTRACT

The study entitled "Seed production potential, dormancy and seed storage behaviour in *Sida* spp." was undertaken at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2012-2014. The study aimed to assess seed production potential and characterize dormancy in three *Sida* species (*Sida alnifolia*, *Sida acuta* and *Sida cordifolia*) and to standardize ideal pre-treatment for improving germination and to study seed storage behaviour in *Sida alnifolia*.

Flowering and fruiting in *Sida* species was staggered and started four months after planting. Flowering and fruiting was earliest in *Sida acuta* and latest in *Sida alnifolia*. Maximum seed rain and seed production potential was exhibited by *Sida acuta.* The fruits and seeds were biggest in *Sida cordifolia*.

Physical characterization of dormancy by analysis of seed coat thickness and embryo size of three species revealed that hard thick seed coat comprising endoderm, mesoderm, periderm and presence of thick corrugated impermeable cuticle was a major limiting factor restricting the exchange of water and gases. Acid scarification brought out reduction in all layers of the seed coat with maximum reduction in cuticle thickness. Upon storage, the total thickness of the seed coat and individual layers was slightly reduced but embryo area, perimeter and cuticle thickness increased slightly.

The physiological conditions tried such as exposure of seeds to different temperatures, light and dark and exposure to stress by hydration and dehydration failed to promote germination in fresh seeds. Irrespective of the species, seeds whether fresh or stored, also failed to germinate without pre-treatment.

Germination studies under field conditions indicated that seeds exposed to natural weathering showed a spontaneous response on receipt of rain and the

germination was completed in ten days with highest germination (74.50 per cent) in *Sida acuta* followed by *Sida alnifolia* (67.50 per cent) and least in *Sida cordifolia* (42.50 per cent).

Biochemical analysis of seeds revealed that species differed in the content of total extractives, total phenol and total alkaloid and *Sida acuta* recorded higher contents of total extractives and total alkaloid whereas total phenol content was higher in *Sida alnifolia.* Analysis of the seed leachates indicated species difference in the content of minerals and electrical conductivity. *Sida acuta* recorded the highest potassium and EC values.

Among the eighteen pre-treatments tried for improving germination, chemical scarification using Conc. H_2SO_4 was significantly superior and recorded higher germination per cent and speed of germination. Untreated seeds failed to germinate. Scarification of seeds with Conc. H_2SO_4 for higher duration of 30 minutes was selected as the best treatment considering improvement in water imbibition, germination per cent, speed of germination, growth and vigour of seedling and significant reduction in the intensity of dormancy.

Storage studies revealed that upon storage, germination, vigour of seedling and reduction in dormancy factors were at a positive side compared to fresh seeds. Storage of seeds under cooler atmosphere either refrigerator or freezer was found to improve the water imbibition, content of minerals, germination and vigour of seedling compared to ambient storage.

The present investigations could thus establish that seeds of *Sida* species exhibit coat imposed dormancy and require two months of after ripening period, posing problems in germination. Pre-treatment of seeds with Conc. H_2SO_4 for 30 minutes is suggested to bring down dormancy and improve germination and vigour of seedlings. Exposing the seeds to natural weathering is a simple way of improving germination.

The seeds can be effectively stored under cooler conditions in refrigerator / freezer for one year or longer which also lowered dormancy and improved germination and vigour of seedlings.