PROPAGATION STUDIES IN SARPAGANDHA, *RAUWOLFIA SERPENTINA* (L.) BTH.EX KURZ.

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July, 2013

PROPAGATION STUDIES IN SARPAGANDHA *RAUWOLFIA SERPENTINA* (L.) BTH.EX KURZ

Thesis submitted to,

DR. BALASAHEB SAWANT KONKAN KRISHI VIDYAPEETH,
DAPOLI DIST. RATNAGIRI, MAHARASHTRA STATE.

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (FORESTRY)

In

Medicinal and Aromatic plants
(Forest Product Utilization)

by

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Under the guidance of

Dr. D. N. Mokat
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This is to certify that, the thesis entitled **"PROPAGATION STUDIES IN SARPAGANDHA, *RAUWOLFIA SERPENTINA* (L.) BTH.EX KURZ."** is a record of independent bonafied research work carried out by **PARAG PRAKASH SALVI** (FDPM 11-27) at this college during the academic periods 2012 - 2013, under my guidance and supervision for the degree of M.Sc. (Forestry) of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. The said thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or any other similar title.

The source of materials used and all assistance received if any, during the course of investigation have been duly acknowledged.

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Date: July, 2013 *(Parag Prakash Salvi)*

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THESIS ABSTRACT

a)	Title of the thesis	:	**PROPAGATION STUDIES IN SARPAGANDHA, *RAUWOLFIA SERPENTINA* (L.) BTH. EX KURZ.**
b)	Full name of student	:	**Parag Prakash Salvi**
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f)	Major subject	:	**Forest Product and Utilization**
g)	Abstract	:	

The root drug, *Rauwolfia serpentina* (L.) Bth. ex Kurz (Family-Apocynaceae) is used for the treatment of mental illness, hypertension, blood pressure, snake bite, etc. Demand for this root drug has increased in traditional and modern systems of medicine in recent days. However, natural population of this species is under dwindling stage. The plant is categorized as an 'endangered' by IUCN. In one side the demand has increased for the manufacture of drugs, while in another side the plant is naturally under threat. *R. serpentina* is naturally found in Konkan region of Maharashtra in particular and in Maharashtra in general. Farmers of the state also enquire about its seed, planting material, propagation techniques, agro-techniques, etc. Hence the present investigations on propagations were conducted for the mass scale seedling production of *R. serpentina.*

The field experiments were conducted to study various aspects of present study at experimental farm of College of Forestry, Dapoli Dist. Ratnagiri, (Maharashtra) during the year 2012-13. In the present study, observations on different parameters *viz.* seed germination per cent, seedling growth, biomass attributes and survival per cent was recorded. The important findings of the present investigations are summarized below-

In seed propagation, 68.25 per cent germination was recorded maximum in T_1 (scarification) compared to other treatments. In Germination attributes *viz.*

Germination Rate Index (3.18), Mean Daily Germination (2.24), Peak Value of germination (2.24) and Germination Value (5.08) was observed maximum in T_1 (scarification) followed by in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) in which Germination Rate Index (2.56), Mean Daily Germination (1.76), Peak Value of germination (2.14) and Germination Value (3.75). The overall performance of treatment T_1 (scarification) was found better in per cent germination and germination attributes as compared to other treatments.

Propagation through seed: Biomass parameters after 90 DAS- The maximum Fresh shoot weight 0.55 gm, Dry shoot weight 0.09 gm, Dry root weight 0.04 gm, Total fresh weight 0.76 gm and Total dry weight 0.13 gm and fresh root weight was recorded maximum in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) and maximum fresh root weight 0.26 gm was recorded in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) after 90 DAS.

Propagation through Soft wood leafy cutting-: Growth parameters after 90 DAP- The maximum number of shoots 1.74 was recorded in T_2 (soaking cuttings in IBA @ 25 ppm for 30 min). The maximum shoot height 13.07 cm was recorded in T_3 (soaking cuttings in IBA @ 50 ppm for 30 min). The number of leaves 6.69, root length 10 cm and diameter 3.36 mm was recorded maximum in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min). The highest value recorded for number of roots 15.75 in T4 (Soaking cuttings in IBA @ 75 ppm for 30 min)

Propagation through Semi-hard wood cutting-: Growth parameters after 90 DAP- The maximum number of shoots 2.30 was recorded in T_1 (Control) and showed better results. The maximum root length 9 cm, number of roots 13 and diameter 4.50 mm was recorded in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min). The maximum number of leaves 11.64 was recorded in T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) and The maximum shoot height 16.27 cm was recorded in T_4 (soaking cuttings in IBA @ 75 ppm for 30 min) after 90 DAP.

Propagation through Root cutting: Growth parameters after 90 DAP- The maximum number of shoots 1.80 was recorded in T_1 (Control). While treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) showed maximum Shoot height 10.97 cm, Number of leaves 13.55, Root length 10.88 cm and Number of roots 14.25 whereas, Diameter 8.07 mm recorded highest in T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) after 90 DAP.

Propagation through Root cutting: Biomass parameters after 90 DAP- The treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) showed maximum Fresh shoot weight 5.50 gm, Fresh root weight 2.27 gm, Dry shoot weight 1.66 gm, Dry root weight 0.88 gm, Total fresh weight 7.84 gm and Total dry weight 2.55 gm after 90 DAP. Maximum per cent survival (78%) was recorded in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) and also overall performance in growth parameters, Biomass parameters and per cent survival was found maximum in the same treatment.

On the basis of overall results of the experiment, treatment T_1 (scarification) to seed was found better in per cent germination and germination attributes as compared to other treatments. Treatment T_1 (scarification) showed maximum per cent germination 68.25 followed by T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min). In the leafy soft wood cutting treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) was given good results for number of leaves, root length, diameter and biomass parameters. In the propagation through semi-hard wood cutting the treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) showed better results for root33 length, number of roots, diameter and biomass parameters after 90 DAP. Treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) showed good results in the experiment propagation through roots and showed better results as compared to other treatments for shoot height, number of leaves, root length, number of roots and other biomass parameters.

Maximum survival per cent was recorded in propagation through seed with scarification followed by GA_3 200 ppm treatment and propagation through roots. Therefore these two methods are recommended for mass multiplication of *Rauwolfia serpentina.*

Date:

Place: Dapoli **(Parag Prakash Salvi)**

CHAPTER I
INTRODUCTION

Medicinal plants have been the subjects of man's curiosity since time immemorial. Almost every civilization has a history of medicinal plant use. Global estimates indicate that 80 per cent of about 4 million population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. (Talye *et al.,* 2012).

India is a land of varied climatic, ethnic, and linguistic zones. The Indian subcontinent is treasure trove for medicinal plants and also the birthplace of several traditional health-care system *viz*. Ayurveda, Unani, Siddha, etc. (Karan and Karan., 2003). There are about 45,000 plant species in India, most of the endemic and threatened plants are concentrated in the hotspots *viz*. Eastern Himalayas, Western Ghats and Andaman and Nicobar Island. The Traditional Indian System of Medicine has been therefore well developed due to this richness of bio-resources. Interest on indigenous health traditions and demand for herbal remedies are undoubtedly discernible trends worldwide. (Patil and Patil., 2010). Due to varied diversity, usage, availability and distribution found in different parts of India most of world's peoples attract towards Indian Herbs for getting the material for solving the health problems. There are 960 Indian drugs in world trade. *Rauwolfia serpentina, Gloriosa superba, Taxus baccata, Inula racemosa,* etc. are the some of the Indian drugs have consumption is more than 100 MT in the international market. In the other hand these drugs are on the line of rarity in wild state. *Rauwolfia serpentina* is one of them and is categorized as "endangered". Therefore, there is a need to cultivate these valuable threatened drugs on farmers field, forest waste lands, etc.

Rauwolfia serpentina (L.) Bth. ex Kurz, Sarpagandha has attracted worldwide attention and used for centuries in the Ayurvedic and Unani systems of medicines in South Asia (Ilahi *et al.* 2007). Sarpagandha is one of the most important medicinal plants known in ancient Indian medicinal literature since 3000 years back. Plumier in 1703 assigned the name *Rauvolfia* to the genus in honour of a German physician – Leonhart Rauvolf of Augsburg. The genus *Rauvolfia* of

Apocynaceae family comprises over 170 species distributed in the tropical and subtropical parts of the world including 5 species *Rauwolfia serpentina* (L.) Bth. ex Kurz*., Rauwolfia vomitoria* Afz. *, Rauwolfia canescens* Linn., *Rauwolfia densiflora, Rauwolfia beddomei* are native to India. It is also known as in Sanskrit 'Saragandha' and 'Chandra'. It is also popular as *"Madman's medicine"* among tribals. (Trivedi 1995 and Joy *et al.* 1998).

Chopra *et al.* (1933) were first to observe on experimental animals the hypotensive properties of *Rauwolfia* roots. But the attention of western countries was drawn to it only after the isolation and examination of its most important alkaloid, reserpine, by the Swiss scientists, Schlittler and Mueller, in 1952. After that, there was so much demand for its roots. The extraordinary medicinal properties of the *Rauwolfia serpentina* have brought this Indian drug plants to the attention of the medical world (Dutta *et al.*1963)

Its dried root is the economical part which contains a number of alkaloids of which contain more than 50 alkaloids *viz.* deserpidine, ajmaline, neoajmalin, serpentine, ajamalacine, reserpine, rescinnamine are pharmacologically important. The root is a sedative property and is used to control high blood pressure and certain forms of insanity; In Ayurveda, it is used for the treatment of insomnia, epilepsy, asthma and painful delivery. It is used in snake-bite, insect stings, and mental disorders. (Joy *et al.* 1998; Panday and Mandal 2010)

Rauwolfia serpentina (L.) Bth.Ex Kurz. is an erect perennial shrub that normally grows up to 15-45 cm, but it can be grow up to 90 cm under cultivation. Roots are vertical, tapering up to 15 cm thick at the crown and long giving a serpent-like in appearance. Roots greenish-yellow externally and pale yellow inside, extremely bitter in taste. Leaves are in whorls of 3-4, elliptic-lanceolate or obovate, pointed at apex. Flowers numerous borne on terminal or axillary cymose inflorescence. Corolla tubular, 5-lobed, 1-3 cm long, whitish-pink in colour. Fruit drupe, obliquely ovoid and purplish black in colour at maturity with stone containing 1-2 ovoid wrinkled seeds. (Plate.1) The plant is cross-pollinated. (Sulochana, 1959).

Its ideal habitats receive an annual rainfall of 1500-3500 mm and the annual mean temperature is 10-38°c. It grows up to an elevation of 1300- 1400 m from

mean sea level. It can be grown in open as well as under partial shade conditions. It grows on a wide range of soils. Medium to deep well drained fertile soils and clay-loam to silt-loam soils rich in organic matter are suitable for its cultivation. It requires slightly acidic to neutral soils for good growth (Sulochana, 1959; Dutta *et al.*1963).

The plant is distributed widely in sub-Himalayan tract from Punjab eastward to Nepal, Sikkim and Bhutan, Assam, lower hills of Gangetic plains, Eastern and Western Ghats, Central India and in the Andamans. (Trivedi and Kumari 2011)

The International Union for the Conservation of Nature and Natural Resources (IUCN) has assigned an endangered status to *Rauwolfia serpentina;* Indian Government has prohibited the exploitation of wild growing plants in forest and its export since 1969. (Trivedi 1995; Joy *et al.* 1998 and Panwar and Guru 2011). In addition to India, Thailand is the chief exporter of *Rauwolfia* alkaloids followed by Zaire, Bangladesh, Sri Lanka, Indonesia and Nepal. (Joy *et al.* 1998).

Demand for medicinal plants is increasing in both developed and developing countries. The Republic of India is the world's leading producer and consumer of *Rauwolfia* root with estimated annual domestic consumption ranging from 2,00,000 to 5,00,000 kg. *Rauwolfia* root, which is mainly wild harvested but also produced somewhat from cultivated sources, is used in the Ayurveda, Siddha and Unani systems of medicine, as well as in folk and homoeopathic medicine (Sajem *et al*. 2008 and Brinkmann, 2011).

In India, nearly 9,500 registered herbal industries and a multitude of unregistered cottage-level herbal units depend up on the continuous supply of medicinal plants for manufacture of herbal medical formulations based on Indian System of Medicine 960 medicinal plant species formed the source of 1289 botanical raw drugs which are traded in the country. Out of which more that 90 per cent of the medicinal plants are collected directly from the forest. This shows the importance of medicinal plant collection from forest (Ved and Goraya, 2007).There are many other potential causes of rarity in medicinal plant species, such as habitat specificity, narrow range of distribution, land use disturbance, introduction of non- native, habitat alternation, climatic changes, heavy livestock grazing, explosion of human

population, fragmentation and degradation of population bottleneck and genetic drift.(Sajem *et al.* 2008)

Germination of seeds is lower in *R. serpentina*. Moreover, collection of seeds from wild sources is laborious and costly, in as much as the plants grow sporadically and the seeds ripen a few at a time. If the ripe seeds are not collected in time, they drop off on the ground and are lost. For these reasons seeds are not easily available from wild sources (Paul *et al* 2008).

The growing demand of *Rauwolfia* roots and its alkaloids have led to domestication studies bringing it into regular agriculture. Several research institutions in India have carried out such studies making its cultivation possible though farmers friendly cultivation practices. At present, it is cultivated in small areas, scattered far and wide in the states of Uttar Pradesh, Bihar, Tamil Nadu, Orissa, Kerala, Assam, West Bengal and Madhya Pradesh. (Trivedi 1995 and Dutta *et al.*1963).

Therefore, for large scale cultivation of this important highly valuable species, production of quality planting material is essential and propagation studies for the production of QPM'S are basis and hence the present work has been proposed with following objective.

1. To standardize propagation methods by root cutting, stem cutting and seed for production of seedlings.

CHAPTER II

REVIEW OF LITERATURE

Research is a continuous process and the review of literature is one of the important aspects in research. It helps the researcher to get acquainted with the subject matter and channelizes research efforts in a desirable direction. Efforts were made to review the relevant literature that, has direct or indirect bearing with the study. The reviewed literature has been presented according to different variables studied. Suitable assumptions have been made on the basis of review of literature. For better understanding the different aspects pertaining to the past work on the propagation of *Rauwolfia* and other species have been presented as under

Propagation by root cutting-

Badhwar *et al*. (1956) elaborated method of propagation and their effect on root production in *Rauwolfia serpentina*. Fresh root cuttings having size 1 to 2 inches in length suitable for propagation and showed better result if planted horizontally, covered with about half an inch of soil. Cuttings with a diameter ranging from above 0.1 to 0.5 inch diameter, above 0.1 inch in diameter planted during March to June showed 52 to 79 per cent survival. They further observed that, the most of the cuttings start sprouting after about 1 to 2 months after planting.

Hedayatullah (1959) conducted experiment on culture and propagation of *Rauvolfia serpentina* Benth at east Pakistan and noted 36 per cent success with root cuttings.

Kwong *et al*. (1972) studied the effect of different harvest times on the root weight and alkaloid content of 2 year old *Rauwolfia serpentina* plants. They also reported that, it is usually propagated by root cutting for commercial cultivation.

Alamgir and Ahamed (2005) investigated growth and phytochemical studies in *Rauwolfia serpentina* and reported that, the cuttings from root-stem junction revealed the highest root formation (78 per cent) followed by root cuttings (62 per cent) and stem cuttings (42 per cent). Per cent rooting and propagule development was reported maximum 83.53 per cent in the treatment 50 ppm IBA. They also mentioned crude alkaloid contents significantly increased only under the higher concentration of N level.

Pandey and Mandal (2010) conducted an experiment on influence of propagation techniques and harvesting time on root yield and alkaloid contents of *Rauwolfia serpentina.* In their experiment 3-5 cm long root cuttings were planted in 1-2 cm deep beds filled with sand in first week of May. They observed that, the cuttings were sprouted within three weeks with 40 to 85 per cent success.

Propagation by stem cutting-

Nair (1955) studied the *Rauwolfia serpentina* (L.) Bth. ex Kurz. its importance and cultivation. He found that, 90 to 95 per cent success in propagule development from stem cuttings, planted between 15 June and 15 July, and only 30 to 50 per cent success even after treatment with plant hormone Seradix B with stem cuttings.

Badhwar *et al*. (1956) carried out a study on *Rauwolfia serpentina* (L.) Bth. ex Kurz.–Method of propagation and their effect on root production. In the study stem cuttings were prepared from woody twigs were about 6 to 9 inches long having 3 internodes. They observed that, the cuttings prepared from tender green twigs have not been successful. They further observed that the cutting start sprouting 3 to 4 days after planting, they actually strike root after about $2^{1}/_{2}$ months, producing 2 to 3 very thin rootlets.

Chandra (1956) carried out an experiment on inducing rooting in stem cuttings of *Rauwolfia canescens* L. and reported that the *R. canescens* can be raised by vegetative means with hard wood cutting after the treatment with hormones (IAA and NAA solutions). Without treatment with hormones the stem cutting did not strike roots in case of *R. canescens* and such cutting dried within a month and half. The 30 ppm solution of hormones appear to be suitable for initiating the production of roots in the species of *Rauwolfia*. The B-Indolyl acetic acid (B-I.A.A.) was superior as it has given higher percentage.

Hedayatullah (1959) carried out a study on culture and propagation of *Rauvolfia serpentina* (L.) Bth. ex Kurz. at east Pakistan and noted 12 per cent success with stem cuttings.

Pal *et al.* (1995) reported vegetative propagation by root branch cuttings in *Rauwolfia serpentina* (L.) Bth. ex Kurz. and stated that semi hard wood cutting and softwood leafy cutting are easy to root. Treatment with IBA and 1-NAA suppressed

the rooting. In the control treatment, IBA 10 mg per liter and IBA 100 mg per liter showed maximum per cent rooting (66.7) while mean number of per cutting was reported in control treatment followed by IBA 10 mg per litter. Mean root length per cutting was reported in IBA 10 mg per litter which was maximum (1.7 cm) in the treatment IBA 10mg followed by control treatment (1.6 cm).

Alamgir and Ahamed (2005) carried out a study on growth and phytochemical investigation of *Rauvolfia serpentina* (L.) Bth. ex Kurz. propagule they reported that to increase the potential further, only stem cuttings were tried with growth regulators. Effect of IBA, NAA, their combinations and 2,4-D in different concentrations on root formation in stem cuttings leading to propagule development of *Rauvolfia serpentina* was investigated. It is evident that, IBA at low concentration stimulated root formation and 50 ppm IBA gave maximum root formation in cuttings 83 per cent. With the further increase of concentration of IBA (100, 200 ppm) root formation decreased and at 500 ppm an inhibitory effect on propagule development compared to control was noted. NAA only at low concentration (10 ppm) stimulated root formation and propagule development up to 66 per cent. At higher concentrations (50, 100, 500 ppm) NAA inhibited the rooting activity of the cuttings. IBA and NAA were used in combination and at low concentration no synergistic effect was observed. IBA and NAA in combination at low concentration range (5 + 5, 25 + 25, 50 + 50 ppm) revealed only 50 per cent root formation and propagule development activity, but at higher concentration range (250 + 250 ppm) IBA and NAA had inhibitory effect. The highest activity 100 per cent was observed at 5 ppm 2,4-D, and at 50 ppm and it decreased root formation down to 33 per cent at 100 ppm. It totally inhabited rooting activity of the cuttings and propagule development.

Singh *et al.* (2005) conducted experiment on seasonal rooting response in leafy stem cuttings and petiole cuttings of *Rauvolfia serpentina* under mist conditions and they reported that the seed of this species show very poor germination percentage i.e 20-50 per cent and therefore macro-propagation technique is the best suitable and convenient method for large-scale multiplication of elite planting stocks. Through this techniques maximum percentage of rooting. i.e 95.43 per cent was reported in the cuttings which were planted in the month of May 2003 followed by cuttings planted in the month of December 2002. The

rooting were started form these cuttings after 67 days. The leaf petiole cuttings planted in the month of June 2003 and rooting was started after 25 days of planting and 30.22 per cent rooting was observed treated with commercial grade rootex. The complete plants developed from these cuttings 5 months after shifting.

Ehiagbonare and Joseph (2007) studied regeneration of *Rauvolfia vomitoria* by stem cutting by using quick dip, drip down and total immerse method. They was reported 80 per cent rooting in immerse method followed by 60 per cent in drip down method and 70 per cent rooting in quick dip method.

Nimbalkar (2007) studied the propagation of Karonda (*Carissa congesta*) by hard wood cuttings and observed 47.33 per cent sprouting in the treatment IBA 3000 ppm quick deep method.

Dhuria (2008) studied the rooting behavior of 'Sarpagandha' (*Rauwolfia serpentina* (L.) Bth. ex Kurz.), using branch cuttings under mist conditions, and observed that, the regeneration of this species with seed is difficult. Therefore, vegetative propagation through branch cuttings in mist condition has been attempted using three auxins (IAA, IBA and NAA) as rooting hormones. Leafy branch cuttings (18-20 cm long), collected from 8-10 months old plants of *Rauwolfia serpentina* (L.) were treated with different concentrations of Indole Acetic Acid (IAA), Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) on their 2 cm basal portion for 1-2 minutes. Cuttings were planted in impure sand medium, inside a mist chamber possessing congenial micro-climatic conditions of 25-30^{o}c temperature and 70 per cent relative humidity. NAA was observed as the best rooting hormone for the rooting of branch cuttings of *Rauwolfia serpentina* at lower concentrations (500-1000 ppm). The number of roots was found to be maximum at higher concentrations of the hormone (3000 ppm) of IAA and IBA.

Basnet and Dey (2009) conducted the experiment of vegetative propagation of *Rauwolfia serpentina* (L.) Bth. ex Kurz. at Darjeeling West Bengal and reported that, the method of planting and cutting type significantly influenced the rooting parameters. The highest rooting percentage was recorded from the root cuttings planted horizontally and stem cuttings prepared from the terminal half of the stem with apical shoot. This may offer an alternative means of rapid

multiplication to produce quality planting materials, selected from genetically superior plants with high extractable reserpine content for large scale cultivation.

Baul *et al*. (2010) reported effect of indole-3-butyric acid (IBA) on stem cuttings in *Holarrhena pubescens.* They recommended 0.1 per cent IBA or without IBA for rooting juvenile leafy stem cuttings, followed by application of fertilizer for subsequent growth of stockings in polythene bags.

An experiment was conducted by Pandey and Mandal (2010) on influence of propagation techniques and harvesting time on root yield and alkaloid contents of *Rauwolfia serpentina* (L.) Bth. ex Kurz. at Tropical Forest Research Institute, Jabalpur. In their experiment 15 to 20 cm long stem cuttings with 3 to 4 nodes were planted in the nursery in May-June. The cuttings were dipped in 100 ppm solution of IBA before planting. The lower end of the cuttings was planted in the bed However; the opposite end of cuttings was sealed by wax. The beds were kept by giving light irrigation daily. They observed 40 to 65 per cent success.

Hussain *et al.* (2012) tested the effect of auxins on stem cuttings and air layers of *Embelia ribes* Burm. an important medicinal plant of India and observed that, the semi hardwood stem cuttings were collected from a 6-8 year old plants form Bonaccord forest in Thiruvananthapuram district and treated with different concentration of auxin- *viz.* Indole Butyric Acid (IBA), Indole Acetic Acid (IAA), Naphthalene Acetic Acid (NAA)(250, 500, 1000 and 2000 ppm). There were no remarkable results on root initiation in stem cuttings and air layers from aged plants in both control and treatment. The 6-8 years old plants were further focused with auxins to achieve maximum rooting by stem cuttings/air layering. Among the auxins, IBA in 1000 ppm induced more rooting than IAA and NAA. A moderate level of IBA concentration (1000 ppm) was found more effective than terms of rooting percentage, number of roots, length of roots as well as the survival rate of the rooted cuttings than lower and higher concentrations of IBA (250 and 2000 ppm).

Propagation by seed-

Abrol and Handa (1956) tested the propagation of *Rauwolfia serpentina* (L.) Bth. ex Kurz.in Jammu and Kashmir state which was first tried without any success the treatments like soaking in cold water, hot water, alkali treatment and mechanical

scarification also failed to give any fruitful results. Therefore presowing treatment of seeds with gibbrellic acid was tried and it was found to be very effective to overcome the germination problem.

Badhwar *et al*. (1956) carried out a study on *Rauwolfia serpentina* The method of propagation and their effect on root production and concluded that, the germination percentage of 25 to 50. The recommended the commercial cultivation of *Rauwolfia* by seeds could not be adopted due to difficulties in germination.

Nayar (1956) studied the propagation and culture of *Rauwolfia serpentina* (L.) Bth. ex Kurz. by seeds. He found that, the seeds of *Rauwolfia serpentina* germinate irregularly and sporadic; The germination of the seeds is not always as low as 10 per cent; It appears that, for propagation by seed is a better method.

Santapau (1956) studied the botanical aspects of *Rauvolfia serpentina* Benth. and reported that, merits of propagation and germination of seeds was poor and in general it was difficult to get more than 10 per cent germination.

Sobti *et al*. (1957) In their experiment on propagation of *Rauvolfia serpentina* in Jammu and Kashmir. reported three types of seeds in *Rauvolfia,* viz., light, medium and heavy, based on float and sink method and observed that the light seeds which floated on water were mostly empty seeds of shrivelled embryous and showed only 2 per cent germination. On the other hand the germination rate in heavy seeds was found to be 20 percent.

Hedayatullah (1959) stated that the seed germination of *Rauwolfia serpentina* was quit erratic which ranged from 8 to 48 per cent with an average of 19 per cent. Thus, propagation through cuttings alone and in combination with hormone treatment may be helpful.

Dutta *et al*. (1961 a) Observed the germination tests on ripe seeds of *Rauwolfia serpentina* (L.) Bth. ex Kurz. collected monthly from September to January from Field Reasearch Station showed that the seeds collected in November give the highest percentage of heavy seeds as well as the highest percentage of germination averaging to 77 and 47 per cent respectively.

Dutta *et al*. (1963 b) carried out a study on cultivation of *Rauwolfia serpentina* in India and they found 62.77 per cent germination in freshly collected heavy seed lot, harvested during October to November under Jammu condition.

Torne (1964) reported that the dry seeds of *Rauwolfia serpentina* Benth were irradiated with a series of gamma-ray doses ranging from 2,500r to 15,000r at the Atomic Energy Establishment, Trombay and observations on germination of the seeds indicated that the irradiation helped in increasing the percentage of germination from 20.5 in control to 74.5 and 73.0 in seeds with dosages, 2,500 r and 5,000 r, respectively.

Gupta and Rajendra (1968) studied the commercial cultivation of *Rauwolfia serpentina* (L.) Bth. ex Kurz. its need for quality seeds and reported that, on an average 378 heavy or 536 light seed consisted of atrophied embryos and hence germinate very rarely. Light and heavy seeds can easily be separated by simple water floatation. Germination of heavy seeds during May-June after soaking them in water for 24 hours was observed that 20 to 40 per cent success.

Singh and Motilal (1970) tested the effect of gibberellic acid (GA_3) and temperature on seed germination of *Rauvolfia serpentina* (L.) Bth. ex Kurz. and observed that the GA was applied at 250, 500, 750 and 1000 mg/l. The 500 mg level increased germination from 40 (control) to 89.3 percent, and survival was 100 per cent.

Sarin Y. K. (1982) carried out a study on cultivation and utilization of *Rauvolfia serpentina* (L.) Bth. ex Kurz. in India and found that plant can be propagated by seeds but the germination percentage of seeds was very poor and variable (20 to 50%) and often as low as 10 per cent, making propagation by seeds was difficult.

Adeola and Dada (1986) conducted the experiment pretreatment effects on seed germination of *Acacia nilotica* Linn and they observed that the mechanical scarification was the most effective pretreatment as germination percentage obtained was higher, the progress was more homogeneous in time. However, manual abrasion of the seeds was most time consuming pretreatment. It is also proved that for most of the Acacias species nicking produced the highest energy of germination.

Padma *et al*. (1994) carried out a study on the effect of scarification treatments on the germination of *Leucaena leucocephala, Albizzia lebbeck* and *Samanea samon* and found that the hot water soaking (80^0 c) for 5 minutes improved

germination in *Leucaena leucocephala,* but not in *Albizzia lebbeck* and *Samanea samon.*

Bhuyar *et al*. (2000) in his experiment of seed germination studies in sarpagandha (*Rauvolfia serpentina* (L.) Bth. ex Kurz.) observed that, seeds, prior to germination, were subjected to the treatments *viz.* T_1- untreated (control); T_2 cold water (24 h); T_3 hot water (80^0c for 5 min) then cooled down to room temperature; T_4 sulfuric acid (SA; 2 min); T_5 SA (5 min); T_6 SA (10 min); T_7 gibberellic acid (GA) (500 ppm for 24 h); T_8 GA (1000 ppm for 24 h); T_9 potassium nitrate (0.2% for 24 h). Out of the 8 treatment T_3 produced the highest number of normal seedlings (26.66), germination percentage (53.33) and seedling vigour index (428.77), and the tallest seedlings (8.04 cm). Significant results were also obtained for germination percentage and seedling length in treatment T_4.

Gupta (2003) carried out a study regarding seed germination and dormancy breaking techniques for indigenous medicinal and aromatic plants and she observed that, the dormancy breaking was evaluated in 39 medicinal and aromatic plants from India. Most of the species showed low to nil germination. To break such dormancy the various treatments like scarification, hot water treatment, cold stratification to seeds or growth regulator treatments were given to seeds prior to germination. Enhanced seed germination up to 80-95 per cent was obtained with sand paper scarification. Acid scarification, cutting/piercing the seed coat, pre-soaking of seeds for *Rauwolfia serpentina* and GA3 [gibberellic acid] treatment and 0.2 per cent KNO_3 treatment for *Costus speciosus* and *Ocimum sanctum [Ocimum tenuiflorum]*.

Bahar (2007 a) experiment on pod and seed characteristics and effect of pretreatment on seed germination of *Acacia robusta* Burchell reported that the scarification of seed testa may be more beneficial than heat treatment in *Enterolobium cyclocarpum*

Ehiagbonare and Joseph (2007) conducted a study on regeneration of *Rauwolfia vomitoria* at department of Biological sciences, Nigeria and they observed that the maximum value obtained from the seed germination studies was 50 per cent.

. Paul *et al* (2008) conducted different pre-sowing seed treatment on per cent seed germination by using certain physical and chemical treatments *viz.* Mechanical scarification, hot water, sulphuric acid treatment, hydrochloric acid treatment, heat

treatment and pre-sowing seed treatment with chemicals at department of botany, North Bengal University . They reported that the seed treatment with concentrated sulphuric acid up to 30 minutes increased 30 per cent germination to some extent. However, the duration of soaking in sulphuric acid for better germination was different in different plants species. Seed scarification with sand paper increased germination percentage (48%). they further observed that grinding of seeds with stone or nicking with a needle was not effective. Pre-sowing seed treatment with chemicals did not improve germination.

Mishra *et al.* (2010) revealed that the germinability and seedling survival studies of *Rauwolfia serpentina* (L.) Bth. ex Kurz. were carried out under ex-situ conditions for optimization of seed germination emplaying various concentrations of GA_3 and mechanical scarification treatment. Among various treatments scarified seed treated with GA3 300 ppm were found to be most effective and showed maximum of 62.67 per cent germination.

Pandey *et al.*(2010) conducted an experiment on influence of propagation techniques and harvesting time on root yield and alkaloid contents of *Rauwolfia serpentina* (L.) Bth. ex Kurz. in their experiment seeds were soaked in water for 24 hours before sowing. Only heavy seeds were selected by floating them in water. Presoaked seeds were sown in nursery beds at 6-8 cm apart in rows in shallow furrows during 1st week of May. The furrows were then covered with a fine mixture of soil and FYM. Beds were kept moist by light watering. They observed that the germination started after 20-25 days and continued up to 30 to 40 days. Germination percentage varied from 15 to 55 per cent.

Trivedi and Kumari (2011) worked on ethno-botanical and germinational aspects of *Rauvolfia serpentina* (L.) Bth. ex Kurz and reported that the seed coat is hard and seeds showed seed coat dormancy. Unscarified seeds did not germinate at all while freshly harvested scarified seeds showed 54 per cent germination. Scarification by conc. H_2So_4 for 2-20 minutes was of no use. Seeds in response to moisture stress established that availability of water is directly proportional to seed germination. In response to growth regulators. The scarified seeds showed 95.2 per cent germination at 25 ppm and 100 ppm of cytokinin. 90.4 per cent at 100 ppm at IAA and 77.4 per cent at same concentration of gibberellic acid.

Bahar (2012 b) reported that the *Enterolobium contortisiliquum* seed have physical dormancy due to hardiness of testa. Suitable pretreatment to break the dormancy was investigated and observed that, the germination was enhanced from 5.87 per cent (untreated) to 93.17 per cent (mechanical scarification). Although mechanical scarification treatment gives early, uniform and highest germination but it is not practicable in large scale purpose.

Panwar and Guru (2012) studied influence of Gibberellic acid and seed coat removal on the seed germination behaviour of *Rauvolfia serpentina* (L.) Bth. ex Kurz. under control environment and they reported that seed germination was as low as 20.47 per cent in case of intact seeds and was improved upto 40.2 per cent after removal of the seed coat. Supplementing the medium with gibberellic acid (500 ppm), germination improves 52 per cent in intact seeds while 74.58 per cent germination in the seeds without seed coat GA_3 at 700 ppm was found as the optimal concentration and 78.89 per cent germination was achieved.

CHAPTER III

MATERIALS AND METHODS

The present investigation was undertaken to study the Propagation studies in Sarpagandha, *Rauwolfia serpentina* (L.) Bth.ex Kurz. The details of the materials used and methods followed in the present investigation are described in this chapter under the following headings.

3.1 Location

The study was carried out at the research farm of College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri district of Maharashtra having Geo coordinates of $17^0$45' N latitude and $73^0$12' E longitude with an elevation of 250 m MSL.

3.2 Climatic details of study area

The climate of the study area is warm and humid with an annual rainfall ranging from 3000 to 3500 mm. The minimum temperature is 12^0 C and the maximum temperature is 34^0 C during the study period. The relative humidity (RH) ranges from 64.5 to 98.5 per cent. The monthly weather data during the study period from September 2012 to April 2013 has obtained from meteorological observatory, Dr. B. S. K. K. V., Dapoli. The study area is located in the lateritic soil belt.

3.3 Experimental details

3.3.1 Materials

For the present study, the planting material of *Rauwolfia serpentina* was procured from authentic sources and their proper identification was done with the help of plant taxonomist. Preparation of cutting branches and roots was collected from about 2-3 year old plants growing in the college research farm onset of winter.

3.3.2 Methodology

Under this head, objective wise detail methodology is presented for to standardize propagation methods by root, stem and seed for production of seedlings.

The apical soft portion and the lower semi hard wood zones were separated. The apical portion was made in to bi-nodal softwood leafy cuttings. Each cutting was retained only one pair of completely unfolded leaves. The lamina of each leaf was

prunned with the help of sharp scissors to half its size. The lower portions of the branches was defoliated and made in to 10-15 cm long semi hard wood cuttings.

In case of roots, larger than pencil size roots was selected for propagation studies. Root cuttings in 3 cm in length with a 2 nodes was prepared and used for study.

All cutting was soaked in 0.1 per cent Bavistin suspension in water for 15 minutes. The upper end of cuttings was sealed by wax. After that, different treatments *viz.* 25, 50, 75, and 100 ppm Indole Butyric Acid (IBA) for 30 minutes and one treatment were kept as a control. Immediately after that, the cutting was planted in poly bags having size 4X7 cm. Before that the poly bags were filled with a mixture of soil and FYM in the ratio 2:1. The poly bag was kept under partial shed in net house and water was provided to avoid desiccation. The same method was followed for root cutting.

To standardize propagation methods by root cutting, stem cutting experiment was consisting of 5 treatments *viz.* 25, 50, 75, and 100 ppm Indole Butyric Acid (IBA) including control with 4 replications consisting of 25 seedlings each arranged in Randomized Block Design (RBD) under 50 per cent shade net (Plate 2).

For propagation through seed, fresh mature seed was procured from authentic source. The durt, foreign matter, unhealthy seed was removed manually. Individual seeds were rubbed against sand paper. Then seed soaked in hot water at 80^0C for 5 minutes then cooled down to room temperature. The seed was treated with different treatments 100, 200, 300, 400 ppm Gibberellic acid (GA_3). Untreated seed was treating as a control. To standardize propagation method by seed experiment was consisting of 5 treatments including control with 4 replications consisting of 100 seedlings each arranged in Completely Randomised Design (CRD) under shade net during February to May 2013.

3.4 Observations recorded

A monthly observations on following parameters were recorded for seedlings of different treatments up to three months.

3.4.1 Seedling Growth parameters:

a) Per cent sprouting: The number of sprouts per seedling was counted for all the seedlings used for the study and is expressed in numbers.

b) Number of shoot: The number of shoot per seedling was counted for all the seedlings used for the study and is expressed in numbers.

c) Seedling height (cm): The height of seedling was measured in centimeters from the base of seedlings to growing tip, using the measurement scale.

d) Number of leaves: The number of leaves per seedling was counted for all the seedlings used for the study and is expressed in numbers.

e) Collar diameter (mm): The Collar diameter was measured at basal position of the seedling by using digital caliper and is expressed in millimeters.

f) Root length (cm): After uprooting, the length of taproot of each seedling was measured from the collar region to the tip of the tap root by using measurement scale.

g) Number of roots (cm): The number of root per seedling was counted for seedlings used for the study and is expressed in numbers.

3.4.2 Seedling biomass parameters:

For recording biomass of seedlings, one seedling per treatment for each replication was uprooted, washed and observations were recorded.

Fresh and dry weight of shoot and root

Individual seedling was cut into two parts. Fresh weight of shoot, root and leaves was recorded using weighing balance and then these samples were dried in hot air oven at 60^0 c for 1 day. Later, dry weights of samples were measured using weighing balance and expressed in grams.

3.4.3 Diseases pest incidence:

Diseases and pests were recorded and identified with the help of Entomologist and pathologist.

3.4.4 Propagation through seed

a) Germination Count:

The number of seeds germinated was counted every day up to 80 days from the date of sowing. The emergence of plumule above the soil was taken as the indicator of seed germination. Furthermore, data was computed into per centage using the following formula.

$$\textbf{b) Germination Per Centage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Various germination attributes like germination rate index (GRI), mean daily germination (MDG), peak value of germination (PV) and germination vigour (GV) were calculated using standard formulas (Czabator, 1962) as described below:

c) Germination Rate Index = (G1/T1+G2/T2+---------+Gn/Tn)

Where, G_1, G_2, G_n are number of seeds germinated on the first (T_1), second (T_2) and n^{th} day (T_n).

$$\textbf{d) Mean Daily Germination } (\%) = \frac{\text{Cumulative per cent germination}}{\text{Total number of days counted (80 days)}}$$

$$\textbf{e) Peak Value of Germination} = \frac{\text{Total germination (\%)}}{\text{The day on which highest germination recorded}}$$

f) Germination Value = Mean Daily Germination x Peak Value

3.4.5 Seedling Quality Parameters

Following quality indices were calculated using standard formulas using final growth and biomass data:

Shoot to Root Ratio: It was calculated using shoot length upon root length of particular month and expressed as ratio.

Sturdiness Quotient: It was recorded using the following formula (Thompson, 1985).

Final Shoot height (cm)/ Final Basal Diameter (mm)

Quality Index: It was recorded using the following formula (Dicknos *et. al.,* 1960)

$$QI = \frac{\text{Total Dry weight of seedling (g)}}{SQ + \left[\frac{\text{Shoot and leaves dry weight (g)}}{\text{Root dry weight (g)}}\right]}$$

Relative Growth Rate (RGR): It was expressed as grams of dry matter produced per day by a gram of existing dry matter. This was calculated by following formula as described by Williams (1946).

$$RGR = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{t_2 - t_1}$$

Where, W_1 & W_2 are dry weight at the time, t_1 and t_2 respectively.

Net Assimilation Rate (NAR)

It indirectly indicates the rate of photosynthesis. It is expressed as grams of dry matter production per cm^2 of leaf area in a day. This method was proposed by Williams (1946).

$$NAR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{(\text{Log}_e L_2 - \text{Log}_e L_1)}{(L_2 - L_1)}$$

Where, L_1 and W_1 are leaf area and dry weight of plants at time t_1 and L_2 and W_2 are leaf area and dry weight of plants at time t_2.

3.6 Statistical analyses

The data pertaining to all the growth, biomass and quality indices were analyzed statistically using different statistical software like SAS program on Computer. ANOVA (Analysis of Variance) table was constructed for interpretation of data for each parameter statistically.

CHAPTER IV

RESULTS AND DISCUSSION

Experiments were conducted to investigate the propagation studies in Sarpagandha, *Rauwolfia serpentina* (L.) Bth. ex Kurz. During the study the observations were recorded for different parameters related with seedling growth and biomass attributes. The results obtained during the present studies are presented and discussed in this chapter. For better understanding the experimental data have been grouped and presented under the following heads.

4.1.1 Propagation through seed.

4.1.1.a Effect of pre-sowing treatments (GA_3 soaking) on seed germination of *Rauwolfia serpentina.*

4.1.1.b Effect of GA_3 on growth parameters and biomass of *Rauwolfia serpentina* propagated through seed 60 DAS.

4.1.1.c Effect of GA_3 on growth parameters and biomass of *Rauwolfia serpentina* propagated through seed 90 DAS.

4.1.2 Propagation through soft wood leafy (apex) cutting.

4.1.2.a Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 30 DAP.

4.1.2.b Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 60 DAP.

4.1.2.c Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 90 DAP.

4.1.3 Propagation through semi-hard wood (stem) cutting.

4.1.3.a Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 30 DAP.

4.1.3.b Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 60 DAP.

4.1.3.c Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 90 DAP.

4.1.4 Propagation through root cutting.

4.1.4.a Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 30 DAP.

4.1.4.b Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 60 DAP.

4.1.4.c Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 90 DAP.

4.1 Results

4.1.1 Propagation through seed.

4.1.1.a Effect of pre-sowing treatments (GA_3 soaking) on seed germination of *Rauwolfia serpentina.*

In the present investigation, the different pre-sowing treatments *viz* scarification (control), soaking seeds in GA_3 solution @ 100, 200, 300 and 400 ppm solution for 30 minutes along with scarification were undertaken. Seeds with respect to seed germination and its attributes like germination rate index (GRI), mean daily germination (MDG), peak value of germination (PV) and germination value (GV) were studied. The result in respect of the seed germination with different pre-sowing treatments showed the significant as well as the non-significant variation as mentioned under (Table 1).

It was observed from the data presented in the Table 1 in respect of all pre-sowing treatment, the treatment T_4 i.e. (scarification + soaking seeds in 300 ppm GA_3 for 30 min) required minimum number of day for first germination recorded in (16.00) days as compared to all other treatments. It was followed by T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) and T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min). However, treatment T_1 consisting scarification treatment (control) as well as treatment T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) required more number of days (19) for the first germination.

From the above data it was observed that, treatment T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) is the best treatment in respect of pre-sowing treatment regarding day of first germination followed by T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) treatment as compared to other treatments.

Table 1: Effect of pre-sowing treatments (GA_3 soaking) on seed germination of *Rauwolfia serpentina*

Pre-sowing treatments		Day of first germination	Day of highest germination	Per cent Germination	GRI	MDG	PV	GV
T_1	Scarification (Control)	18.50	75.00	68.25	3.18	2.24	2.24	5.08
T_2	Scarification + Soaking seeds in GA3 @ 100 ppm for 30 min.	18.50	74.50	48.50	2.28	1.57	2.24	3.67
T_3	Scarification + Soaking seeds in GA3 @ 200 ppm for 30 min.	16.75	74.50	52.00	2.56	1.76	2.14	3.75
T_4	Scarification + Soaking seeds in GA3 @ 300 ppm for 30 min.	16.00	76.75	43.75	2.17	1.47	1.70	2.68
T_5	Scarification + Soaking seeds in GA3 @ 400 ppm for 30 min.	17.00	72.50	38.25	1.77	1.23	1.46	1.94
Mean		17.35	74.65	50.15	2.39	1.65	1.95	3.42
SEm (±)					0.28	0.19	0.28	0.73
CD (P = 0.05)					0.87	0.58	NS	NS

CD=Critical Difference; GRI= Germination rate index; MDG = Mean daily germination; PV = Peak value; GV= Germination value

The data in respect of the days of highest germination showed that, the minimum number 72.50 i.e.73 days were observed in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) followed by T_1 scarification (control) 75 days, T_2 scarification + soaking seeds in 100 ppm GA_3 for 30 min) and T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) 74.50 i.e.75 days treatment respectively. However, T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) treatment required more number 76.75 i.e. 77 days for the highest germination.

It is seen from the data presented in Table 1 and depicted in Fig.1, the germination percentage was ranged from 38.25 to 68.25 per cent with mean value of 50.15 in respect of all pre-sowing treatment (Plate.3).

The minimum per cent germination (38.25%) was observed in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) treatment and the maximum per cent germination (68.25 %) were observed in T_1 treatment consisting scarification (control) treatment.

From the above data it is observed that, the T_1 (scarification) treatment is always better followed by T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) treatment (52%) as compared to other pre-sowing treatment regarding per cent germination.

The data given in Table 1 showed significant results in respect of all pre-sowing treatment consisting T_1 scarification (control) to T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min).

The Growth rate index was observed minimum (1.77) in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) treatment and maximum Growth rate index was observed in (3.18) T_1 treatment i.e. scarification (control). It has been observed from data that T_1 scarification (control) showed the statistically significant result as compared to all other treatment except T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) treatment (2.56).

The effect of pre-sowing treatment on Mean daily germination (MDG) presented in Table1 showed significant result in respect of all pre-sowing treatment consisting T_1 scarification (control) to T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min). The Mean daily germination (MDG) was observed minimum in

(1.23) T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) while (2.24) T_1 scarification (control) showed maximum Mean daily germination (MDG).

T_1 scarification (control) and T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) were at par with each other. T_1 scarification (control) was significantly different from T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min), T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) and T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min).

From the above data indicated that T_1 scarification (control) is always better for pre-sowing treatment followed by T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) regarding Mean daily germination (MDG). GA_3 usually promotes germination but in this case the no effect on seed germination rather it reduced the rate as compared to control. It indicated that higher days may not be suited.

The peak value (PV) was in range of 1.46 (T_5 - scarification + soaking seeds in 400 ppm GA_3 for 30 min) to 2.24 (T_1 - scarification i.e. control) with 1.95 mean. Treatment T_1 (2.24) i.e. scarification (control) and T_2 (2.24) i.e. scarification + soaking seeds in 100 ppm GA_3 for 30 min was superior over all other treatment it was followed by T_3 (2.14) i.e. scarification + soaking seeds in 400 ppm GA_3 for 30 min.

Similar trend was also recorded for germination value (GV) and it ranges from 1.94 (T_5 - scarification + soaking seeds in 400 ppm GA_3 for 30 min) to 5.08 (T_1 - scarification i.e. control) with 3.42 mean.

The overall result showed that seeds treated in T_1 scarification (control) performed better with respect to per cent germination, GRI, MDG, PV and GV as compared to all other treatments used as well as the GRI and MDG were statistically significant.

4.1.1.b Effect of GA_3 on growth parameters and biomass of *Rauwolfia serpentina* propagated through seed 60 DAS.

Considering the data of plant growth and biomass of *Rauwolfia serpentina* with respect to influence of different GA_3 concentrations on the seedling growth like shoot height, root length, number of roots, number of leaves, diameter as well as

Table 2: Effect of GA_3 on growth parameters and biomass of *Rauwolfia serpentina* propagated through seed 60 DAS

Pre-Sowing treatment		Growth parameters					Biomass parameters					
		Shoot Height (cm)	Root Length (cm)	Number of Roots	Number of Leaves	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)
T_1	Scarification (Control)	5.37	4.88	17.88	5.15	1.56	0.33	0.08	0.05	0.02	0.41	0.07
T_2	Scarification + Soaking seeds in GA3 @ 100 ppm for 30 min.	5.93	5.02	22.18	5.48	1.56	0.37	0.09	0.06	0.02	0.45	0.08
T_3	Scarification + Soaking seeds in GA3 @ 200 ppm for 30 min.	5.54	4.55	22.23	5.48	1.47	0.35	0.08	0.06	0.02	0.43	0.08
T_4	Scarification + Soaking seeds in GA3 @ 300 ppm for 30 min.	6.33	4.94	20.55	5.65	1.44	0.32	0.08	0.06	0.02	0.40	0.07
T_5	Scarification + Soaking seeds in GA3 @ 400 ppm for 30 min.	6.98	5.44	21.13	5.65	1.50	0.39	0.10	0.07	0.02	0.49	0.08
Mean		6.03	4.96	20.79	5.48	1.51	0.35	0.09	0.11	0.02	0.44	0.08
SEm(±)		0.33	0.37	1.22	0.29	0.06	0.03	0.01	0.01	0.11	0.04	0.01
CD (P = 0.05)		1.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

seedling biomass like fresh shoot, root weight and dry shoot, root weight at 60 DAS are presented here (Table 2).

There was a significant variation among five treatments for T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) showed significantly highest shoot height. It ranges from 5.37 cm to 6.98 cm with 6.03 cm mean. Treatment T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) was significantly superior as compare to all other treatments except treatment T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min)

All the growth parameters except shoot height shows non-significant variation in which root length showed maximum in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) i.e.5.44 cm and minimum in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) i.e.4.55 cm.

In number of roots, maximum number recorded 22.23 i.e. 22 in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) and minimum number 17.88 i.e 18 in T_1 scarification (control) likewise number of leaves showed in treatment T_4 and T_5 having similar (5.65) and minimum in (5.15) T_1 scarification (control), also Diameter of *Rauwolfia serpentina* showed in T_1 and T_2 having similar (1.56 mm) and lowest in T_4 (1.44 mm).

Similar in biomass parameter *viz.* fresh shoot, fresh root weight, dry shoot, dry root weight, total fresh weight and total dry weight having non-significant variation in which fresh shoot weight showed range between 0.32 gm to 0.39 gm in the treatment T_4 and T_5 respectively. While, fresh root weight showed highest in the treatment T_5 (0.10 gm) and lowest in T_1, T_3 and T_4 (0.08 gm) was recorded.

However, dry shoot weight showed maximum difference in between T_4 (0.30 gm) to T_1 (0.05 gm). Interestingly all treatment showed similar dry root weight having (0.02 gm). In total fresh weight ranges from 0.40 gm in T_4 to 0.49 gm in T_5 In total dry weight T_2, T_3 and T_5 showed (0.08 gm) T_1 and T_4 showed (0.07 gm) was recorded.

The overall result showed that the pre-sowing seed treatment of T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) performed better with respect to other treatments regarding growth and biomass parameters.

4.1.1.c Effect of GA_3 on growth parameters and biomass of *Rauwolfia serpentina* propagated through seed 90 DAS.

In the given Table 3, effect of GA_3 on growth parameters and biomass of *Rauwolfia serpentina* propagated through seed 90 DAS in growth parameters like root length, number of roots, number of leaves, diameter showed non-significant variation. Similarly in case of seedling biomass i.e. fresh shoot weight, fresh root weight and dry shoot, dry root weight, total fresh and total dry weight showed non-significant variation. Considering in case of vigour indices like Sturdiness quotient, Quality index, Shoot to Root ratio, Relative Growth Rate, and Net Assimilation Rate showed non-significant variation.

In case of growth parameters shoot height was in range of 5.76 cm to 6.23 cm in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) and T_1 scarification (control) respectively and root length ranges from 6.14 cm to 7.84 cm in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) and T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) respectively. However, number of roots showed maximum (25.65) in T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) whereas, the minimum (20.85) in T_1 scarification (control), similarly, number of leaves showed maximum (7.20) in T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) whereas, the minimum in (6.28) in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min). In the case of diameter, highest value (1.73 mm) recorded in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) and lowest value (1.64 mm) recorded in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) and T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) respectively.

The data presented in biomass parameters, fresh shoot weight ranges between 0.47 in treatment T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) to 0.55 gm in treatment T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min), in fresh root weight T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) showed maximum value (0.26 gm) and T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) showed minimum value (0.17 gm). However, dry shoot weight was recorded lower values (0.08) in all the treatments except T_3 (scarification

Table 3: Effect of GA_3 on growth parameters and biomass of *Rauwolfia serpentina* propagated through seed 90 DAS

Pre-Sowing treatment		Growth parameters					Biomass parameters						Vigour indices				
		Shoot Height (cm)	Root Length (cm)	Number of Roots	Number of Leaves	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)	SQ	QI	S:R	RGR	NAR
T_1	Scarification (Control)	6.23	6.84	20.85	6.38	1.68	0.53	0.25	0.08	0.04	0.76	0.12	3.76	2.90	1.02	0.01	0.00
T_2	Scarification + Soaking seeds in GA3 @ 100 ppm for 30 min.	6.19	6.14	24.35	6.28	1.64	0.52	0.26	0.08	0.03	0.76	0.12	3.79	3.06	1.06	0.01	0.00
T_3	Scarification + Soaking seeds in GA3 @ 200 ppm for 30 min.	6.10	7.84	22.10	7.00	1.73	0.55	0.22	0.09	0.04	0.76	0.13	3.56	3.03	0.93	0.01	0.01
T_4	Scarification + Soaking seeds in GA3 @ 300 ppm for 30 min.	6.08	6.69	25.65	7.20	1.69	0.54	0.20	0.08	0.03	0.74	0.12	3.64	3.37	1.12	0.01	0.01
T_5	Scarification + Soaking seeds in GA3 @ 400 ppm for 30 min.	5.76	6.46	24.08	6.33	1.64	0.47	0.17	0.08	0.03	0.64	0.10	3.56	3.09	0.98	0.00	0.00
Mean		6.07	6.79	23.41	6.64	1.67	0.52	0.22	0.08	0.03	0.73	0.12	3.66	3.09	1.02	0.01	0.00
SEm(±)		0.29	0.48	1.54	0.27	0.04	0.05	0.05	0.01	0.01	0.07	0.01	0.16	0.45	0.07	0.00	1.18
CD (P = 0.05)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

SQ= Sturdiness Quotient, QI= Quality index, S:R = Shoot to root ratio, RGR = Relative growth rate, NAR = Net assimilation rate.

+ soaking seeds in 200 ppm GA_3 for 30 min) treatment i.e. 0.09 gm, While, dry root weight was observed maximum (0.04 gm) in treatment T_1 scarification (control) and T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) respectively and in minimum (0.03 gm) in T_1, T_2 and T_3 respectively, In case of total fresh weight, values ranges between 0.64 in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) to 0.76 gm in T_1, T_2 and T_3 respectively and total dry weight showed maximum value (0.13 gm) in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) and minimum value (0.10 gm) in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min).

Similarly, in vigour indices data showed that, sturdiness quotient was calculated maximum (3.79) in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) and minimum (3.56) in T_3 and T_5, However, Quality index recorded highest value (3.37) in T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) and lowest value (2.90) in T_1 scarification (control). While, shoot to root ratio ranges from 0.93 in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) to 1.12 in T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min), Whereas, Relative growth rate (0.01g g^{-1}day-^{1}) calculated highest value (0.01) for all treatments except T_5 lowest value (0.00). In Net Assimilation Rate T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) and T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) recorded maximum (0.01) and minimum (0.00) in T_1, T_2 and T_5.

Present findings corroborate with results of research work conducted by Panwar and Guru (2012) they reported seed germination was low as 20.47 per cent in case of intact seeds and was improved up to 40.2 per cent after removal of seed coat. Supplementing the medium with GA_3 500 ppm germination improves 52 per cent in intact seed while 74.58 per cent germination in the seeds without seed coat they achieved 78.89 per cent germination for the treatment GA_3 at 700 ppm however, in the present investigation maximum per cent germination was observed in treatment T_1(Scarification). Followed by T_3 (Scarification + Soaking seeds in GA_3 @ 200 ppm for 30 minutes).

Singh and Motilal (1970) reported 52 per cent germination while treating the intact seeds with GA_3 (500 ppm). Paul and Basu (2008) reported 32 per cent germination in the treatment scarification (Sand paper). However, in the present

investigation maximum per cent germination (68.25) was observed in the treatment T_1 (Scarification).

Mishra *et al.* (2010) reported 62.67 per cent germination in the treatment scarified seeds with GA_3 300 ppm. Trivedi and Kumari (2011) reported freshly harvested scarified seeds showed 54 per cent germination and also mentioned the response to growth regulators the scarified seed showed 95.2 per cent germination at 25 and 100 ppm cytokinin, 90.4 per cent at 100 ppm IAA and 77 per cent at 100 ppm gibberellic acid.

4.1.2 Propagation through soft wood leafy (apex) cutting.

4.1.2.a Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 30 DAP.

In the present study, results showed that the effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 30 DAP. It was observed that, the non-significant variation in different growth parameters and biomass parameters (Table 4).

Number of shoot having ranges from 1.00 to 1.17 in treatment T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) and T_1 (control) respectively. The shoot height ranges from 10.99 cm to 12.17 cm in T_1 (control) and T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) respectively, However, number of leaves showed maximum value (3.80) in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) and minimum (3.00) in T_1 (control). In root length, highest (2.98 cm) was observed in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) and lowest (0.18 cm) in T_1 (control). Whereas, number of roots ranges from 1.75 to 6.00 in T_1 (control) and T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) respectively. However, diameter was measured maximum (3.35 mm) in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) and minimum (3.07 mm) in the treatment T_1 (control).

Similarly, in biomass parameter, fresh shoot weight was recorded maximum value 1.97 gm in (T_5) and minimum value 1.36 in T_4 (soaking cuttings in IBA @ 75 ppm for 30 min). However, fresh root weight showed highest value (0.39 gm) in T_4 and lowest (0.25 gm) in T_3. Whereas, dry shoot weight ranges from 0.38 to 0.56 gm in T_4 and T_5 respectively. While, dry root weight measured maximum (0.14 gm) in T_5 and minimum (0.08 gm) in T_2 (soaking cuttings in IBA @ 25 ppm for 30 min).

Table 4: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 30 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters					
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)
T_1	Control	1.17	10.99	3.00	0.18	1.75	3.07	1.71	0.26	0.49	0.09	2.10	0.58
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.08	12.00	3.24	0.50	2.25	3.20	1.52	0.32	0.41	0.08	1.92	0.48
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.00	12.17	3.73	0.35	2.75	3.25	1.83	0.25	0.54	0.11	2.09	0.64
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.08	11.53	3.62	1.63	3.75	3.31	1.36	0.39	0.38	0.13	2.08	0.50
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.03	11.52	3.80	2.98	6.00	3.35	1.97	0.38	0.56	0.14	2.36	0.69
Mean		1.07	11.64	3.48	1.13	3.30	3.24	1.67	0.32	0.47	0.11	2.11	0.58
SEm (±)		0.06	0.09	0.27	0.82	1.60	0.12	0.26	0.10	0.10	0.04	0.29	0.12
CD (P = 0.05)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

However, total fresh weight ranges between 2.08 to 2.36 gm in T_4 and T_5 respectively.

In case of total dry weight, maximum value observed (0.69 gm) in T_5 and minimum (0.48 gm) in treatment T_2.

From the above data indicated that the T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) better than other treatments regarding growth and biomass parameters.

4.1.2.b Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 60 DAP.

The effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings at 60 DAP showed significant results in root length, fresh shoot weight, dry root weight and total fresh weight (Table 5).

Among the growth parameters number of shoot showed maximum and minimum ranges between 1.09 to 1.34 in (T_5) and (T_2) respectively. However, shoot height recorded maximum value (12.44 cm) in T_3 and minimum (11.19 cm) T_1. Whereas, number of leaves showed range between 4.05 to 5.01 in T_2 and T_5 respectively. While, number of roots were recorded maximum (13.75) in T_5 and minimum (3.50) in T_1. However, diameter was measured highest (3.36 mm) in T_5 and lowest (3.00 mm) in T_1 ranges from (3.36 to 3.00 mm).

Data showed in Table 5 revealed that, the root length was recorded for 60 DAP in range of 0.43 cm (T_1) to 9.38 cm (T_5) with mean value 5.29 cm. Whereas, the maximum root length of 9.38 cm was recorded in treatment (T_5) followed by 7.38 cm (T_4) and 0.43 cm minimum in (T_1). Treatment (T_5) was significantly superior over all other treatment.

Similar trend was also recorded in biomass parameters fresh shoot weight observed significant variation, It was highest in T_5 (1.91 gm) and lowest in T_2 (0.88 gm) with mean value of (1.29 gm). Treatment T_5 and T_4 were at par with each other, T_5 was significantly different with T_3, T_1 and T_2 respectively.

Table 5: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 60 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters					
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)
T_1	Control	1.32	11.19	4.14	0.43	3.50	3.00	1.00	0.31	0.38	0.07	1.15	0.45
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.34	12.34	4.05	4.50	8.75	3.16	0.88	0.18	0.29	0.08	1.06	0.37
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.12	12.44	4.53	4.75	10.75	3.23	1.20	0.33	0.29	0.13	1.52	0.41
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.20	11.76	4.60	7.38	12.25	3.28	1.46	0.31	0.44	0.10	1.82	0.54
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.09	12.16	5.01	9.38	13.75	3.36	1.91	0.59	0.43	0.22	2.72	0.65
Mean		1.21	11.98	4.47	5.29	9.80	3.21	1.29	0.34	0.37	0.12	1.65	0.48
SEm(±)		0.10	0.31	0.24	0.57	2.31	0.10	0.16	0.10	0.07	0.03	0.21	0.08
CD (P = 0.05)		NS	NS	NS	1.75	NS	NS	0.48	NS	NS	0.09	0.66	NS

The fresh root weight observed maximum in treatment T_5 (0.59 gm) whereas, minimum in T_2 (0.18 gm) and dry shoot weight recorded maximum (0.44 gm) in T_4 and minimum (0.29 gm) in treatment T_2 and to T_3

In dry root weight showed significant variation ranged from 0.07 to 0.22 gm in (T_1) and (T_5) respectively with mean value of 0.12. Treatment (T_5) was significantly superior over all different treatments.

Similarly, in total fresh weight showed significant variation in treatment ranges in between 1.06 to 2.72 gm in (T_2) and (T_5) with mean value 1.65. The Treatment (T_5) was significantly superior over all other treatments.

Total dry weight of treatment T_5 recorded maximum value (0.65 gm) and minimum value in treatment T_2 (0.37 gm).

From the above data it is revealed that, the Treatment (T_5) was superior over all other different pre-sowing treatments regarding growth and biomass parameters.

4.1.2.c Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 90 DAP.

In the present study, effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 90 DAP was studied. The parameters like number of shoots, shoot height, number of leaves, number of roots and diameter were recorded. Result showed that there was non-significant variation among treatments but in root length different treatments showed significant variation (Table 6).

In case of biomass, parameters like fresh shoot weight, fresh root weight, dry root weight, total fresh weight, and total dry weight were observed. The data showed significant result in respect of all pre-sowing treatments on biomass parameters.

In case of growth parameters, number of shoot recorded maximum (1.74) in T_2 and minimum (1.18) in T_5. While, shoot height ranges between 11.72 to 13.07 cm in (T_1) and (T_3) respectively. However, number of leaves recorded highest value (6.69) in T_5 and lowest value (5.04) in T_2. Whereas, number of roots ranges between 6.00 to 15.75 in (T_1) and (T_4) respectively. In the case of diameter, maximum value (3.36 mm) in treatment T_5 and minimum (2.93 mm) in T_1. Interestingly, root length showed significant variation. It was maximum in T_5 (10.00 cm) whereas minimum

Table 6: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through soft wood leafy cuttings 90 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters						Survival (%)
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)	
T_1	Control	1.43	11.72	5.38	1.20	6.00	2.93	1.10	0.24	0.29	0.08	1.34	0.37	35.00
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.74	12.77	5.04	4.78	9.25	3.21	1.32	0.26	0.32	0.09	1.59	0.41	42.00
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.27	13.07	6.16	5.25	9.75	3.25	1.44	0.22	0.50	0.07	1.65	0.57	34.00
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.30	12.18	5.82	8.50	15.75	3.23	1.86	0.41	0.45	0.14	2.27	0.59	56.00
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.18	12.76	6.69	10.00	15.00	3.36	2.12	0.57	0.71	0.19	2.84	0.90	65.00
Mean		1.38	12.50	5.82	5.95	11.15	3.20	1.57	0.34	0.45	0.11	1.94	0.56	46.40
SEm(±)		0.14	0.37	0.47	0.72	2.43	0.12	0.18	0.06	0.07	0.02	0.22	0.08	-
CD (P = 0.05)		NS	NS	NS	2.22	NS	NS	0.56	0.20	0.22	0.07	0.69	0.26	-

in T_1 (1.20 cm) with mean value 5.95. Treatments T_5 and T_4 were at par with each other, T_5 was significantly different from T_3, T_2 and T_1.

However, in biomass parameters the data showed great variation. In fresh shoot weight the recorded maximum value was (2.12 gm) in treatment T_5 and minimum value is (1.10 gm) in treatment T_1 with mean value 1.57. Whereas, T_5 and T_4 were at par with each other, T_5 was significantly different from T_3, T_2 and T_1 respectively.

However, fresh root weight showed significant variation ranges between 0.57 to 0.22 gm in T_5 and T_3, with mean value 0.34. It was observed that the treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) showed the statistically significant result as compared to all other treatment except T4 (soaking cuttings in IBA @ 75 ppm for 30 min) i.e. 0.41 gm.

Interestingly, dry shoot weight and dry root weight, total fresh weight and total dry weight showed significant variation among the treatments.

Whereas, dry shoot weight showed maximum (0.71 gm) in T_5 and minimum in T_1 (0.29 gm) with mean value 0.45. However, dry root weight ranges from 0.07 gm to 0.19 gm in T_3 and T_5 respectively with 0.11 mean value. Similarly, Total fresh weight recorded highest (2.84 gm) in T_5 and lowest (1.34 gm) in T_1 with mean value 1.94 and total dry weight showed range in between 0.37 to 0.90 gm in (T_1) to (T_5) respectively.

It was observed from the data of dry shoot weight, treatment T_5 and T_3 were at par with each other, treatment T_5 was showed significantly difference from T_4, T_2 and T_1. Dry root weight showed that the treatment T_5 and T_4 were at par with each other. However, T_5 was showed significantly, difference from T_2, T_1 and T_3 respectively. Total fresh weight was found maximum in T_5 treatment i.e. (soaking cuttings in IBA @ 100 ppm for 30 min.) and showed statistically significant result as compared to all other treatment except T_4 i.e. (soaking cuttings in IBA @ 75 ppm for 30 min).

Interestingly, in total dry weight T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) which was significantly superior over rest of the treatments.

It was seen from the data presented in table 6 that the percent survival was ranges from 65 to 34 percent with mean value of 46.40 percent in respect of all pre-sowing treatment.

The data observed from table 6 was found to be maximum percent survival in T_5 (65%) whereas minimum in T_3 (34%) was recorded (Fig.2).

From above data result showed that, the T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) is better for pre sowing treatment regarding growth and biomass parameters as well as per cent survival (Fig 2.and Plate 4).

4.1.3 Propagation through semi-hard wood (stem) cutting.

4.1.3.a Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 30 DAP.

In the present study, results showed that growth parameters likewise number of leaves, root length and number of roots observed significant variation in different treatment (Table 7).

Result showed that, the number of shoot having ranges between1.23 to 1.60 in treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) and T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) respectively. Whereas, shoot height showed maximum value (13.95 cm) in T_5 and minimum (12.46 cm) in T_1 (control). Highest diameter was observed in T_5 (4.50 mm) whereas it is minimum (4.16 mm) in T_2 (soaking cuttings in IBA @ 25 ppm for 30 min).

However, number of leaves ranges from 3.96 (T_1) to 5.52 (T_5) with mean value 4.89. Whereas, maximum number of leaves (5.52) recorded in treatment T_5 followed by T_3. Where, T_5, T_3 and T_4 (soaking cuttings in IBA @ 75 ppm for 30 min) were at par with each other, T_5 was significantly different from T_2 and T_1 respectively.

Similarly, in root length maximum value was observed in T_5 (5.88 cm) whereas minimum in T_1 (0.38 cm) with mean value 2.73. In the treatment T_5 was significantly superior overall remaining treatments.

Similar trend was also recorded in number of roots ranges from 2.25 to 8.50 in T_1 and T_5 respectively. The treatments T_5, T_4 and T_3 were at par with each other and T_5 was significantly different from T_2 and T_1 respectively.

Table 7: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 30 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters					
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)
T_1	Control	1.38	12.46	3.96	0.38	2.25	4.20	1.57	0.19	0.59	0.08	1.77	0.67
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.53	12.74	4.63	0.88	2.50	4.16	2.09	0.34	0.87	0.12	2.45	0.99
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.60	12.91	5.26	2.75	6.25	4.40	2.81	0.53	1.05	0.17	3.35	1.23
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.27	13.50	5.07	3.75	7.25	4.28	1.96	0.39	0.58	0.14	2.35	0.70
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.23	13.95	5.52	5.88	8.50	4.50	1.68	0.36	0.54	0.11	2.08	0.65
Mean		1.40	13.11	4.89	2.73	5.35	4.31	2.02	0.36	0.73	0.12	2.40	0.85
SEm(±)		0.10	0.42	0.15	0.45	1.30	0.15	0.56	0.14	0.23	0.04	0.67	0.26
CD (P = 0.05)		NS	NS	0.47	1.39	3.99	NS	NS	NS	NS	NS	NS	NS

In case of biomass parameters like fresh shoot weight, dry root weight, dry shoot weight, dry root weight, total fresh and total dry weight did not showed significant variation among different treatments.

In that, fresh shoot weight recorded maximum (2.81 gm) in T_3 and minimum (1.57 gm) in T_1, similarly fresh root weight ranges from 0.19 to 0.53 gm. However, dry shoot weight showed maximum (0.87 gm) in T_2 whereas minimum (0.54 gm) in T_5. However, dry root weight ranges between 0.08 to 0.17 gm with mean value 0.12. While, total fresh weight showed maximum (3.35 gm) in T_3 and minimum (1.77 gm) in T_1. The total dry weight recorded highest value in T_2 (0.99 gm) and lowest in T_5 (0.65 gm).

4.1.3.b Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 60 DAP.

Data presented in Table 8 showed that, the effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 60 DAP. Growth parameter *viz.* shoot height, number of leaves and root length, number of root showed significant variation. In case of biomass the treatments did not showed significant variation.

In case of growth parameters, number of shoot ranges from 1.50 to 1.78 in T_4 (soaking cuttings in IBA @ 75 ppm for 30 min) and T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) respectively. The diameter was recorded highest in treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) (4.49 mm) and lowest recorded in T_1 (control) (4.29 mm).

However, shoot height was maximum recorded in treatment T_5 (14.84 cm) and minimum in T_2 (soaking cuttings in IBA @ 25 ppm for 30 min) (12.92 cm) with mean value 13.99. Treatment T_5, T_4 and T_3 were at par with each other, however T_5 was significantly different from T_1 and T_2 respectively.

Whereas, number of leaves were recorded maximum in T_3 (7.97) and minimum in T_1 (5.43) with mean value 6.82. Treatment T_3, T_5 and T_4 were at par with each other. T_3 is significantly different from T_2 and T_1

Table 8: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 60 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters					
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)
T_1	Control	1.66	12.98	5.43	0.50	2.50	4.29	3.72	0.65	1.18	0.15	4.39	1.33
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.77	12.92	5.87	1.75	3.50	4.20	3.27	1.57	1.17	0.26	4.82	1.42
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.78	14.36	7.97	4.13	7.25	4.48	3.25	1.20	0.84	0.40	4.43	1.25
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.50	14.83	7.09	5.38	8.50	4.34	1.92	1.79	0.44	0.41	3.70	0.85
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.51	14.84	7.72	6.50	12.25	4.49	2.53	2.63	0.80	0.55	5.16	1.35
Mean		1.64	13.99	6.82	3.65	6.80	4.36	2.94	1.57	0.88	0.35	4.50	1.24
SEm(±)		0.12	0.38	0.29	0.93	1.06	0.16	0.54	0.49	0.22	0.13	0.51	0.20
CD (P = 0.05)		NS	1.17	0.89	2.85	3.26	NS	NS	NS	NS	NS	NS	NS

However, root length was recorded highest value in T_5 (6.50 cm) whereas lowest in T_1 (0.50 cm) with 3.65 mean value. Treatment T_5, T_4 and T_3 were at par with each other, however T_5 was significantly different from T_1 and T_2 respectively.

Similarly, number of roots, ranges between 2.50 (T_1) to 12.25 (T_5) with mean value 6.80. The treatment T_5 is significantly superior over all other treatments.

In case of biomass parameters, fresh root weight recorded maximum (3.72 gm) in T_1 and minimum (1.92 gm) in T_4. However, fresh root weight ranges from 0.65 gm to 2.63 gm in T_1 and T_5 respectively. While, dry shoot weight was recorded maximum (1.17 gm) in T_2 and minimum in (0.44 gm) in T_4. Whereas, dry root weight was recorded highest (0.55 gm) in T_5 and lowest (0.15 gm) in T_1 and total fresh weight ranges from 3.70 gm to 5.16 gm in T_4 and T_5. Whereas total dry weight was recorded maximum in T_2 (1.42 gm) and minimum (0.85 gm) in T_4.

4.1.3.c Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 90 DAP.

The data given in Table 9 showed that, the parameter *viz*. shoot height, number of leaves and number of root showed significant variation among treatments. As well as in biomass parameters i.e. fresh root weight and total dry weight observed significant variation.

Considering the data of plant growth parameter, number of shoot having maximum value in T_1 (2.30) and minimum value in T_5 (1.78) and root length was in range of T_5 9 cm to 3.13 cm in T_1.Whereas, diameter measured maximum in T_5 (4.50 mm) and minimum (4.03 mm) in T_2 respectively.

However, shoot height showed maximum value in treatment T_4 (16.27 cm) whereas minimum value in T_2 (13.06 cm) with mean value 14.64. Treatment T_4, T_5 and T_3 were at par with each other. The treatment T_4 was significantly different from T_1 and T_2.

The number of leaves recorded maximum value in treatment T_3 (11.64) and minimum in T_2 (7.84) with mean value 9.90. Treatment T_3, T_5 and T_4 were at par with each other, T_3 was significantly different from T_1 and T_2.

Table 9: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through semi-hard wood cuttings 90 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters						Survival (%)
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)	
T_1	Control	2.30	13.29	7.98	3.13	4.00	4.16	2.55	0.29	0.83	0.12	2.85	0.94	43.00
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	2.28	13.06	7.84	4.38	8.25	4.03	3.21	0.69	1.11	0.56	3.99	1.40	55.00
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	2.27	15.21	11.64	6.38	8.00	4.40	3.31	0.55	1.02	0.24	3.89	1.26	50.00
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.87	16.27	10.64	8.00	9.00	4.24	2.79	0.92	0.76	0.27	3.68	1.03	55.00
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.78	15.37	11.38	9.00	13.00	4.50	5.45	1.84	1.86	0.61	7.31	2.46	65.00
Mean		2.10	14.64	9.90	6.18	8.45	4.27	3.46	0.86	1.11	0.36	4.34	1.42	53.60
SEm(±)		0.22	0.53	0.44	1.46	1.46	0.16	0.87	0.30	0.27	0.16	1.04	0.32	-
CD (P = 0.05)		NS	1.64	1.36	NS	4.50	NS	NS	0.93	NS	NS	NS	0.98	-

Similarly, number of roots observed highest in T_5 (13) and lowest in T_1 (4) with mean value 8.45. Treatment T_5 and T_4 were at par with each other, T_5 was significantly different from T_2, T_3 and T_1 respectively.

Similar trend was also recorded in biomass parameters. Fresh root weight ranges from 0.29 gm to 1.84 gm in (T_1) and (T_5) respectively with mean value 0.86. T_5 and T_4 were at par with each other, T_5 was significantly different from T_2, T_3 and T_1 respectively.

Similarly, total dry weight showed maximum in treatment T_5 (2.46 gm) whereas minimum in T_1 (0.94 gm) with mean value 1.42. Treatment T_5 showed significantly superior to all other treatment.

However, fresh shoot weight ranges from 2.55 gm to 5.45 gm in T_1 and T_5 respectively and dry shoot weight showed maximum value (1.86 gm) in T_5 and minimum in T_4 (0.76 gm). Whereas, dry root weight recorded highest value (0.61 gm) in T_5 and lowest (0.12 gm) in T_1 similarly, total fresh weight ranges from 2.85 gm to 7.31 gm in T_1 and T_5 respectively.

Whereas, per cent survival was recorded maximum in treatment T_5 (65%), followed by T_4 and T_2 (55%) each, whereas minimum observed in T_1 (43%) with mean value 53.60 (Fig.3).

The overall result showed that, the pre-sowing treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) performed better with respect shoot height, number of root , fresh root weight and total dry weight compared to all other treatments (Plate.5).

Alamgir and Ahamed (2005) reported propagation through stem cuttings. They communicated the root-stem junction revealed the highest root formation and propagule development activity (78%) and it was followed by root cuttings (62%) and stem cuttings (42%). IBA at low concentration stimulated root formation and 50 ppm IBA gave maximum root formation in cuttings 83 per cent. In the present investigation highest percentage of survival in soaking basal portion of cuttings in IBA at 100 ppm for 30 minutes was observed.

The present results corroborate with the findings of Pandey and Mandal (2010). The cuttings of *Rauwolfia serpentina* were dipped in 100 ppm solution of IBA before planting. The lower end of the cuttings was planted in the bed However; the opposite end of cuttings was sealed by wax. The beds were kept by giving light irrigation daily. They observed 40 to 65 per cent success. However, in the present investigation 65 per cent survival was observed.

The results showed contradictory with the result of Pal *et al*. (1995). They reported 66.7 per cent rooting in control and IBA 10 mg/l. However in the present investigation 100 ppm IBA showed maximum rooting per cent (65%).

Dhuria (2008) reported NAA is the best rooting hormone for the rooting of leafy branch cutting of *R. Serpentina* at lower concentrations (500 -1000 ppm). The number of roots was found to be maximum at higher concentration of the hormone (300 ppm) of IAA and IBA.

Nimbalkar (2007) studied vegetative propagation and reported 38.89 per cent rooting after 90 days after sprouting in the treatment IBA 3000 ppm in *Carissa congesta* Wt.

4.1.4 Propagation through root cutting.

4.1.4.a Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 30 DAP.

The effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 30 DAP are presented in Table 10. The result showed significant variation among growth parameters like shoot height, number of leaves, root length and diameter. Similarly biomass parameters like dry shoot weight, dry root weight, total fresh weight and total dry weight showed significant variation among all treatments.

The shoot height was observed maximum in the treatment T_5 (3.93 cm) and minimum in T_1 (3.07 cm) with 3.45 mean value. T_5 showed significantly superior to all other treatment.

Table 10: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 30 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters					
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)
T_1	Control	1.24	3.07	4.73	0.63	3.25	5.60	0.73	0.12	0.27	0.06	0.86	0.33
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.25	3.29	5.82	2.25	5.50	5.96	2.09	0.44	1.02	0.17	2.55	1.19
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.19	3.50	5.61	3.13	7.00	7.59	1.45	0.65	0.51	0.22	2.12	0.73
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.13	3.48	5.50	6.00	6.75	7.10	1.34	0.27	0.44	0.08	1.64	0.52
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.32	3.93	7.25	7.75	7.50	7.77	2.27	0.50	0.76	0.17	2.81	0.92
Mean		1.23	3.45	5.78	3.95	6.00	6.80	1.57	0.39	0.60	0.14	1.99	0.74
SEm(±)		0.05	0.11	0.44	0.69	1.66	0.43	0.25	0.13	0.12	0.04	0.31	0.14
CD (P = 0.05)		NS	0.34	1.37	2.12	NS	1.32	0.78	NS	0.37	NS	0.97	0.42

However, number of leaves observed maximum value in treatment T_5 (7.25) and minimum in T_1 (4.73) with mean value 5.78. Treatment T_5 showed significantly superior to all other the treatment.

Similarly, root length recorded highest value (7.75 cm) in treatment T_5 and minimum (0.63 cm) in T_1 with mean 3.95. Treatment T_5 and T_4 were at par with each other, T_5 was significantly different from T_3, T_2 and T_1.

In diameter, it was observed in range between 5.60 mm to 7.77 mm in T_1 and T_5 respectively with mean value 6.80. Treatment T_5, T_3 and T_4 were at par with each other, T_5 was significantly different from T_2 and T_1 respectively.

However, number of shoots were recorded maximum (1.32) in T_5 and minimum (1.13) in T_4, while number of roots recorded highest value in T_5 (7.50) and lowest in T_1 (3.25).

In case of fresh shoot weight, it was measured maximum in the treatment T_5 (2.27 gm) and minimum in T_1 (0.73 gm) with mean value 1.57. Treatment T_5 and T_2 were at par with each other, treatment T_5 showed significantly different from T_3, T_4 and T_1.

However, fresh root weight recorded highest value in treatment T_3 (0.65 gm) and lowest in (0.12 gm) in T_1 treatment. Similarly, dry root weight having range from 0.06 gm to 0.22 gm in (T_1) and (T_3) each.

Whereas, dry shoot weight showed maximum value in T_5 (0.76 mm) and minimum in T_1 (0.27 mm) with mean value 0.60. Treatment T_2 and T_5 were at par with each other, treatment T_2 shows significantly different from T_3, T_4 and T_1.

Interestingly, total fresh weight ranges from 0.86 gm to 2.81 gm in (T_1) and (T_5) with respect of mean value 1.99. Treatment T_5, T_2 and T_3 were at par with each other, T_5 was significantly different from T_4 and T_1.

However, total dry weight was recorded highest value in treatment T_2 (1.19 gm) whereas lowest value in T_1 (0.33 gm) with mean 0.74. Treatment T_2 and T_5 were at par with each other T_2 was significantly different from T_3, T_4 and T_1 respectively.

4.1.4.b Effect of IBA on growth parameters and biomass of *serpentina* propagated through root cutting 60 DAP.

The data presented in Table 11 effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 60 DAP. It showed that, the shoot height, number of leaves, root length and diameter observed significant variation among treatment. However, in biomass parameters *viz.* fresh root weight, dry root weight, total fresh weight and total dry weight also showed significant variation among treatments.

In case of growth parameters, shoot height ranges from 4.08 to 7.34 cm (T_1) and (T_5) each with mean value 5.39. T_5 was significantly superior over all the treatment.

The number of leaves observed maximum value (9.76) in treatment T_5 and minimum (6.02) in T_1 with mean 7.91. Treatment T_5, T_4 and T_3 were at par with each other. T_5 was significantly different from T_2 and T_1.

However, root length showed highest value in T_5 (10.25 cm) and minimum in T_1 (2.88 cm) with overall mean 6.91. Treatment T_5, T_4 and T_3 were at par with each other. T_5 was significantly different from T_2 and T_1.

Similar trend was observed in diameter having maximum in treatment T_5 (8.07 mm) to minimum (5.49 mm) with mean 6.97. T_5, T_3 and T_4 were at par with each other and T_5 was significantly different from T_2 and T_1.

Number of shoots ranges between 1.23 to 1.63 in (T_4) and (T_3). Whereas, number of roots recorded maximum (12.50) in T_5 and minimum (4.75) in T_1.

Considering the data of biomass, fresh root weight showed significant variation having maximum in treatment T_5 (4.18) to minimum in T_2 (0.91) with mean value 1.86. Treatment T_5 and T_4 were at par with each other, treatment T_5 was showed significantly different from T_3, T_1 and T_2.

However, dry root weight showed highest value in T_5 (1.30 gm) and lowest in T_1 (0.21 gm) with mean value 0.70. T_5 and T_4 were at par with each other, T_5 was significantly different from T_3, T_2 and T_1 respectively.

Table 11: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 60 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters					
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)
T_1	Control	1.36	4.08	6.02	2.88	4.75	5.49	0.67	0.97	0.20	0.21	1.38	0.35
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.39	4.57	7.16	5.23	7.00	6.19	2.33	0.91	0.91	0.42	3.24	1.33
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.63	4.98	8.26	7.25	8.75	7.69	3.22	0.98	0.75	0.60	4.20	1.36
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.23	6.00	8.36	8.93	9.00	7.40	3.38	2.27	0.95	0.99	5.65	1.94
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.38	7.34	9.76	10.25	12.50	8.07	3.70	4.18	1.25	1.30	7.88	2.55
Mean		1.40	5.39	7.91	6.91	8.40	6.97	2.66	1.86	0.81	0.70	4.47	1.50
SEm(±)		0.05	0.26	0.68	1.29	3.34	0.47	0.78	0.69	0.24	0.20	0.81	0.26
CD (P = 0.05)		NS	0.80	2.08	3.98	NS	1.46	NS	2.11	NS	0.61	2.49	0.80

Whereas, total fresh weight showed maximum in treatment T_5 (7.88 gm) and minimum in T_1 (1.38 gm) with mean 4.47. T_5 and T_4 were at par with each other, T_5 was significantly different from T_3, T_2.

The total dry weight was ranges between 1.33 to 2.55 gm in (T_2) and (T_5) respectively with mean value 1.50. Treatment T_5 and T_4 were at par with other, T_5 was significantly different from T_3, T_2 and T_1 respectively.

In fresh shoot weight, maximum value recorded (3.70 gm) in T_5 and minimum (0.67 gm) in T_1. Whereas, dry shoot weight ranges from 0.20 gm to 1.25 gm in (T_1) and (T_5) respectively.

4.1.4.c Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 90 DAP.

The data presented in Table 12 showed that, the effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 90 DAP. In that table growth parameters *viz.* shoot height, number of leaves and diameter observed significant variation among treatment (Plate 6). In case of biomass parameters showed all treatments were non- significant.

Number of shoots were observed maximum (1.80) in control (T_1) treatment and minimum (1.44) in T_4 (soaking cuttings in IBA @ 75 ppm for 30 min). Interestingly, highest shoot height was observed in T_5 (10.97 cm) and lowest in T_2 (soaking cuttings in IBA @ 25 ppm for 30 min) treatment (5.50 cm) with mean 7.42. T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) was significantly superior compared to all other treatment.

However, number of leaves was recorded maximum in T_5 (13.55) and minimum in T_1 (8.69) with mean 11.22. Treatment T_5, T_3 and T_4 were at par with each other. T_5 was significantly different from T_2 and T_1.

Whereas, root length of *Rauwolfia serpentina* (L.) having non-significant with highest value in treatment T_5 (10.88 cm) and lowest in T_1 (6.00 cm). However, number of roots recorded maximum in treatment T_5 (14.25) and lowest in T_1 (5.50) Interestingly, diameter of *Rauwolfia serpentina* having range between 5.78 to 8.07 mm in (T_1) and T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) respectively with

Table 12: Effect of IBA on growth parameters and biomass of *Rauwolfia serpentina* propagated through root cutting 90 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters						Survival (%)
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)	
T_1	Control	1.80	5.57	8.69	6.00	5.50	5.78	1.97	0.70	0.76	0.25	2.67	0.81	47.00
T_2	Soaking cuttings in IBA @ 25 ppm for 30 min.	1.60	5.50	8.75	8.00	7.50	6.34	4.75	1.68	1.42	0.63	6.43	2.09	58.00
T_3	Soaking cuttings in IBA @ 50 ppm for 30 min.	1.68	6.96	12.97	9.00	9.00	8.07	5.07	1.54	1.50	0.52	6.94	2.03	61.00
T_4	Soaking cuttings in IBA @ 75 ppm for 30 min.	1.44	8.12	12.11	9.50	10.75	7.29	3.81	1.53	1.20	0.57	5.53	1.58	68.00
T_5	Soaking cuttings in IBA @ 100 ppm for 30 min.	1.57	10.97	13.55	10.88	14.25	8.03	5.50	2.27	1.66	0.88	7.84	2.55	78.00
Mean		1.62	7.42	11.22	8.68	9.40	7.10	4.22	1.54	1.31	0.57	5.88	1.81	62.40
SEm(±)		0.08	0.30	0.63	1.09	2.17	0.51	1.17	0.45	0.42	0.17	1.55	0.55	-
CD (P = 0.05)		NS	0.93	1.95	NS	NS	1.56	NS	NS	NS	NS	NS	NS	-

mean 7.10. Treatment T_3, T_5 and T_4 were at par with each other. T_3 was significantly different from T_2 and T_1.

In case of biomass parameters, fresh shoot weight showed highest value (5.50 gm) in treatment T_5 whereas treatment T_1 recorded lowest value (1.97 gm). Whereas, fresh root weight recorded maximum (2.27 gm) in T_5 and minimum (0.70 gm) in T_1. Similar trend was observed in dry shoot weight ranges from 0.76 to 1.66 gm in (T_1) and (T_5). Whereas, dry root weight measured highest value (0.88 gm) in T_5 and lowest (0.25 gm) in T_1, Similarly, total fresh weight was recorded maximum (7.84 gm) in T_5 and minimum (2.67 gm) in T_1. Whereas, total dry weight was measured maximum in T_5 (2.55 gm) and minimum in T_1 (0.81 gm).

Interestingly, in case of per cent survival, it was recorded maximum (78%) in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) followed by T_4 (68%) and T_3 (61%) each, whereas minimum observed in T_1 (47%) with mean value 62.40 (Fig. 4).

The present results corroborate with the findings of Alamgir and Ahamed (2005) they reported 2,4-D at 5 ppm had the highest positive impact on root formation and propagule development (100 %), followed by IBA (83 % at 50 ppm, 66.83 % at 10 ppm) and NAA (66% at 10 ppm). In the present investigation maximum survival (65%) was observed in soaking basal portion of cuttings in IBA at 100 ppm for 30 minutes.

The results are thus in agreement with the findings of Badhwar (1956) who concluded fresh weight of green roots 1-2 inches in length are suitable and are best planted horizontally with different treatments. Hedayatullah (1959) reported 36 per cent success with root cutting of *Rauwolfia serpentina.* However, in the present investigation 78 per cent survival was recorded. It was highest as compared to propagation through soft wood leafy cutting (65%) and semi-hard wood cutting (65%).

CHAPTER V

SUMMARY AND CONCLUSIONS

The root drug *Rauwolfia serpentina* (L.) Bth. ex Kurz. (Family-Apocynaceae) is used for the treatment of mental illness, hypertension, blood pressure, snake bite, etc. Demand for this root drug has increased in traditional and modern systems of medicine in recent days. However, natural population of this species is under dwindling stage. The plant is categorized as an 'endangered' by IUCN. In one side the demand has increased for the manufacture of drugs, while in another side the plant is naturally under threat. *R. serpentina* is naturally found in Konkan region of Maharashtra in particular and in Maharashtra in general. Farmers of the state also enquire about its seed, planting material, propagation techniques, agro-techniques, etc. Hence the present investigations on propagations were conducted for the mass scale seedling production of *R. serpentina.*

The field experiments were conducted to study various aspects of present study at experimental farm of College of Forestry, Dapoli Dist. Ratnagiri, (Maharashtra) during the year 2012-13. In the present study, observations on different parameters *viz.* seed germination per cent, seedling growth, biomass attributes and survival per cent was recorded. The important findings of the present investigations are summarized below-

- In seed propagation, 68.25 per cent germination was recorded maximum in T_1 (scarification) compared to other treatments. In Germination attributes *viz.* Germination Rate Index (3.18), Mean Daily Germination (2.24), Peak Value of germination (2.24) and Germination Value (5.08) was observed maximum in T_1 (scarification) followed by in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) in which Germination Rate Index (2.56), Mean Daily Germination (1.76), Peak Value of germination (2.14) and Germination Value (3.75). The overall performance of treatment T_1 (scarification) was found better in per cent germination and germination attributes as compared to other treatments.
- **Growth parameters at 90 DAS**- Shoot height 6.23 cm was maximum in T_1 (scarification) followed by 5.76 cm in T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min) and root length 7.84 cm recorded maximum in T_3

(scarification + soaking seeds in 200 ppm GA_3 for 30 min) followed by 6.14 cm in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min). However, number of roots was recorded maximum 25.65 in T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) whereas, the 20.85 minimum in T_1 scarification (control). Number of leaves showed maximum 7.20 in T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) whereas, the minimum 6.28 was recorded in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min). Maximum diameter 1.73 mm was recorded in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) followed by 1.64 mm in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) and T_5 (scarification + soaking seeds in 400 ppm GA_3 for 30 min).

- **Propagation through seed: Biomass parameters after 90 DAS-** The maximum Fresh shoot weight 0.55 gm, Dry shoot weight 0.09 gm, Dry root weight 0.04 gm, Total fresh weight 0.76 gm and Total dry weight 0.13 gm and fresh root weight was recorded maximum in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min) and maximum fresh root weight 0.26 gm was recorded in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) after 90 DAS.

- **Propagation through seed: Vigour indices-** Sturdiness Quotient was recorded maximum 3.79 in T_2 (scarification + soaking seeds in 100 ppm GA_3 for 30 min) and Quality index and Shoot to Root ratio was recorded highest in T_4 (scarification + soaking seeds in 300 ppm GA_3 for 30 min) 3.37 and 1.12 respectively. Maximum Relative Growth Rate and Net Assimilation Rate 0.01 were recorded in T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min).

- **Propagation through Soft wood leafy cutting-: Growth parameters after 90 DAP-** The maximum number of shoots 1.74 was recorded in T_2 (soaking cuttings in IBA @ 25 ppm for 30 min). The maximum shoot height 13.07 cm was recorded in T_3 (soaking cuttings in IBA @ 50 ppm for 30 min). The number of leaves 6.69, root length 10 cm and diameter 3.36 mm was recorded maximum in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min). The highest value recorded for number of roots 15.75 in T4 (Soaking cuttings in IBA @ 75 ppm for 30 min)

- **Propagation through Soft wood leafy cutting-: Biomass parameters after 90 DAP-** The maximum Fresh shoot weight 2.12 gm, Fresh root weight 0.57 gm, Dry shoot weight 0.71 gm, Dry root weight 0.19 gm, Total fresh weight 2.84 gm and Total dry weight 0.90 gm was recorded in treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) at 90 DAP**.**

  Propagation through Semi-hard wood cutting-: Growth parameters after 90 DAP- The maximum number of shoots 2.30 was recorded in T_1 (Control) and showed better results. The maximum root length 9 cm, number of roots 13 and diameter 4.50 mm was recorded in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min). The maximum number of leaves 11.64 was recorded in T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) and The maximum shoot height 16.27 cm was recorded in T_4 (soaking cuttings in IBA @ 75 ppm for 30 min) after 90 DAP.

- **Propagation through Semi-hard wood cutting-: Biomass parameters after 90 DAP-** The maximum Fresh shoot weight 5.45 gm, Fresh root weight 1.84 gm, Dry shoot weight 1.86 gm, Dry root weight 0.61 gm, Total fresh weight 7.31 gm and Total dry weight 2.46 gm was recorded in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min). Whereas, the maximum 65 per cent survival was recorded in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min).

- **Propagation through Root cutting: Growth parameters after 90 DAP-** The maximum number of shoots 1.80 was recorded in T_1 (Control). While treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) showed maximum Shoot height 10.97 cm, Number of leaves 13.55, Root length 10.88 cm and Number of roots 14.25. Whereas, Diameter 8.07 mm recorded highest in T_3 (soaking cuttings in IBA @ 50 ppm for 30 min) after 90 DAP.

- **Propagation through Root cutting: Biomass parameters after 90 DAP:** The treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) showed maximum Fresh shoot weight 5.50 gm, Fresh root weight 2.27 gm, Dry shoot weight 1.66 gm, Dry root weight 0.88 gm, Total fresh weight 7.84 gm and Total dry weight 2.55 gm after 90 DAP. Maximum per cent survival (78%) was recorded in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) and also overall performance in growth parameters, Biomass parameters and per cent survival was found maximum in the same treatment.

- On the basis of overall results of the experiment, treatment T_1 (scarification) to seed was found better in per cent germination and germination attributes as compared to other treatments. Treatment T_1 (scarification) showed maximum per cent germination 68.25 followed by T_3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min). In the leafy soft wood cutting treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) was given good results for number of leaves, root length, diameter and biomass parameters. In the propagation through semi-hard wood cutting the treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) showed better results for root length, number of roots, diameter and biomass parameters after 90 DAP. Treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) showed good results in the experiment propagation through roots and showed better results as compared to other treatments for shoot height, number of leaves, root length, number of roots and other biomass parameters.
- Maximum survival per cent was recorded in propagation through seed with scarification followed by GA_3 200 ppm treatment and propagation through roots. Therefore, these two methods are recommended for mass multiplication of *Rauwolfia serpentina.*

CHAPTER VI

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Appendix 2

ABBREVIATIONS AND SYMBOLS USED

%	-	Per cent
@	-	At the rate
CD	-	Critical difference
cm	-	Centimeter
Fig.	-	Figure
g	-	Gram
i.e.		that is
mg	-	Milligram
m	-	Meter
mm	-	Millimeter
NAR	-	Net assimilation rate
No.	-	Number
et al.	-	and others
RGR	-	Relative growth rate
Sr. No	-	Serial number
SEm	-	Standard error of mean
T	-	Treatment
Viz	-	namely
w.r.t	-	with respect to
MSL	-	Mean Sea Level
ANNOVA	-	Analysis of variance
Anon.	-	*Anonymous*
ºC	-	Degree Celsius
DF	-	Degree of freedom
Dr. BSKKV	-	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
Dist.	-	District
etc	-	et cetra
e.g.	-	Example
NS	-	Non significant
Sig	-	Significant
S.E.(m)	-	Standard error of mean

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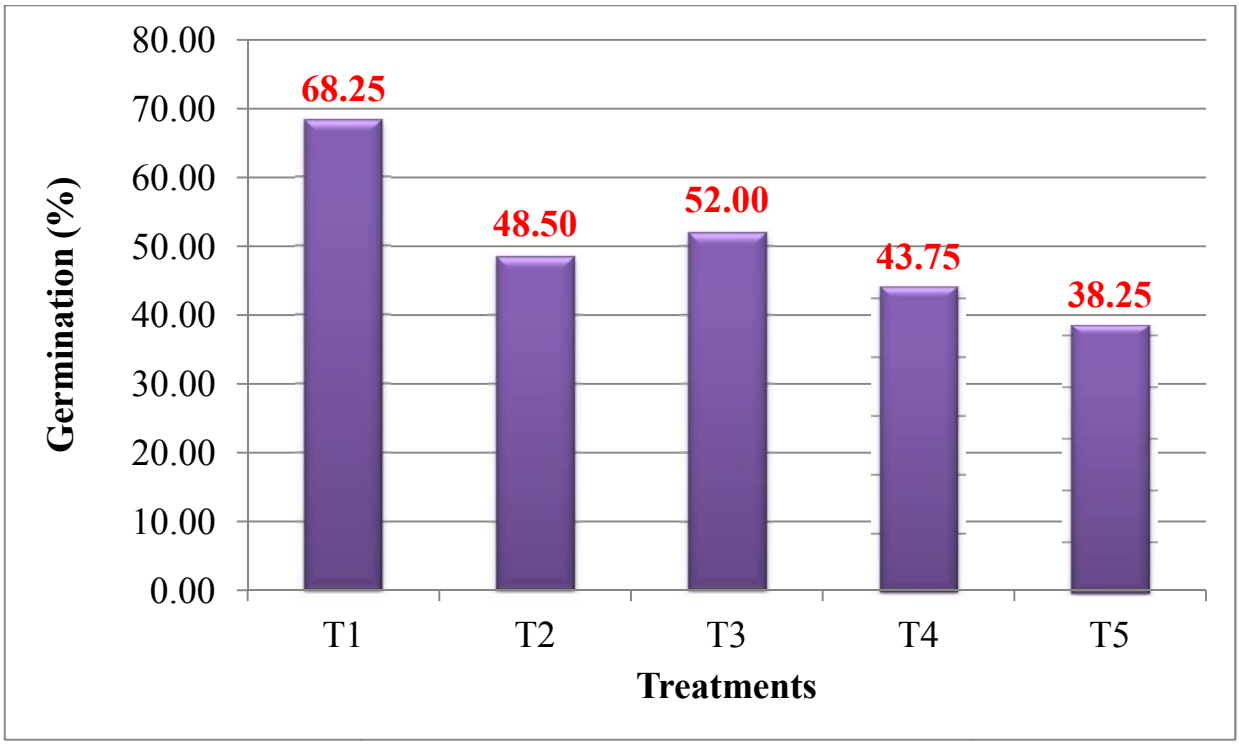

Fig 1: Effect of pre-sowing treatments (GA_3 soaking) on seed germination of *Rauvolfia serpentina* (L.) Bth. ex Kurz.

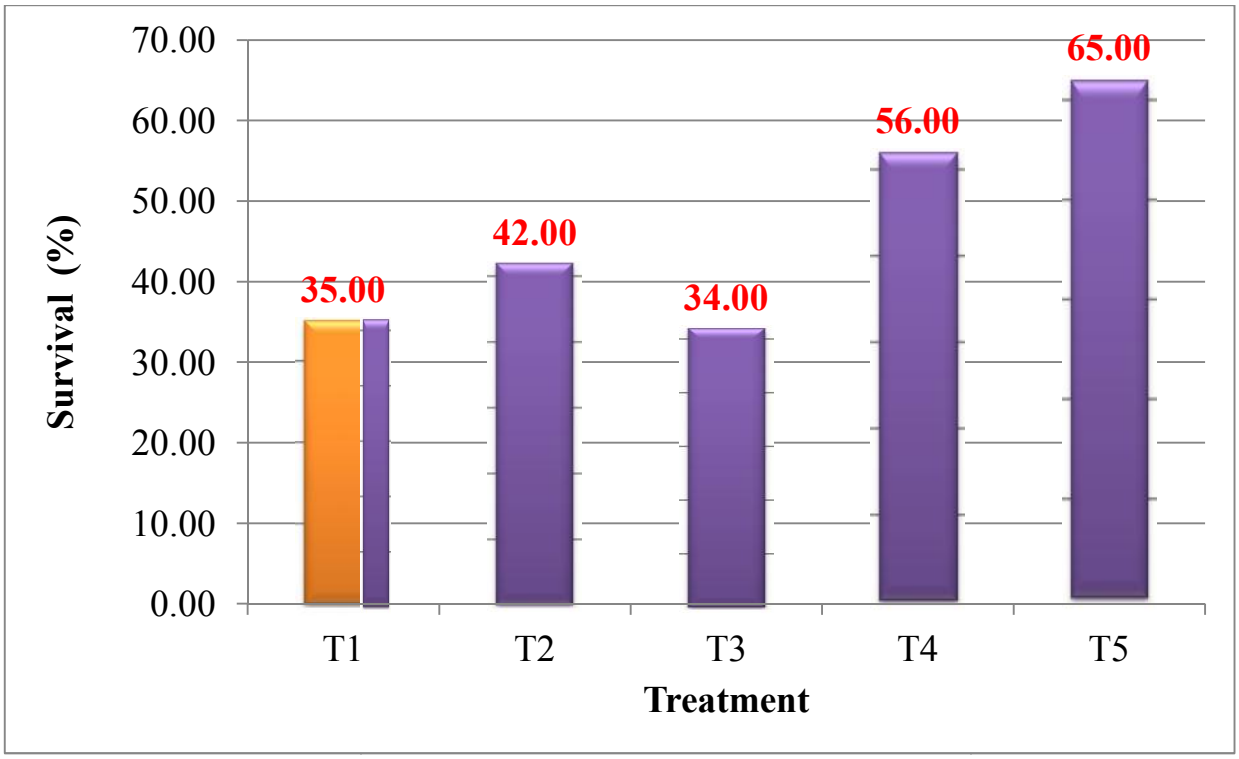

Fig 2: Effect of IBA on survival of *Rauvolfia serpentina* (L.) Bth. ex Kurz. propagated through soft wood leafy cuttings 90 DAP

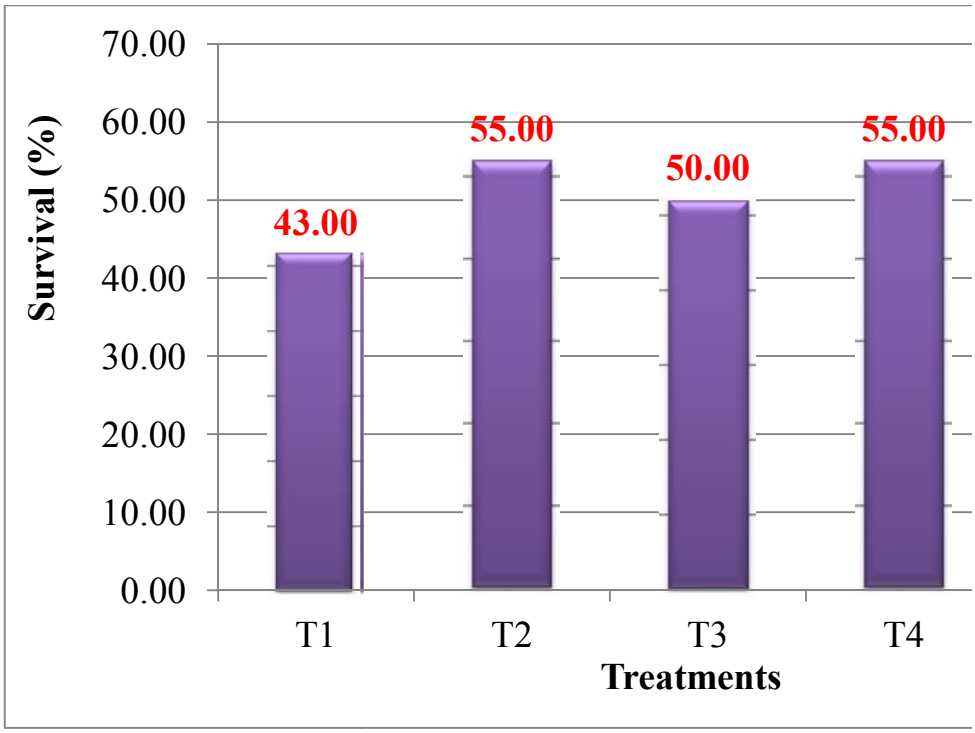

Fig 3: Effect of IBA survival of *Rauvolfia serpentina* (L.) Bth. ex Ku through semi-hard wood cuttings 90 DAP

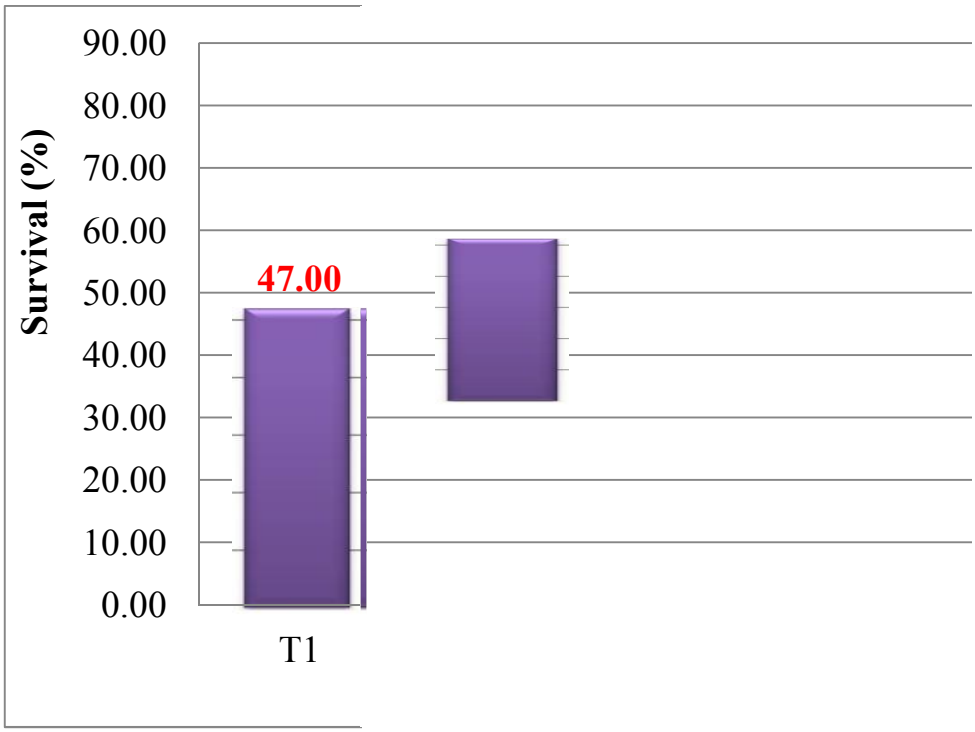

Fig 4: Effect of IBA on survival of *Rauvolfia serpentina* (L.) Bth. ex K through root cutting 90 DAP