“Effect of PGR on Growth and Quality of Madhunashini - (*Gymnema sylvestre* R.Br.)”

A

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BY

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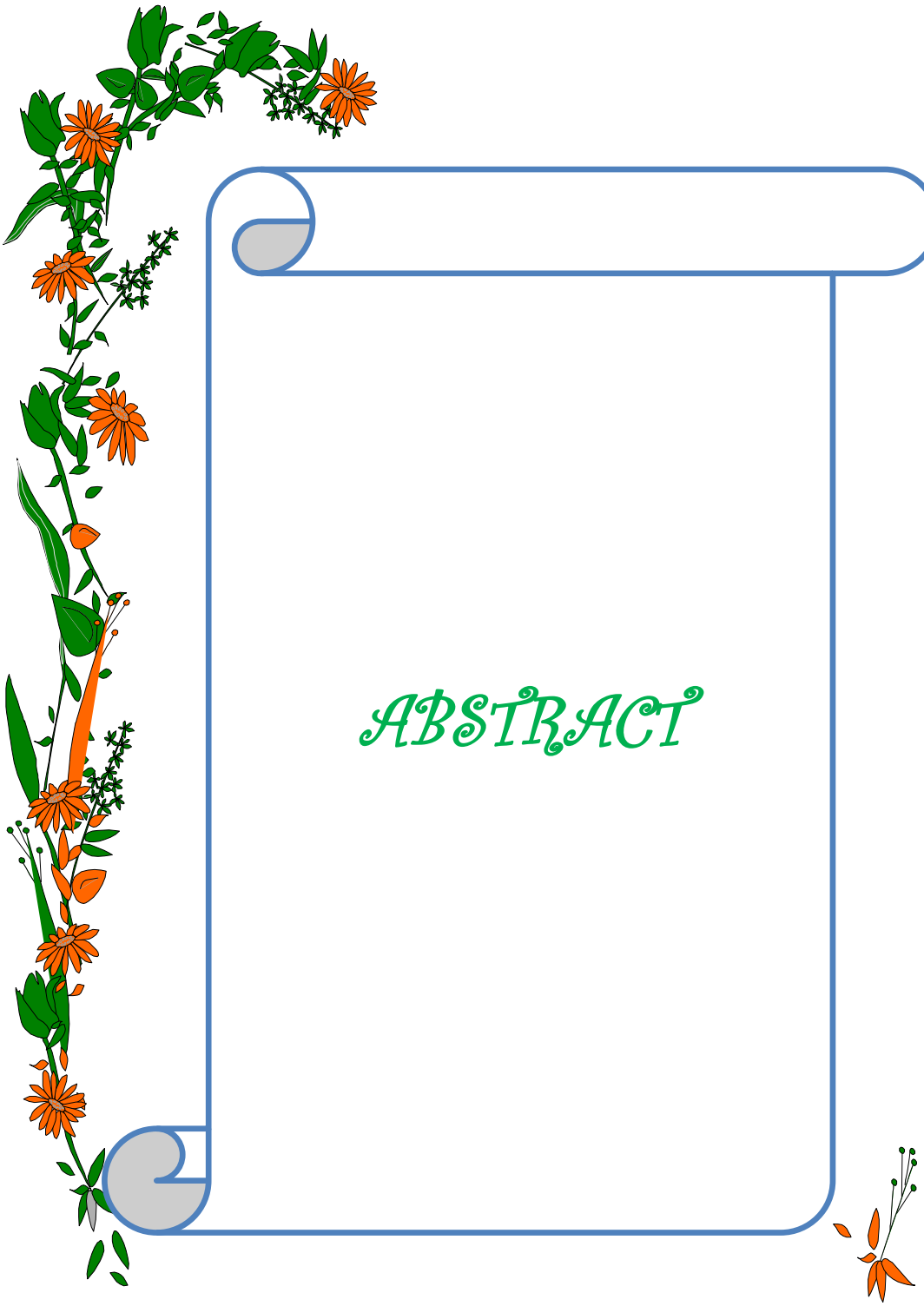
FAMILY
&
THOSE WHO ARE
SUFFERING FROM



ABSTRACT

"Effect of PGR on Growth and Quality of Madhunashini - (*Gymnema sylvestre* R.Br.)"

Student: **Major Advisor:**

CHAVDA J. R. **Dr. B. S. Desai**

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ABSTRACT

The present investigation entitled "Effect of PGR on Growth and Quality of Madhunashini (*Gymnema sylvestre* R.Br.)" was conducted at Model Nursery on Medicinal and Aromatic Plants, Navsari Agricultural University, Navsari, AES Zone-III (South Gujarat Heavy Rainfall Zone) during the month of July 2014 to December 2014

Influence of two auxins i.e IAA and IBA, either alone (500 ppm, 1000 ppm and 1500 ppm) or in combination (250 ppm IBA + 250 ppm IAA; 500 ppm IBA + 500 ppm IAA and 750 ppm IBA + 750 ppm IAA) were investigated for enhancing rooting success and growth of *G. sylvestre* cuttings. The experiment was laid out in CRD. Overall, there were ten treatments with three repetitions and each repetition consisted of thirty cuttings. The planting medium comprised of Red earth soil, FYM and Vermicompost in the ratio of 2:1:1 in 7' x 9' (15 x 20 cms) sized perforated polythene bags.

Among the various concentrations of auxins i.e IBA, IAA alone or in combinations, treatment T_5 [IAA-1000 ppm] took minimum days required for sprouting (19.67). The same treatment also showed better plant height and shoot length at 30, 60, 90 and 120 DAP with values of (8.67 cm, 47.83 cm, 111.07 cm and 117.91 cm) and (5.72 cm, 45.00 cm, 102.80 cm and 110.61 cm), respectively; whereas, treatment T_4 [IAA-500 ppm] was found superior with respect to larger leaf area (24.09 cm^2) and main root girth (7.69 mm) in comparison to the other treatments applied.

Maximum number of branches / plant and number of leaves/ branch at 30, 60, 90 and 120 DAP was recorded in treatment T_7 [IBA 250 ppm + IAA 250 ppm] with values of (0.50, 1.36, 2.27 and 3.67) and (2.67, 12.67, 30.27 and 38.87), respectively. Lengthiest main root was obtained in treatment T_8 [IBA 500 ppm + IAA 500 ppm] with value of (25.69 cm). Cuttings without any auxin treatment (Treatment T_{10}) produced lowest or minimum values for all parameters under study.

Finally, it can be concluded that out of all the combinations of IAA and IBA applied either individually or in combination, 1000 ppm IAA i.e Treatment T_5 has better influence on plant growth and it was at par with other treatments T_4, T_7 and T_8 found best for rooting and leaves character. Hence, IAA-1000 ppm can be used for large scale propagation of *G. sylvestre* through cutting.

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CERTIFICATE

This is to certify that the thesis entitled "Effect of PGR on Growth and Quality of Madhunashini - (*Gymnema sylvestre* R.Br.)" by CHAVDA JAYENDRASINH RAJENDRASINH in partial fulfilment of the requirements for award of the degree of MASTER OF SCIENCE (FORESTRY) in the subject of MEDICINAL AND AROMATIC PLANTS in Navsari Agricultural University is a record of bonafide research work carried out by him under my guidance and supervision and that the thesis has not been previously formed the basis for the award of any degree, diploma or has been published for other similar title. All the assistance and help received during the course of the investigation have been duly acknowledged by him.

Place: Navsari **(B. S. Desai)**

Date: 29/05/2015 **Major Advisor**

DECLARATION

This is to declare that the whole of the research work reported here in the thesis for the partial fulfilment of the requirements for the degree of Master of Science (Forestry) in the subject of Medicinal and Aromatic Plants by the undersigned is the result of investigation done by him under guidance and supervision of Dr. B. S. Desai, Assistant Professor, Department of Basic Sciences and Humanities, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari and no part of this work has been submitted for any other degree so far.

Place: Navsari

Date: 29/05/2015 **(CHAVDA J. R.)**

Countersigned by

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Place: Navsari

Date: 29 /05 /2015 **(CHAVDA J. R.)**

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ABBREVATIONS

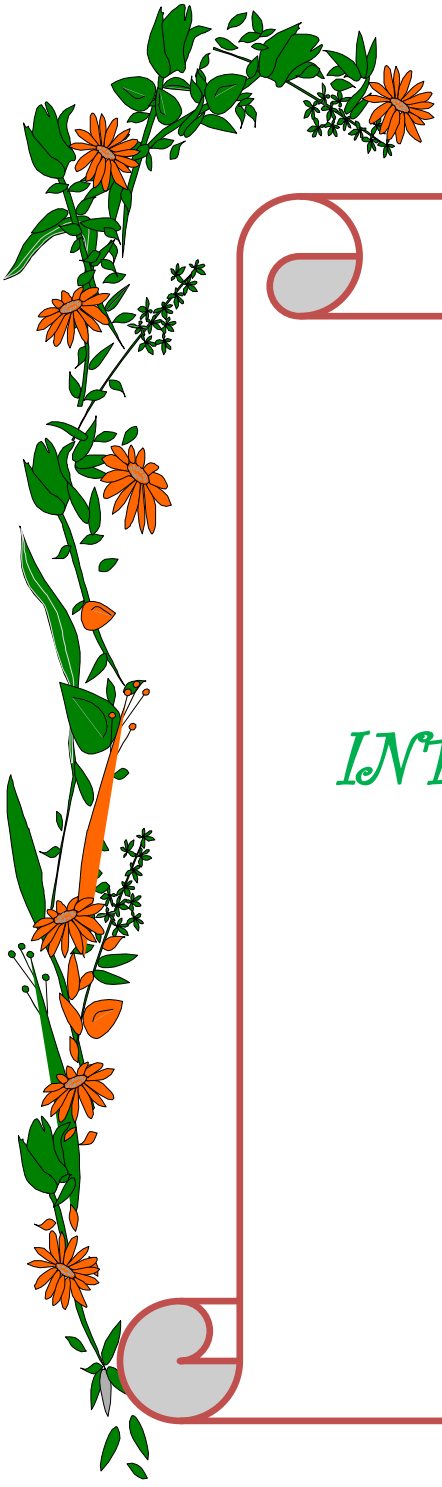
SR. NO.	SHORT FORM	FULL NAME
1.	µM	Micro molar
2.	2,4-D	2,4- Dichlorophenoxy Acetic Acid
3.	2ip	2-isopentenyladenine
4.	*A.J. Agric. food chem.*	Asian Journal of Agriculture and Food Chemistry
5.	*Acta Physiol. Plant.*	Acta Physiologiae Plantarum
6.	AES	Agro Ecological Zone
7.	*Am. J. Physiology*	American Journal of Physiology
8.	ANOVA	Analysis of Variance
9.	*Asian J. Pharm. Clin. Res.*	Asian Journal of Pharmaceutical and Clinical Research
10.	AYUSH	Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy, New-Delhi.
11.	BA	6-benzyladenine
12.	*Bangladesh J. Bot.*	Bangladesh Journal of Botany
13.	BAP	6-benzylamino purine
14.	BMI	Body Mass Index
15.	*Brain Res.*	Brain Research
16.	BSI	Botanical Survey of India, Kolkata.
17.	BVN	Bavistin
18.	C.D.	Critical difference
19.	C.V.	Coefficient of variance
20.	*Chem. Pharm. Bull.*	Chemistry and Pharmaceutical Bulletin
21.	Cm	Centimetre
22.	cm^2	Centimetre square
23.	CRD	Completely Randomized Design

24.	*Crop. Res.*	Crop Research
25.	CSIR	Council of Scientific and Industrial Research, New Delhi
26.	DAP	Days after planting
27.	DAS	Days after sowing
28.	*Diabetes Obes. Metab.*	Diabetes, Obesity and Metabolism
29.	*Diabetes Res. Clin. Pract.*	Diabetes Research and Clinical Practices
30.	DPPH	2, 2-diphenyl-1-picrylhydrazyl.
31.	DW	Distilled water
32.	e.g	As for example
33.	et al.	et allii, and others
34.	*Ethno-Med.*	Ethnomedicine
35.	EtOH	Ethyl alcohol
36.	Fig.	Figure
37.	FLS	Flowering
38.	FRLHT	Foundation for Revitalization of Local Health Traditions, Banglore.
39.	FRS	Fruiting
40.	FYM	Farm Yard Manure
41.	g/l	Gram per litre
42.	GA_3	Gibbrellic Acid
43.	Gm	Gram
44.	GoI	Government of India
45.	GSFDC	Gujarat State Forest Development Corporation
46.	HPLC	High Performance Liquid Chromatography
47.	Hrs	Hours
48.	i.e	That is
49.	IAA	Indole-3-Acetic Acid

50.	IBA	Indole-3-Butyric Acid
51.	ICAR	Indian Council of Agricultural Research, New-Delhi.
52.	ICMR	Indian Council of Medical Research, New-Delhi.
53.	*Indian J. Biochem. Biophys.*	Indian Journal of Biochemistry and Biophysics
54.	*Indian J. Exp. Biol.*	Indian Journal of Experimental Biology
55.	*Indian J. Pharmacol.*	Indian Journal of Pharmacology
56.	*Int. J. Green Pharmacy*	International Journal of Green Pharmacy
57.	*Int. J. Pharmacol.*	International Journal of Pharmacology
58.	*J. Asian Nat. Prod. Res.*	Journal of Asian Natural Products Research
59.	*J. Biosci. Res.*	Journal of Biosciences Research
60.	*J. Ethnopharmacol.*	Journal of Ethnopharmacology
61.	*J. Herb Med. Toxicol.*	Journal of Herb and Medical Toxicology
62.	*J. Med. P. Res.*	Journal of Medicinal Plant Research
63.	*J. Nat. Prod.*	Journal of Natural Products
64.	*J. Pharm. chem.*	Journal of Pharmaceutical Chemistry
65.	*J. Pharm. Sci.*	Journal of Pharmaceutical Sciences
66.	*J. Trop. For*.	Journal of Tropical Forestry
67.	*J. Vet. Med. Sci*.	Journal of Veterinary and Medical Sciences
68.	*Karnataka J. Agric. Sci.*	Karnataka Journal of Agricultural Sciences
69.	Kg	Kilogram
70.	Km	Kilometre
71.	Km / h	Kilometre per hour
72.	KN / KIN	Kinetin
73.	LDL	Low density lipoprotein

74.	M	Meter
75.	M.C. %	Moisture content
76.	Max.	Maximum
77.	Mg	Milligram
78.	mg/l	Milligram per litre
79.	Min	Minute
80.	Min.	Minimum
81.	Ml	Millilitre
82.	Mm	Millimetre
83.	Mm	Milli molar
84.	MS	Murashige and Skoog
85.	MSL	Mean sea level
86.	NAA	1-Napthalene Acetic Acid
87.	NaOH	Sodium hydroxide
88.	*Nat. Prod. Sci.*	Natural Products Science
89.	NBRI	National Botanical Research Institute, Lucknow
90.	NMMP	National Mission on Medicinal Plants, New Delhi
91.	NMPB	National Medicinal Plants Board, New Delhi
92.	NPK	Nitrogen, Phosphorus, Potassium
93.	NR	Nitrate reductase
94.	NTFP's	Non Timber Forest Products
95.	*Pak. J. Biol. Sci.*	Pakistan Journal of Biological Sciences
96.	PGR	Plant Growth Regulator
97.	*Physiol. Mol. Biol. Plants*	Physiology and Molecular Biology of Plants
98.	*Plant Biotechnol. Rep.*	Plant Biotechnological Reports
99.	Ppm	Parts per million
100.	R & D	Research and Development

101.	R.H.	Relative humidity
102.	S. Em ±	Standard Error of mean
103.	*Science Pub.*	Science Publication
104.	*South Indian Hort.*	South Indian Journal of Horticulture
105.	T	Treatment
106.	t / ha	Tonnes / hectare
107.	TC	Total Cholesterol
108.	TDZ	Thidiazuron
109.	TERI	The Energy and Resources Institute, New-Delhi.
110.	TFRI	Tropical Forest Research Institute, Jabalpur.
111.	TG	Triglyceride
112.	TMP	Trimethoprim
113.	TN	Tamil Nadu
114.	TNAU	Tamil Nadu Agricultural University
115.	*Turky J. Biol.*	Turkish Journal of Biology
116.	USDA	United States Developement Agency
117.	v/v	Volume/volume
118.	Viz.	Namely
119.	VLDL	Very low density lipoprotein
120.	w.r.t.	With respect to
Symbols		
1.	%	Percent / Percentage
2.	/	Per
3.	°	Degree
4.	'	Minute
5.	˚C	Celsius/Centigrade
6.	<	Less than
7.	>	Greater than
8.	≤	Less than or equal to
9.	₹ .	Rupees



INTRODUCTION

I. INTRODUCTION

1.1. Importance of the study undertaken:

Gymnema sylvestre R.Br. commonly known as “Gudmar” or “Madhunashini” is one of the potential anti diabetic medicinal climber belonging to Asclepiadaceae and its native to India with chromosome complementation (2n=22). Most importantly, it is one of the 32 medicinal plants prioritized by NMPB under NMMP for *ex-situ* and *in-situ* conservation. Almost 49 species of *Gymnema* is identified globally.

Its leaves contain Gymnemic acid and Gymnemasides. As already mentioned above besides its anti diabetic properties, this plant also posses other important therapeutic uses *viz*., bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic, emetic, diuretic, stomachic, stimulant, anthelminthic, laxative, cardiotonic, expectorant, antipyretic and uterine tonic. It is useful in dyspepsia, renal and vesicle calculi, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis and leucoderma (Rani *et al*. 2012). The present study is undertaken to throw some insight on its growth and rooting behaviour under South Gujarat conditions (Nursery level).

1.2. History:

Gymnema has a long history of therapeutic use in India's Ayurvedic system of medicine. Indians first used *Gymnema* to treat diabetes almost 2,000 years ago. Today, *Gymnema* is used for diabetes (Joseph and Ellen, 2005) metabolic syndrome, weight loss and cough. It is also used to cure malaria and as an antidote against snake bite. It is digestive, stimulant, laxative, appetite, suppressant and a diuretic agent. The primary application of this plant was for diabetes; a condition once described as "honey urine" and is continued to be recommended today in India. In the 1920’s, preliminary scientific studies found some evidence

that *Gymnema* leaves can reduce blood sugar levels, but nothing much came of this observation for decades (American Botanical Council P.O box 201660). Even today also *Gymnema* is one of the popular medicine in the United States as a supportive treatment to cure diabetes (Kerry, 2002).

1.3. Taxonomical classification:

Kingdom- Plantae

Subkingdom- Tracheobionta

Super division -Spermatophyta

Division- Magnoliophyta

Class- Magnoliopsida

Subclass- Asteridae

Order -Gentianales

Family- Asclepiadaceae

Genus- *Gymnema* R. Br.

Species- *sylvestre* (Kirtikar and Basu, 1998).

1.4. INFORMATION ABOUT PLANT:

1.4.1. Global Distribution:

Gymnema Sylvestre R. Br. is a highly valuable medicinal herb, and is widely distributed in India, Malaysia, Sri Lanka, Australia, Indonesia, Japan, Vietnam, Tropical Africa and the South-Western region of the People's Republic of China (Saneja *et al.* 2010).

1.4.2. Distribution in India:

Gymnema sylvestre R. Br. is native of India and is found in forests of Western Ghats, Konkan, Madhya Pradesh, Chattisgarh, Bihar, Tamilnadu, and Karnataka (Deshpande, 2005). *Gymnema* grows in open woods and bush lands at an elevation of 100-1000 m. The plants are found largely in Deccan Peninsula (Anonymous, 1985). It is widely

distributed and commonly found in the hills of Orissa and Deccan peninsula (Singh, 2013).

1.4.3. Botanical Description:

1.4.3.1. Genus: *Gymnema* R.Br.

The generic name is derived from Greek words, **Gymnos** = **naked** and **Klados** = **a branch**; referring to the appearance of branches during winter season (Gymnocladus, 2010).

The genus comprises of twining shrubs. Leaves are opposite. Flowers are small and are arranged in crowded lateral umbellate cymes. Calyx is 5-partite; corolla is sub rotate to the middle or beyond it, lobes thick and overlapped to right of bud. Staminal column arises from the base of the corolla. Style is often exerted beyond the anthers. The follicles are smooth with comose seeds.

About 25 species occur in Paleo-tropical regions, South Africa and Australia, of which 10 species are known to occur in India.

1.4.3.2. Species: *Gymnema sylvestre* R. Br.

It is a large, stout, much branched and woody climber with densely appressed hairy branchlets. Leaves are 3.2-5.0 x 1.3-3.0 cm, rarely pubescent above, thinly coriaceous and elliptic or obovate-acute. Flowers are yellow, small in crowded umbelliform cymes; corolla sub-rotate with thick lobes and fleshy coronal processes on the throat. The follicles are 6.0- 7.5 x 0.7-0.8 cm, slender and glabrous; one follicle often suppressed. Seeds are about 1.2 cm long, narrowly obovoid-oblong and flat with a broad thin wing and pale brown in colour. Overall habit along with flowering and fruiting of *Gymnema sylvestre* R. Br. are depicted in Plate 1.

1.4.4.3. Synonyms:

1. *Asclepias geminata* Roxb.
2. *Gymnema melicida* Edgew.

Plate No. 1. Overview of Plant Habit – *Gymnema sylvestre* R. Br.

1. *Gymnema sylvestre* R. Br. Source

2. *Gymnema sylvestre* R.Br. Flowering

3. *Gymnema sylvestre* R.Br. Fruits

3. *Marsdenia sylvestris* (Retz.)
4. *Periploca sylvestris* Retz. (Gupta *et al*. 2012).

1.4.4.4. Common / vernacular Names:

Gujarati: Dhubli, Mardashingi, Kaavalee.

Sanskrit: Meshasringi means Ram's Horn. Name derived from plants fruits, Sarpadarushtrika

English: Periploca of the woods, *Gymnema*, Australian Cow plant, Small Indian Ipecacuanha. (Joy *et al.* 1998)

Hindi: Gudmar, Mera-singi

Kannada: Madhunashini

Tamil: Boda-patra

Telugu: Shiru-kurunja

1.4.5. Phenology:

Flowering (FLS): July-August

Fruiting (FRS): October-December.

1.4.6. Plant Parts Used:

Whole plant (seeds, leaves and roots) (Singh, 2013).

1.4.7. Species Diversity in Genus *Gymnema*:

The genus comprises of 49 species, some of which like *Gymnema montanum, Gymnema yunnanense,* and *Gymnema inodorum* have medicinal properties (Persaud *et al.*1999). According to plant list (2010) Table 1.

Table No. 1.1. Different species of genus *Gymnema*.

Sr. No.	Species Name	Sr. No.	Species Name
1.	*Gymnema acuminatum* Wall.	26.	*Gymnema longiretinaculatum* Tsiang
2.	*Gymnema albidum* Decne.	27.	*Gymnema lushaiense* M.A.Rahman &Wilcock
3.	*Gymnema albiflorum* Costantin	28.	*Gymnema macrothyrsa* Warb.
4.	*Gymnema brevifolium* Benth.	29.	*Gymnema maingayi* Hook.f.
5.	*Gymnema calycinum* Schltr.	30.	*Gymnema mariae* Schltr.
6.	*Gymnema chalmersii* Schltr.	31.	*Gymnema micradenium* Benth.
7.	*Gymnema cumingii* Schltr.	32.	*Gymnema molle* Wall. ex Wight
8.	*Gymnema cuspidatum* (Thunb.) Kuntze	33.	*Gymnema montanum* Hook.f.
9.	*Gymnema dissitiflorum* Ridl.	34.	*Gymnema muelleri* Benth.
10.	*Gymnema dunnii* (Maiden & Betche) P.I.Forst.	35.	*Gymnema pachyglossum* Schltr.
11.	*Gymnema elegans* Wight & Arn.	36.	*Gymnema piperii* Schltr.
12.	*Gymnema erianthum* Decne.	37.	*Gymnema pleiadenium* F.Muell.
13.	*Gymnema foetidum* Tsiang	38.	*Gymnema recurvifolium* Blume
14.	*Gymnema glabrum* Wight	39.	*Gymnema rotundatum* Thwaites
15.	*Gymnema griffithii* Craib	40.	*Gymnema rufescens* Decne.
16.	*Gymnema hainanense* Tsiang	41.	*Gymnema schlechterianum* Warb.
17.	*Gymnema hirsutum* Wight & Arn.	42.	*Gymnema spirei* Costantin
18.	*Gymnema hirtum* Ridl.	43.	*Gymnema suborbiculare* K.Schum.
19.	*Gymnema inodorum* (Lour.) Decne.	44.	*Gymnema syringaefolium* (Decne.) Costantin
20.	*Gymnema javanicum* Koord.	45.	*Gymnema thorelii* Costantin
21.	*Gymnema khandalense* Santapau	46.	*Gymnema tricholepis* Schltr.
22.	*Gymnema lacei* Craib	47.	*Gymnema trinerve* R.Br.
23.	*Gymnema lactiferum* (L.) R.Br. ex Schult.	48.	*Gymnema uncarioides* Schltr.
24.	*Gymnema latifolium* Wall. ex Wight	49.	*Gymnema yunnanense* Tsiang
25.	*Gymnema littorale* Blume		

1.5. Uses of *Gymnema sylvestre* R. Br.

1.5.1. Ayurvedic Properties and Traditional value:

Monumental and classical literature of Ayurveda – Sushruta, describes *Gymnema sylvestre,* as a destroyer of madhumeha (glycosuria) and other urinary disorders. It neutralises excess of sugar present in the body in Diabetes mellitus (Anonymous, 1995). The drug is also used in different ayurvedic preparations like Ayaskri, Varunadi, Kasaya, Varunadighrtam, Mahakalyanaka-ghrtam (Joy and Thomas, 1998). It is reported to be bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic emetic, diuretic, stomachic, stimulant, anthelmenthic, laxative, cardiotonic, expectorant, antipyretic and uterine tonic. It is useful in dyspepsia, constipation and jaundice, haemorrhoids, renal and vesicle calculi, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis and leucoderma (Nandkarni, 1993; Vaidyaratnam, 1995; Chopra *et al.* 1992 and Saneja *et al.* 2010).

1.5.2. Traditional uses:

Leaf: in gastric troubles; **ETHNIC COMMUNITIES OF RAJASTHAN and DHASAN VALLEY**: Leaf: in diabetes; **ETHNIC COMMUNITIES OF KANDALA (Maharashtra)**: Leaf: in urinary complaints; **GOND TRIBE**: Leaf: in diabetes and stomach ache; **ETHNIC COMMUNITIES OF MADHYA PRADESH**: Leaf: in cornea opacity and other eye diseases; **ETHNIC COMMUNITIES OF GODAVARI DISTRICT (Andhra Pradesh)** : Leaf: in diabetes, glycosuria; **IRULAS TRIBE**: Leaf: in diabetes; **CHARAKA SAMHITA**: removes bad odour from breast milk, aperitive; **SUSHRUTA SAMHITA**: plant useful as purgative, in eye troubles; leaf extract and also the extract of flower is beneficial for eyes; bark useful in the diseases caused by vitiated kapha (phlegm); **BAGBHAT**: root bark useful in piles; **BHAVAPRAKASHA**: it is bitter, appetiser, gastric

stimulant, removes cough, alleviates breathing troubles, useful in curing phlegm, eye troubles, wounds; **RAJA NIGHANTU**: appetiser, removes phlegm, piles, colic pain, cures dropsy, useful in eye troubles, cardio tonic, beneficial in respiratory diseases, wounds, detoxicant; fruits are bitter, sialagogue, thermogenic, cures the diseases caused by vitiated kapha (phlegm) or vata (wind); **NIGHANTU RATNAKARAM**: removes cough, vitiated wind, detoxicant, appetiser, useful in eye troubles. **SIDDHA**: an ingredient of 'Cirukuricinver'; **UNANI**: an ingredient of 'Gurmarbuti'. The fresh leaves, when chewed, paralyse the sense of sweet for sometime; for this reason it is called Gurmar, thereby meaning sugar-killer and impression has become prevalent in some parts of the country that it is useful in diabetes mellitus. Chewing fresh leaves also paralyse the taste of bitter for a while (BSI, 2013).

MODERN USE: Aerial parts (50% EtOH extract): spasmolytic, hypoglycaemic, *in vitro* antiviral against influenza A2 virus (BSI, 2013).

1.5.3. Ethnobotanical and Medicinal uses:

There are over four hundred different tribal and other ethnic groups in India. Each tribal group is having their own tradition, folk language, beliefs and knowledge about the use of natural resources as medicines. The Indian subcontinent has given to the medicinal world, systems of medicines such as *Ayurveda*, *Yunani* and *Siddha*. Based on such systems, we can find not only new remedies; but also new lead molecules of therapeutic values. Most of the drugs from plant sources are secondary metabolites, which are less significant in plant metabolism; but are postulated to play a major role in the plant defence mechanism. However, not much difference is seen in the basic metabolic processes in plants as well as animals (Ramachandran *et al.*, 2003). Fever is treated with an oral administration of half an ounce to an ounce (one part in 10) of leaves. Swollen glands are treated with an external application of

triturated leaves mixed with castor oil. In Sri Lanka, the plant is utilized to cure bone fractures. The jungle Irulas inhabitants (Nagari Hills of the North Arcot district, Bombay and Gujarat from India) have the habit of chewing a few green leaves of *G. sylvestre* in the morning in order to keep their urine clear and to reduce glycosuria. Bourgeois classes of Bombay and Gujarat also chew fresh leaves for the same effect. In Bombay and Madras, 'Vaids' are known to recommend the leaves in the treatment of furunculosis and madhumeha. The juice obtained from root is used to treat vomiting and dysentery and plant paste is applied with mother milk to treat mouth ulcer. *Gymnema* preparations have shown to possess anti-allergic activity (Porchezhian and Dobriyal, 2003). Plant has Gymnemic acid as a major constituent of the leaves that inhibits glucose absorption in the small intestine (Shimizu *et al.* 1997).

"Gurmarin" one of the major constituents of the leaves too suppress sweet taste of sensation (Pierce, 1999; Flier, 2001; Imoto *et al.* 1992 and Masayuki *et al.* 1997). Root powder and paste of the fresh root is applied externally on wounds as an antidote against snakebite (Miyasaka and Imoto, 1995 and Gomes *et al.* 2010). The potassium salt of gymnemic acid, which is a triterpenoid glycoside isolated from *Gymnema sylvestre* inhibits ATPase in, *Naja naja* (Indian cobra) venom and *Vipera russelli* (viper) venom (Gomes *et al.* 2010, Kini and Gowda, 1982). Inhibition occurs due to competitive binding between *Gymnema*te and ATP. The inhibition of organic compounds like histamine, triggering responses to foreign particles shows that these pectic substances have anti-allergic activities (Sawabe *et al.* 1992).

Traditional healers from diverse parts of India use this plant in various ailments. The leaves are given in gastric troubles in Rajasthan. Traditional healers of Maharashtra prescribe it in urinary problems, whereas in Madhya Pradesh it is used cure stomach-ache. Tribal's and

local healers apply the leaf extract in cornea opacity and other eye disease. In Andhra Pradesh, plant is used in glycosuria. In Eastern Africa, pounded leaves are rubbed into scarification in the side to heal stitch.

In Tanzania, pounded cooked roots in food are taken to treat epilepsy. In Angola, leaf and stem preparations are taken to treat cancer. In Botswana, pounded cooked roots or root powder are applied externally to treat boils. In Madagascar an infusion of the leafy twigs is taken to treat gonorrhoea (Kirtikar and Basu, 1998; Agnihotri *et al.* 2004 and Ekka and Dixit, 2007). This effect lasts up to about 2 hours; the herb actually reduces cravings for sugar by blocking sugar receptors in the tongue. It might neutralise the excess of sugar present in the body in Diabetes mellitus (Anonymous, 1995).

Mechanisms such as the stimulating or regenerating effect on β cells or extrapancreatic effects are proposed for the hypoglycemic action of these herbs, such as *Momordica charantia, Pterocarpus marsupium*, and *Trigonella foenum greacum*, have been reported to be beneficial for treating type 2 diabetes (Saxena and Vikram, 2004).

Ethnobotanical uses along with few reported ethnoveterinary uses of *Gymnema sylvestre* R.Br. are as listed below.

A. Leaves are administered in diarrhoea and fever among cattles mainly in (Telugu) Andhra Pradesh. Locally called as "Podapathri" in Andhra Pradesh (Jain, 1999).

B. Two species of *Gymnema viz. G. hirsutum* and *G. sylvestre* are widely used in ethnomedicines. Both the species are utilized by tribes of Maharashtra, Madhya Pradesh, Andhra Pradesh, Sikkim, Bengal, Bihar and Orissa. *G. sylvestre* is also used as a remedy by tribes of Uttar Pradesh, Punjab, Haryana, Rajasthan, Gujarat and towards south in Kerala, Karnataka and Tamilnadu. *G. hirsutum* leaves are mainly used to cure anasarca, bronchitis, colic, constipation, dropsy, dysentery,

dysuria, fever, impotency, sores and syphilis. Similarly, *G. sylvestre* leaves are taken as remedy for diabetes, gastric disorders and glycosuria. Roots and leaves in combination are applied as remedy for snake bite, stomach-ache and urinary complaints (Jain, 1991).

1.5.4. Pharmacological activities:

1.5.4.1. Antiobesity Study:

G. sylvestre helps to promote weight loss possibly through its ability to reduce cravings for sweets and control blood sugar levels. It has been reported that the gurmarin peptide block the ability to taste sweet or bitter flavours and thus reduces sweet cravings (Pierce, 1999 and Ninomiya and Imoto, 1995). A standardized *Gymnema sylvestre* extract in combination with niacin-bound chromium and hydroxycitric acid has been evaluated for antiobesity activity by monitoring changes in body weight, body mass index (BMI), appetite, lipid profiles, serum leptin and excretion of urinary fat metabolites. This study showed that the combination of *G. sylvestre* extract and hydroxycitric acid, niacin bound chromium can serve as an effective and safe weight loss formula that can facilitate a reduction in excess body weight and BMI while promoting healthy blood lipid levels (Preuss *et al*. 2004).

1.5.4.2. Antidiabetic Activity:

The first scientific confirmation of *G. sylvestre* use in human diabetics came almost a century back when it was demonstrated that the leaves of *G. sylvestre* reduce urine glucose in diabetics (Charpurey, 1926). In an animal study, (Paliwal *et al.* 2009) have investigated that gurmar leaf powder had positive and encouraging effects over blood glucose levels. No adverse effect was observed on the health status of the subjects concluding that gurmar powder is effective in lowering the fasting as well as postprandial blood glucose levels (Paliwal *et al*. 2009). Moreover, the antihyperglycemic action of a crude saponin fraction and

five triterpene glycosides derived from the methanol extracts of *G. sylvestre* was noticed (Sugihara *et al.* 2000).

1.5.4.3. Hypolipidaemic Activity:

The administration of leaf extracts to hyperlipidaemic rats for two weeks have been found to show reduction in elevated serum triglyceride (TG), total cholesterol (TC), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) – cholesterol in dose dependent manner. The efficiency of this drug was almost similar to that of a standard lipid lowering agent-clifibrate (Bishayee and Chaterjee, 1994 and Rachh *et al*. 2004).

1.5.4.4. Antimicrobial Activity:

The ethanolic extract of *G. sylvestre* leaves showed good antimicrobial activity against *Bacillus pumilis, B. subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* and no activity were found against *Proteus vulgaris* and *Escherichia coli* (Satdive *et al*. 2003). The aqueous and methanolic extract of *Gymnema sylvestre* leaves also showed moderate activity against the three pathogenic *Salmonella* species (*Salmonella typhi, S. typhimurium* and *S. paratyphi*). Out of the two extracts used, aqueous extract showed higher activity against the *Salmonella* species (Pasha *et al*. 2009). Ethanolic, Chloroform and Ethyl acetate extracts of the aerial parts of *G. sylvestre* is also reported to have antibacterial effects against *Proteus vulgaris, Escherichia coli, Pseudomonas aeroginosa, Klebsella pneumoniae* and *Staphylococcus aureus* (Paul and Jayapriya, 2009).

1.5.4.5. Anti-Inflammatory Activity:

The aqueous extract of *G. sylvestre* leaves was investigated for evaluation of anti-inflammatory activity in rats at a dose of 200, 300 and 500 mg / kg in carrageenin-induced paw oedema and cotton pellet method. The aqueous extract at 300 mg / kg decreased the paw oedema

volume by 48.5% within 4 h after administration, while the standard drug phenylbutazone decreased the pawoedema volume by 57.6% when compared with the paw oedema volume of control. The aqueous extract at the dose of 200 mg / kg and 300 mg / kg produced significant reduction in granuloma weight, when compared to control group (Malik *et al*. 2008).

1.5.4.6. Free Radical Scavenging Activity:

In vitro, inhibitory effects of DPPH radicals and LDL oxidation were found with aqueous extract of *G. sylvestre* require 32.1 μl, for scavenging 50% of the DPPH radicals (Ohmori *et al*. 2005).

1.5.4.7. Clinical Applications:

The primary clinical application of this botanical is as an antidiabetic agent. *Gymnema* has been the object of considerable research since the 1930s, with promising results for type 1 and 2 diabetes. i.e Diabetes mellitus and Diabetes insipidus.

In a controlled study, a standardized *Gymnema* extract was given to 27 type 1 diabetics at a dose of 400 mg daily for 6-30 months. Thirty-seven others continued on insulin therapy alone and were tracked for 10-12 months. Insulin requirements were decreased by about one-half and the average blood glucose decreased from 232 mg / dL to 152 mg / dL in the *Gymnema* group. The control group showed no significant reduction in blood sugar or insulin requirement. In addition, there was a significant decrease in glycosylated haemoglobin (HbA1c) after 6-8 months of *Gymnema* treatment when compared to either the pre-treatment levels or the control group (Shanmugasundaram, *et al.* 1990).

Twenty-two type 2 diabetics were administered 400 mg *Gymnema* extract daily for 18-20 months in addition to their oral hypoglycaemic medications. This group experienced significant decrease in average blood sugar and HbA1c, and an increase in pancreatic release

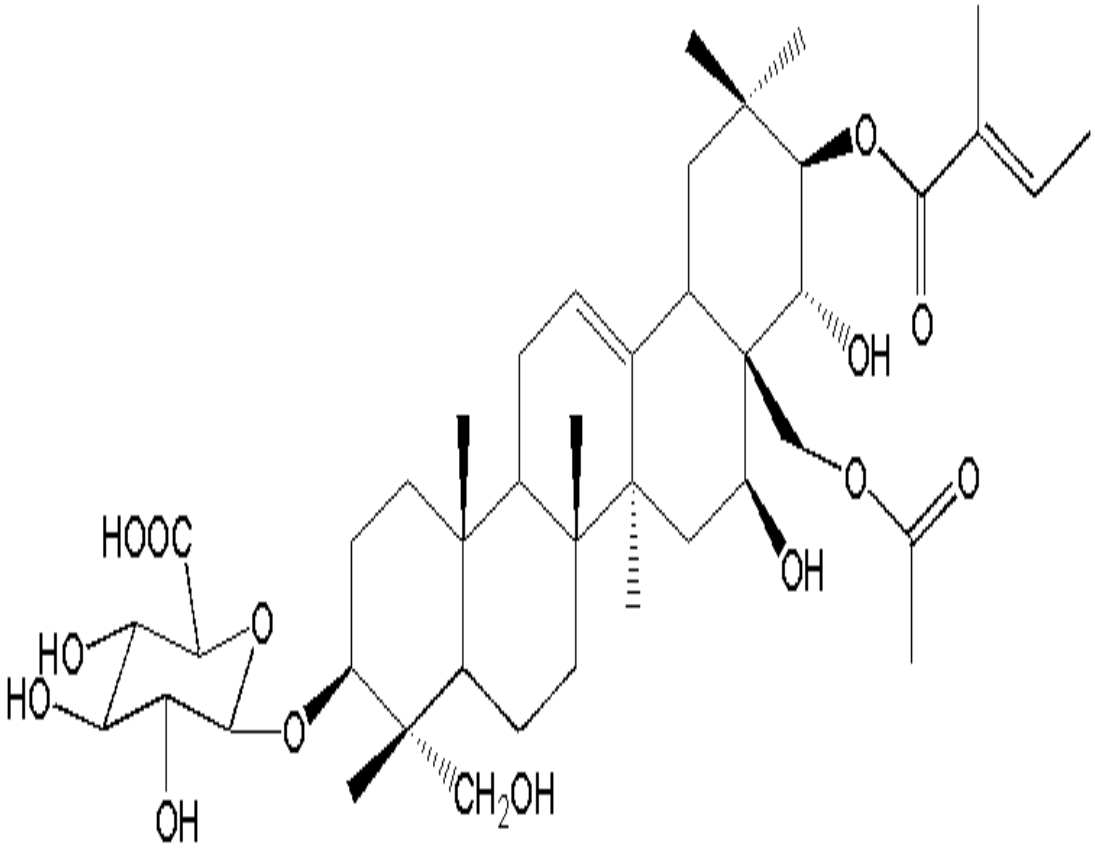
of insulin. Medication dosages were decreased, and five were able to discontinue drugs entirely (Baskaran, *et al.* 1990). Numerous animal based studies have confirmed the hypoglycemic effect of *Gymnema sylvestre* (Srivasta, *et al.* 1985; Okabayashi, *et al.* 1990 and Venkatakrishna, *et al.* 1981).

1.6. Phytochemistry:

The leaves of *G. sylvestre* contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are Gymnemic acids and saponins, while dammarene saponins are Gymnenmasides (Khramov *et al*. 2008; Yoshikawa *et al*. 1992; Dateo and Long 1973). Besides this, other plant constituents are flavones, anthraquinones, pentatriacontane, α and β - Chlorophylls, Phytin, Resins, D-Quercitol, Tartaric acid, Formic acid, Butyric acid, Lupeol, β amyrin related glycosides and stigmasterol. The plant extract also gives positive test for alkaloids. Leaves yield acidic glycoside and anthroquinones and their derivatives (Shah, 2010). The leaves also posses resins, albumin, chlorophyll, carbohydrates, Tartaric acid, Formic acid, Butyric acid, anthraquinone derivatives, inositol, alkaloids, organic acid (5.5%), parabin, calcium oxalate (7.3%), lignin (4 .8%) and cellulose (22%) (Sinsheimer *et al.* 1970). Leaves are major source of gymnemic acid as an effective substance (Shah, 2010). The Primary chemical constituents of *Gymnema* includes; Gymnemic acid, Tartric acid, Gurmarine, Calcium Oxalate, Glucose, Stigmastirol, Betain and Cholin. Few new triterpenoid saponins, Gymnenmasins A,B,C and D were also isolated from the leaves of *G. sylvestre* (Suttisri *et al*. 1995; Sahu *et al*. 1996). Three new oleanane type triterpene glycosides were isolated from the leaves. Besides this, six oleanane type saponins were also reported from the leaves (Ye *et al*. 2000, 2001). An aerial part of *Gymnema pentaphyllum*

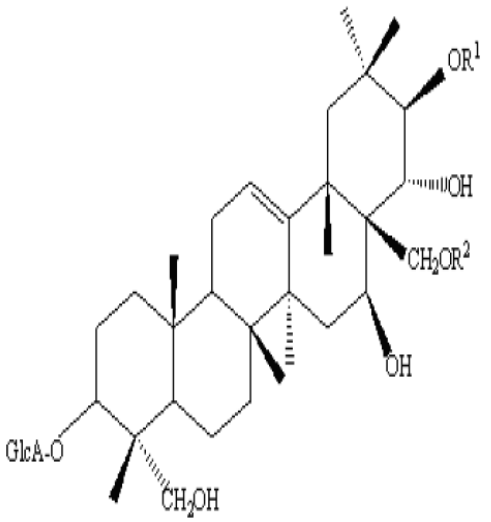
shows the presence of Gypenoside (Norberg *et al.* 2004) (Fig. 1.1 and 1.2)

Fig. No. 1.1: Structure of Gymnemic acid.



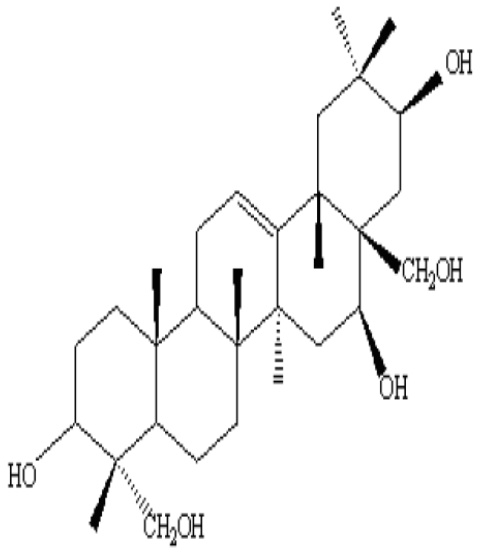
(Anjum and Hasan, 2013)

Fig. No. 1.2: Phytochemicals isolated from *Gymnema sylvestre* R. Br.

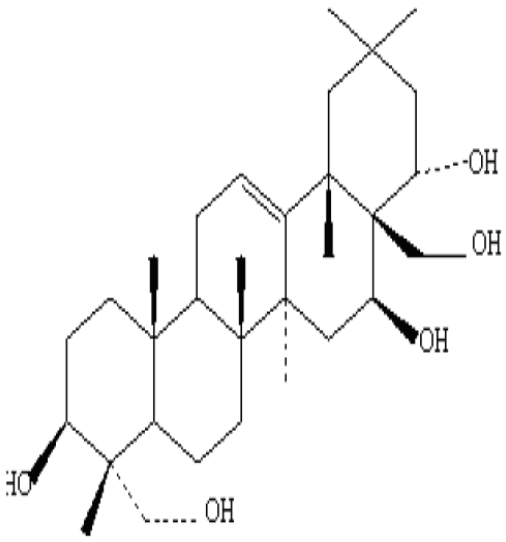


Gymnemic acid

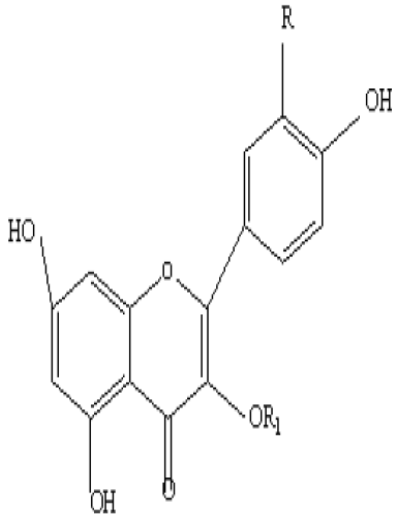
Gymnemic acid types	R^1	R^2
Gymnemic acid I	Tigloyl	Ac
Gymnemic acid II	2-methylbutyroyl	Ac
Gymnemic acid III	2-methylbutyroyl	H
Gymnemic acid IV	Tigloyl	H



Gymnestrogenin



Gymnemanol



R = H, R_1 = beta-O-Glcp-(1--> 4)- alpha-L-Rhap-(1-->6)-beta-O-Galp

Kaempferol 3-O-beta-D-glucopyranosyl-(1-->4)-alpha-L-rhamnopyranosyl- (1-->6)-beta-D glactopyranoside

(Saneja *et al.* 2010)

1.7. AGRO TECHNIQUES:

1.7.1. Nursery technique:

1.7.1.1. Raising propagules:

Styrofoam trays or poly bags are filled with soil, sand, and FYM in 1:2:1 ratio and terminal or axillary cuttings are planted in them. Vermicompost may be used in place of FYM. February to March is the best season for planting the cuttings in nursery, especially in North Indian conditions. The cuttings are placed under humid conditions in shade houses or mist chambers for development of roots. Rooting is initiated within a month of planting. Seed setting is poor in this species and the seeds show a maximum germination percentage of 50%–55 % when sown in soil mixed with vermicompost.

1.7.1.2. Propagule rate and pre-treatment:

About 6700 rooted cuttings are required for plantation in 1 hectare of land. At 80% survival, about 8400 cuttings would be required. The stem cuttings are dipped in IBA (100 ppm) for six minutes before planting in the nursery to promote rooting.

1.7.2. Planting in the field:

1.7.2.1. Land preparation and fertilizer application:

The field is ploughed to turn the soil and make it weed-free. About 10 tonnes of FYM is mixed with the soil as a basal application at the time of land preparation.

1.7.2.2. Transplanting and optimum spacing:

The period between June and August is best for transplanting the rooted plants in the field. An optimum spacing of 1 m × 1.5 m is recommended for a crop stand of about 6700 plants per hectare. The rooted cuttings/seedlings may be planted by crow bar method.

1.7.2.3. Intercropping system:

When the plants are young, green gram can be grown as an intercrop. Alternatively, the crop can be raised beneath the tree species that serve as host or staking for these twinners.

1.7.2.4. Intercultural and maintenance practices:

About 10–12 t / ha of FYM or 250 kg of NPK (nitrogen, phosphorus, potassium in equal quantities) is applied as basal dose at the time of land preparation. An additional equal dose may be added every year for maximizing biomass production.

1.7.2.5. Irrigation practices:

Irrigation is required at least once in a week during summer season. Frequency may also depend on the soil moisture in winter. It may be limited to one per month.

1.7.2.6. Disease and pest control:

An aphid (*Aphis* sp.) is observed to attack the apical tender parts of the plant during rainy season. However, if the *G. sylvestre* – crop damage is not severe, no control measures are required. Use of chemical pesticides should be avoided since leaves are to be regularly plucked for harvest.

1.7.3. Harvest management:

1.7.3.1. Crop maturity and harvesting:

Leaves that are about 30–40 days old can be plucked for use, and harvesting can be done every three months. However, better yield is obtained after one year of growth.

1.7.3.2. Post-harvest management:

Leaves are dried in shade and the dried leaves are packed in polythene bags. The moisture content of the dry leaves should be less than 8% to prevent deterioration.

1.7.3.3. Yield and cost of cultivation:

About 1250 kg of dry-weight leaves can be obtained per hectare every three months. The approximate per hectare cost of cultivation is accounts to Rs 25,000 / ha.

1.7.4. Market trend – 2006/07:

1.7.4.1. Market price: Rs 50 / kg (dry leaves)

1.7.4.2. Market demand: 1 t / year (Anonymous, 2008).

- Recent market price during 2014-2015 is Rs. 45-90 / Kg (dry leaves with < 8 % M.C. %).
- Market demand in India is about 0.5 -1.0 t / year (Rathod and Rajput, 2015).

1.8. Propagation:

It can be propagated both by seeds as well as vegetative means.

1.8.1. Seed Propagation:

Flowering and fruiting takes place during July-December. Seeds are collected during February-April. In June, seeds mixed with fine sand are directly sown into polybags. The suitable soil medium for polybags is (FYM: Sand: Clay in the ratio of 2:1:1). About 40 percent germination is reported to occur in within 7 to 10 days.

1.8.2. Vegetative Propagation:

1.8.2.1. Through cutting:

Semi-hardwood, young, terminal shoot cuttings of 15 to 20 cm length are planted either at the place near by a tree or in polybags. The latter is transplanted after sufficient rooting takes place. Cuttings sprout in 6 days and about 60 percent of the cuttings sprout. Planting of cuttings with the onset of rains, is preferable, provided, care is taken to avoid water logging at the planting site.

1.8.2.2. Through apical shoots:

The apical shoots can also be propagated in a mist chamber. They are covered with soil and a light weight is imposed on them. Roots emerge in about 30 to 40 days.

1.9. Pharmacognosy of *Gymnema sylvestre*:

1.9.1. Microscopic characters:

1.9.1.1. Lamina:

The epidermal cells of lamina are square shaped with outer convex wall and thin cuticle, when viewed transversally, epidermal cell surface are interrupted with trichomes, which are uniseriate, multicellular with 2-5 celled, present in abundance on both the surfaces. Single layered closely arranged palisade cells are, present just below the adaxial epidermal layer V.B. are amphicribal and the mesophyll is 3-5 celled thick (Agnihotri *et al.* 2004; Anonymous 2003; Madhurima *et al.* 2009).

1.9.1.2. Stem:

The T.S. of stem is circular in out line. The epidermis is barrel shaped and thick walled. Trichomes are multicellular, uniseriate. The cork is 3-5 layered thick and cortical cells are laterally elongated and collenchymatous. The Phloem is well developed comprising of large sieve plates, companion cells and phloem parenchyma. Xylem is in the form of a continuous cylinder interupted by narrow medullary rays. The epidermis is conspicuous and the pericycle is broad (Agnihotri *et al.* 2004; Madhurima *et al.* 2009).

1.9.1.3. Petiole:

Transverse Section of petiole is horse shoe shaped. The epidermis is barrel shaped single layered, thick walled covered with uniseriate, multicellular non glandular trichomes. The cortex is collenchymatous with 3 amphicribal Vascular Bundles. Well developed phloem consists of sieve tubes, companion cells and phloem parenchyma.

The xylem consists of vessels, tracheids and tracheidal fibres. Starch grains are polygonal, simple or compound in two or many groups (Saneja *et al*. 2010; Agnihotri *et al*. 2004; Madhurima *et al*. 2009).

1.9.1.4. Leaf Powder:

Powdered leaf / leaves are slightly yellowish green in colour, bitter in taste with pleasant aromatic odour. On microscopic examination, it shows thick walled, uniseriate, multicellular trichomes, anomocytic stomata, idioblast with rosette crystals of calcium oxalate, starch grains, remnants of collenchymatous and parenchymatous cells; vessels, tracheids, tracheidal fibres, bast fibres and sieve plates (Agnihotri *et al.* 2004; Zhen *et al.* 2001 and Madhurima *et al.* 2009).

1.9.2. Substitutes and adulterants:

It is commonly adulterated with Bitter melon, Cinnamon, Chromium, Zinc, Biotin, Banana plant and huckleberry or these are used as substitute of *G. sylvestre* (Saxena and Vikram, 2004).

1.9.3. Macroscopic characters:

Leaves are green in colour and stem is hairy and light brown. Leaf is 2-6 × 1-4 cm. dimensions. The leaves are simple, petiolate, rounded to cordate base, margin entire opposite with acute apex, reticulate venation, pubescent on both the surfaces. The odour is characteristics and taste of leaf is slightly bitter and astringent. It also possesses remarkable property of paralyzing the sense of the taste for sweet substances for few hours (Madhurima *et al*. 2009; Agnihotri *et al.* 2004).

1.10. Objectives of the study:

I. To study the effect of different PGR's on growth and quality of *Gymnema sylvestre* R. Br.

II. To study the effect of different PGR's on rooting of *Gymnema sylvestre* R. Br.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Brief review of research work:

During the course of the experiment conducted on Madhunashini – *Gymnema sylvestre* R. Br., following mentioned literature survey was done for proper understanding and thorough insight into the objectives was set during initiation of the research work.

2.1. *Gymnema sylvestre* R.Br.

Rao *et al.* (2000) conducted an experiment on Macro-propagation of medicinal plants *viz*., *Andrographis paniculata* Nees., *Gymnema sylvestre* R.Br., *Hemidesmus indicus* R.Br., *Tinospora cordifolia* Willd. and *Tinospora tomentosa* Colebr. at Regional Forest Research Centre, Rajahmundry, Andhra Pradesh using root inducing hormones (IBA, NAA, IAA). The study indicated that these plants can be successfully propagated without any hormone (>70 % rooting), but the rooting was slow and not profuse. The hormonal influence on rooting of above mentioned medicinal plants along with different concentrations of various rooting hormone was studied. In case of *A. paniculata,* quicker rooting was noticed in IBA-100 ppm. In *G. sylvestre and H. indicus* maximum rooting was observed in IBA- 200 ppm with 96 % in 21 days and 100% in 15 days, respectively, while in the case of *T. cordifolia* and *T. tomentosa,* ideal rooting hormone concentration was IBA- 200 ppm or IBA- 300 ppm and IBA- 200 ppm respectively.

Somashekhar and Sharma (2002) at FRLHT, Banglore worked on vegetative propagation of *Gymnema sylvestre* R. Br. using semi hardwood cuttings of 6-8 inches (10-15 cm) long procured from the

apical shoots. These cuttings were planted in poly bags and transplanted after sufficient rooting. They recorded about 60 % rooting within 6 DAS.

Arunakumara (2004) conducted trials on vegetative propagation of *Gymnema sylvestre* R. Br. at Department of Civil Engineering, University of Moratuwa, Sri Lanka. Three separate experiments were carried out using stem cuttings, double nodal semi-hard wood cuttings were rooted in polybags filled with different rooting media including sand, a mixture of sand and top soil (1: 1), a mixture of sand, top soil and compost (1:1:1) and top soil alone to investigate the effect of media on rooting. Hard wood, semi-hard wood and soft wood cuttings were planted in polybags filled with a mixture of sand, top soil and compost (1:1:1), to determine the effect of maturity of cuttings on rooting. Effect of watering on rooting of cuttings was also investigated using semi-hard wood cuttings planted in polybags containing potting mixture of sand, top soil and compost (l: 1: 1) with three watering frequencies. Results showed that germination percentage of seeds was significantly ($p \leq 0.05$) high (92 %) in coir dust, whereas the lowest germination percentage (28 %) was observed from top soil media. There were no significant ($p \leq 0.05$) differences in germination of seeds throughout the first two months of storage under normal condition. Results of the vegetative propagation studies revealed that a mixture of sand, top soil and compost (1:1:1) was the most suitable rooting media for *Gymnema* cuttings, whereas the semi-hard wood cuttings rooted significantly ($p \leq 0.05$) higher than the hard wood and soft wood cuttings. With regard to the watering frequency, cuttings watered once in two days rooted and performed significantly ($p \leq 0.05$) better than the other treatments.

It was also concluded that *Gymnema sylvestre* R. Br. can be propagated by means of both sexual and asexual methods. Semi-hard wood cuttings, which appear to be more amenable to rooting and potting

mixture of sand, top soil, and compost watered once in two days, showed the best results, while high germination percentage of seeds could be obtained within first two months of storage.

Arunakumara and Subasinghe (2004) studied seed germination dynamics of *Gymnema sylvestre* R. Br., as influenced by sowing media and storage period at Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka. Uniform, viable seeds were placed in Petri dishes filled with different media (i.e sand, coir dust, top soil and 1:1:1 mixture of sand, top soil and coir dust) to study the effect of media on germination. A Petri dish with a moist filter paper was used as a control. The highest germination (92 %) was recorded in coir dust, followed by control (68 %). There was no significant ($P<0.05$) reduction in germination within first 2 months of storage. Authors further concluded that, coir dust is the best media for seed germination of *Gymnema sylvestre* and seeds should not be stored for more than two months to ensure higher germination.

Saraswathy *et al.* (2004) studied the effect of IBA on rooting in *Gymnema sylvestre* R. Br. at Horticultural College and Research Institute, TNAU, Coimbatore. Among the three types of cutting tried, terminal cutting registered higher percentage of rooting i.e. 49 %, 37 % and 34 % during the month of June, July and August, respectively. The terminal cuttings were treated with IBA at 700 ppm, the percentage of rooting recorded ranged from 72 % to 86 %. The rooting percentage ranged between 50 % to 66 % and 10 % to 12 % when middle and basal cuttings were treated with IBA- 700 ppm, respectively.

Saraswathy *et al.* (2004) also studied the *in vitro* propagation of *Gymnema sylvestre* R. Br. at Horticultural College and Research Institute, TNAU, Coimbatore. Stem and nodal segments were used as explants. MS

media fortified with different growth regulators *viz*., Auxins (NAA and IAA), Cytokinins (KN and BAP) and GA_3 were used to determine their effect on *in vitro* propagation of *G. sylvestre*. The maximum culture establishment (64.5 %) and bud break in established culture (35.2 %) was recorded in combination of KN (0.4 ppm) + IAA (2.0 ppm). Whereas highest production of healthy shoots (33.3 %) were obtained with combination of KN (0.4 ppm) + BAP (4.0 ppm), respectively.

Tyagi (2005) reported that the semi-hardwood young cuttings of *Gymnema sylvestre* R. Br. with 15-20 cm length planted with the onset of monsoon are suitable for good macropropagtion. He recorded 60 % sprouting within 6-7 DAS.

Arunakumara *et al.* (2006) conducted research on healthy, double nodded cuttings made from the mature plant stock at Department of Crop Science and Soil Science, University of Ruhuna, Kamburupitiya. Sri Lanka. The cuttings were taken from pre-flowering (T_1), flowering (T_2) and post-flowering (T_3) stages and were stuck into preformed holes in poly bags filled with moistened rooting medium which consisted of sand, top soil and compost (1 :1: 1 by volume). They were placed in a shade house and watered once a day. Assessment for rooting was initiated after 75 DAS. The survival percentage was non significant ($p \sim 0.05$) between cuttings taken from the pre-flowering (92 %) and post-flowering (87 %) stages. No significant ($p \sim 0.05$) differences were recorded in the percentage of callused and rooted cuttings between the treatment (T_1) and (T_3). However, number of roots and length of the longest root per cutting were significantly ($p \sim 0.05$) higher in the treatment (T_1) in comparison to other treatments. Further, treatment (T_2) showed minimum values for all the parameters assessed; indicating that the physiological status of the

stock plant at the time the cuttings are excised is of vital importance for the initiation of rooting.

Gopi and Vatsala (2006) standardized *in-vitro* technique for *Gymnema sylvestre* R. Br. at Shri AMM Murugappa Chettiar Research Centre, Tharamani, Chennai, Tamilnadu. Callus culture were initiated from nodal segments and leaf explants of *Gymnema sylvestre* R. Br. on MS (1962) medium containing basic salts and 30 g/l sucrose supplemented with different concentrations (0.10, 0.25, 0.5, 1.0, 2.0 and 5.0 mg/l) of 2,4-D, NAA, IAA, IBA, KN and BA. Callus induction was observed in 0.5 mg/l of 2, 4-D supplemented medium for both nodal and leaf explants. At the initial stage, some parts of explants enlarged and gave rise to pale yellowish calli after 2-3 weeks of incubation. The harvested cell biomass was subjected to extraction of active principles. In this study, cell biomass extracts were compared with extracts from leaves of naturally growing *Gymnema* plants. HPLC analysis of these extracts showed that the main components of the active principles namely Gymnemic acids and Gymnemagenin were present in sufficiently large amounts in the cultured undifferentiated cells.

Madhavan and Manivannan (2007) studied the effect of PGR's on rooting of *Gymnema* cuttings. Experiment was conducted in the Department of Horticulture, Faculty of Agriculture, Annamalai University. Softwood cuttings of *Gymnema*, 10-15 cm long with 1 cm diameter were treated with IAA (T_1-600, T_2-800, T_3-1000 ppm), IBA (T_4-600, T_5-800, T_6-1000 ppm) and NAA (T_7-600, T_8-800, T_9-1000 ppm), respectively. Treated cuttings were planted in polythene bags containing sterilized sand as rooting medium. The different traits taken for investigation were rooting percentage, number of leaves per cutting, length of shoots, days taken for rooting, days taken for sprouting and

field survival percentage. Out of the all treatments, T_5 (IBA- 800 ppm) performed better recording highest rooting percentage (75.12 %), Days taken for rooting (29.15), Number of leaves / cutting (14.54), shoot length (52.46 cm) and field survival percentage (91.19 %) which was followed by treatment T_7 (NAA 600 ppm).

Karthic and Seshadri (2009) investigated the growth and rooting of *Gymnema sylvestre* R. Br. through Hydroponics at Sri AMM Murugappa Chettiar Research Centre, Taramani, Chennai, India. A plastic tube, with polyethylene cover, containing $1/10^{th}$ strength of MS salts supplemented with IBA at different concentrations (0.5, 1.0, 2.5 mg/l) was studied. Medium containing 0.5 mg/l of IBA produced highest rooting (66 %) with 96 % survival. This protocol will serve as an alternative to the existing *in vitro* and clonal multiplication protocols.

Sharma and Bansal (2010) conducted research on the *Gymnema sylvestre* R. Br. a very important medicinal plant used in many formulations mainly for the treatment of diabetes at Department of Biological Sciences, Rani Durgavati University, Jabalpur, Madhya Pradesh. The extensive use of *G. sylvestre* R. Br. has resulted in its over-exploitation and the plant is rare in many states of India. In present study multiple shoots were regenerated using apical bud as explants. Several concentrations of cytokinins *viz*., KN and BAP were attempted with MS medium for efficient shoot induction. Highest shoot frequency was obtained in MS medium fortified with BAP (4.44 μM) and KN (4.64 μM) with 3 % sucrose. Shoots obtained were rooted using half strength MS media with IAA 85 % of rooted shoots survived in the field.

Sundharaiya *et al*. (2010) studied the effect of growth regulators on the propagation of Sarkaraikolli (*Gymnema sylvestre* R. Br.),

medicinal coleus (*Coleus forskohlii* Briq.) and Thippili (*Piper longum* L.) at Agricultural College and Research Institute, Killikulam. Three types of cuttings (terminal, middle and basal) were taken as treatments. They concluded that among the three different types of cuttings, basal cutting showed higher percentage of rooting (72.54 %), followed by the middle cuttings (47.64 %) in *Gymnema*. Among the growth regulators, IBA-1000 ppm gave higher percentage of rooting (64.93 %) in all the three types of cuttings followed by IBA-500 ppm and NAA-1000 ppm, respectively in comparison to control (44.39 %). In *Coleus,* the terminal cuttings gave higher percentage of rooting (74.62 %), followed by the middle cuttings (62.87 %). Among the treatments of various plant growth regulators, the plants treated with IBA 500 ppm gave higher percentage of rooting (81.75 %) in all the applied three types of cuttings. Among the two types of growth regulators, IBA was found better w.r.t highest survival percentage, maximum number of roots, higher root length and shoot length in comparison to NAA and control in the case of *P. longum***.**

Shrivastava and Singh (2011) worked on the nature of the explants, type of medium, PGR's and anti-oxidant property markedly influenced during *in vitro* propagation of *Gymnema sylvestre* R. Br. (gudmar) at Department of Botany, Govt. Motilal Vigyan Mahavidyalaya, Bhopal. Propagation of plant is often difficult, expensive and even unsuccessful. Tissue culture method offers an alternative means of vegetative propagation, clonal propagation through tissue culture popularly called as micropropagation can be achieved in a short time and space. MS basal medium supplemented with various concentrations of BA- 0.5, 1.0, 1.5 and 2.0 (mg/l), sucrose and agar was used in the study. The effect of BA was found to be highest in the concentration of 1.5 mg/l

recording bud break number up to 10 and at lowest concentration of 0.5 mg/l recording bud break up to 3, respectively.

Lal and Jha (2012) carried out research on softwood, semi hardwood and hardwood cuttings of *Gymnema sylvestre* R. Br. at Biotechnology Laboratory, University Department of Botany, Ranchi University, Ranchi. The cuttings were treated with different concentrations of IAA (1000 ppm, 1500 ppm and 2000 ppm), IBA (1000 ppm, 1500 ppm and 2000 ppm) and NAA (1000 ppm, 1500 ppm, 2000 ppm), respectively using the 'quick dip' method and pregnant cow's urine for 5 minutes plus a control and rooted under mist chamber and open conditions. Data were recorded for rooting percentage, longest root length, number of new leaves and days taken for sprouting. In general, semi hardwood cuttings treated with 1000 ppm IBA gave the highest rooting percentage (68.50 %), longest root length (30.25 cm) and number of new leaves. The hardwood cuttings treated with 1000 ppm IAA and 2000 ppm IBA gave the earliest days taken for rooting and sprouting, respectively. The hardwood cuttings treated with 1500 ppm IAA or 2000 ppm IBA gave the highest field survival. Rooting was slow in NAA and in the absence of hormone treatment i.e control. It was concluded that *Gymnema sylvestre* can be successfully propagated vegetatively by treating with IBA, IAA or NAA, for large-scale multiplication under the circumstances when seed availability is low.

Pandey (2012) carried out field experiments during 2004-2007 to develop some important aspects of cultivation techniques of *Gymnema sylvestre* R. Br. to standardize seed germination, vegetative propagation and cultivation practices of the plant at Non Wood Forest Produce Division, TFRI, Jabalpur, Madhya Pradesh. The study revealed that the species can be cultivated successfully by seeds as well as by rooted

cuttings at a spacing of 50 x 50 cm. It was also observed that pre-treatment of seeds in cold water for 24 h improves the seed germination. For vegetative propagation, hard wood cuttings of 10-15 mm diameter having three nodes were found most suitable. Months of March and July were most suitable for vegetative propagation. Among hormonal treatments, dipping of cuttings in 500 ppm IBA solution for 30 min resulted in maximum rooting (52.50 %). Application of 4000 kg FYM / ha was found promising in terms of growth and yield. Total yield of dry leaves was found to be 1.5 t/ha.

Rani *et al*. (2012) conducted research on the macropropagtion of *Gymnema sylvestre* R. Br. at Department of Botany, Osmania University, College for Women, Koti, Hyderabad. Tender stems and older cuttings treated with IAA-500 ppm gave better rooting and sprouting in comparison to other IAA concentrations used.

Arunakumara *et al.* (2013) gave an exhaustive review on mass multiplication of an important medicinal plant *Gymnema sylvestre* R. Br. at Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka. They concluded that despite many aspects including physiochemical characterization, biological evaluation, and molecular mechanism of action(s) of isolated phytoprinciples have been extensively studied; little attention has so far been paid on propagation and conservation of the species. The natural habitats of the species are tremendously under pressure due to unsustainable wild harvest and the poor natural regeneration. No alternative mode of multiplication is found under natural conditions, thus the species is thoroughly dependent upon the seed germination for survival. However, vegetative propagation through stem cuttings and micro-propagation has proved to be viable sources of planting materials. Since, *in vitro* extraction of principal active ingredients could also be

possible, propagation through tissue culture is commercially attractive too.

2. 2. Other Medicinal Plant species:

Gupta (1989) studied the effect of intermittent mist and auxins on the rooting potentiality of *Hibiscus rosa-sinensis*, L. Cv. 'Snow flake' by semi-hardwood cuttings at Plant propagation and Floriculture Lab., NBRI, Lucknow, U.P. He observed that treatment IBA-4000 ppm induced higher percentage of rooting (80.00%), number of roots (18.25) / rooted cutting also produced longer roots (73.23 mm).

Sehrawat *et al*. (2001) at Maharshi Dayanand University, Rohtak studied the effect of various concentrations of IAA on sprouting of *Rauvolfia serpentina* L. and concluded that, use of 40 ppm IAA is best for higher rate of sprouting. Almost 65 to 70 % stem cuttings sprouted when treated with IAA-40 ppm concentration.

Shukla *et al.* (2001) investigated the vegetative propagation through air layering technique in medicinal plant *Leptophonia reticluata* Retz., known as Jivanti in Ayurveda at Laboratory of Plant Ecology, Department of Botany, Jai Narayan Vyas University, Jodhpur. The results revealed that Ceradik powder treated twigs produced profuse and early rooting with higher survival percentage. In Ceradik treated twigs a 100 % rooting was recorded, whereas in control it was up to 80 %. The mortality rate was very high in control (90 %) as compared to Ceradik (30 %) treated experiments. The survival percentage of air layered twigs after transplanting in the field was 70 % and 10 % in Ceradik treated twigs and control, respectively after six months of setting the experiments. The Ceradik treated plants produced larger sized leaves as compared to those of control.

Alamgir and Ahamed (2005) carried out research on growth and phytochemical investigations on *Rauvolfia tetraphylla* L. propagule. Root formation and successful propagule development from cuttings of root, stem and root-stem junction were 62 %, 42 % and 78 %, respectively. IBA, NAA and 2, 4-D at low concentrations stimulated root formation and propagule development from stem cuttings. 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) and NAA (66% at 10 ppm). IBA and NAA in combination had little positive effect on rooting.

Deshpande (2005) investigated the performance of stem cuttings of *Rauvolfia tetraphylla* L. The hard wood part of the stem was cut into pieces of 20-22 cm each having at least 3 internodes. The lower end of the cuttings was treated with 30, 50 and 70 ppm IAA for 12 to 14 hrs. Although the sprouting was seen within 3-4 days, but rooting initiated 30 to 45 DAS. The cuttings performed best with 50 ppm IAA concentration.

Ramulu *et al.* (2005) standardized method of vegetative propagation for *Hemidesmus indicus* R. Br. - a highly valued Indian medicinal plant commonly called as Indian Ginseng by stem cuttings at Department of Botany, Sri Krishnadevaraya University, Anantpur, Andhra Pradesh. Healthy vines were cut into 10-15 cm bits having 5-10 nodes, and the cut ends were treated with 0.3 % Dithane M-45 fungicide for 30 min. Later, the basal part of the cuttings was dipped in solutions containing IAA, IBA and NAA, each at 500, 1000, 2000 and 5000 ppm for 2 min. Leaves on stem cuttings turned pale and got shaded after 3-4 days and axillary buds sprouted after 5-7 days. These buds grew into young shoots. Among the three PGRs, IAA gave best response, followed by IBA and NAA. The roots produced from the stem cuttings treated with IAA and IBA were healthy, however, the percentage of response was

higher in the IAA-treated cuttings. The percentage of response was very poor in the NAA-treated cuttings, and the roots were short and stout. The shoot development was also stunted. After one month, the cuttings were removed from the root trainers filled with vermiculite and sand (3:1), and planted either in pots or in soil. Approximately 90 % of the transplanted plants survived.

Dhuria (2008) worked on the rooting behaviour of Sarpagandha-*Rauvolfia serpentina* L., using branch cuttings under mist condition at Department of Forestry, Wildlife and Environmental Sciences, Guru Ghasidas University, Bilaspur, Chhattisgarh. He revealed that the IAA treated cuttings gave maximum rooting in 500 ppm concentration (100 %) whereas 2000 ppm and 8000 ppm concentrations resulted in 87.5 % rooting and maximum numbers of roots were recorded at 10000 and 6000 ppm, respectively. Whereas, maximum root length was obtained in 500 ppm treated cuttings. With IBA treatments, maximum (100 %) rooting was obtained in 3000 ppm concentration and 500 ppm, 1000 ppm and 4000 ppm concentrations resulted in 85.7 % rooting and maximum number of roots and root length were recorded in 10000 and 8000 ppm concentrations, respectively. With NAA treated cuttings, maximum rooting was obtained at 500 ppm, 1000 ppm and 4000 ppm concentrations (100 %). Maximum numbers of roots were observed in 2000 ppm, 3000 ppm and 4000 ppm NAA and maximum length of roots were recorded in 500 ppm and 4000 ppm NAA treated cuttings, respectively.

Savithramma *et al*. (2008) carried out research on the effect of auxins on *in-vivo* multiplication of *Decaschistia crotonifolia* Wight & Arn. – an important multipurpose medicinal plant taxa. They observed that the maximum rooting percentage was recorded with IBA, followed

by IAA, whereas minimum percentage (2.5 %) was observed with NAA. IBA-1500 ppm was found as the best concentration for mass multiplication of *D. crotonifolia.*

Bhandari *et al*. (2009) conducted a research on physiological effect of auxins on growth characteristics and productive potential of *Verbascum thapsus* L. - A medicinal plant at Department of Botany, H.N.B. Garhwal University. Effect of foliar spray of different concentration of auxins (IAA-50 ppm, 100 ppm and 200 ppm), 2,4-D (5 ppm, 10 ppm and 20 ppm) on augmentation of growth characteristics was studied. Observations revealed that IAA-50 ppm increased the shoot and root length, number of branches, nodes and leaves; while IAA-200 ppm was found to be the best effective for leaf area and number of flower and fruits. 2, 4-D treated plants exhibited much reduction in number of flowers, indicating the adverse effect of this growth regulator towards growth. IAA-50 ppm resulted in maximum productivity of above ground and underground biomass compartment of *V. thapsus* on annual biomss.

Ingle and Venugopal (2009) studied the effect of different growth regulators on rooting of *Stevia rebaudiana* cuttings. The experiment was carried out during the year 2007 between the months of October-November at Agricultural Research Station, Dharwad. They revealed that the rooting percentage was highest (92 %) with the treatment of IBA - 500 ppm followed by IBA - 400 ppm (90 %). This treatment registered maximum number of roots, longest length of roots, increased length of cuttings and highest number of leaves.

Baque *et al*. (2010) studied growth, secondary metabolite production and antioxidant enzyme response of *Morinda citrifolia* L. a potential nutraceutical drink used in cancer at Research Center for the

Development of Advanced Horticultural Technology, Chungbuk, National University, Cheong- ju, South Korea. Adventitious root affected by auxins and cytokinins. They concluded that the root (fresh weight and dry weight) accumulation was enhanced at 5 mg 1^{-1} IBA and at 7 and 9 mg 1^{-1} NAA. On the other hand, 9 mg 1^{-1} NAA decreased the anthraquinone, phenolic and flavonoid contents more severely than 9 mg 1^{-1} IBA.

Minakshi and Lingakumar (2011) conducted study on the role of IAA and 2, 4-D on growth and biochemical constituents in vegetatively propagated *Mentha arvensis* L. at Ayya Nadar Janki Ammal College, Sivaski. Stem cuttings of uniformed size were selected and pre-treated with IAA and 2, 4-D at various concentrations (5.0, 7.5 and 10.0 ppm). There was an increase in overall vegetative growth measured in terms of shoot and root length, total fresh weight and dry weight, leaf area and leaf number / plant. Both the hormones applied individually resulted in appreciable increase especially at 7.5 ppm. In the present study, 2, 4-D proved to be best less effective than IAA. At low concentration of IAA and 2, 4-D, an increase in growth parameters was obtained. There was a clear hike in dry weight of the seedlings after hormonal treatment indicating the effective role of IAA on apical growth. IAA and 2, 4-D treatment caused an increase in internodal length and leaf area. With respect to unit fresh weight basis, the total chlorophyll content was found to increase at 7.5 ppm IAA.

Mostafa and Abou Alhamd (2011) studied the effect of GA_3 and IAA on growth and accumulation of phytochemical composition in *Balanites aegyptica* L. plants at South Valley University, Qena, Egypt. The study resulted in significant increase in the germination percentage, plant height, number of branches and leaves, total chlorophyll content, dry weight of vegetative growth and protein, carbohydrates, alkaloids,

tannins and saponin content. GA_3-50 ppm and IAA-2000 ppm gave best results by increasing the growth and phytochemical, especially alkaloids, tannins and saponin content.

Sharma and Kumar (2011) studied the effects of PGR's and chemical fertilizers on growth and productivity of two highly valued medicinal plants; *Chlorophytum tuberosum* L. and *Pergularia daemia* Forsk. at Department of Botany, Poddar International College, Jaipur, India. Different concentrations of plant growth regulator affected both these plants. For *C. tuberosum*, higher concentrations of IAA and IBA above 100 ppm resulted in stunted growth as compared to lower concentrations. The best result was obtained at 50 ppm concentration of IBA for *C. tuberosum*. Similarly for *P. daemia*, among the various growth regulator treatments, the best results were obtained by application of IBA-100 ppm. However, IAA-50 ppm resulted in significant overall growth of *P. daemia.*

Sofi *et al*. (2011) studied the effect of plant growth regulators on rooting and sprouting behaviour of cuttings of *Derris indica* (Lamk.) Bennet. / *Pongamia pinnata* (L.) Merr. / *Milletia pinnata*- medicinal tree whose fruit oil is one of the best remedy for skin diseases at ASPEE College of Horticulture and Forestry, NAU, Navsari. They concluded that among the various concentrations of NAA, IBA and their combinations applied as treatments, 2000 ppm IBA proved to be the best treatment for all the aspects of plant characters of hardwood cuttings in *D. indica*.

Yadav *et al.* (2011) conducted a research on role of different rooting hormones in *Adhatoda vasica* L., and *Barleria prionitis* L. at Molecular Biology and Seed Technology Laboratory, Govt. Motilal Vigyan Mahavidyalaya. Without stimulation, rooting in these medicinal plants occurred, but after seven weeks and after stimulation, rhizogenesis

takes up to 2 to 3 weeks. Rootex-c, exhibited a significant stimulating effect on rhizogenesis, and effect of them declined in the order Rootex-a, Rootex-b and Rootex-c, respectively. The percentage of rooting of *A. vasica* and *B. prionitis* was higher in preparations of 30gm / 50ml DW than that in other preparations in both cases of Rootex-a and Rootex-b. Overall, Rootex-c is poor rooting factor in all the types of preparations.

Sevik and Guney (2013) studied the effect of IAA, NAA and GA_3 on rooting and morphological features of *Melissa officinalis* L. stem cuttings at Kastamonu University, Turkey. This study analyzed the potential of producing *M. Officinalis* using stem cuttings. Four different hormones (IAA, IBA, NAA and GA_3) were applied to the cuttings, with and without buds, in two doses (1000 mg/l and 5000 mg/l) and after 60 days, 10 morphological characteristics of newly generated plants were detected and a statistical analysis was carried out. The results revealed that the cuttings with at least one bud must be used in order to produce *M. Officinalis* using stem cuttings. Even though the auxins (IAA, IBA and NAA) do not have an apparent effect on rooting percentage, these hormones were detected to affect the morphological characteristics of the newly generated plants, especially root generation while GA_3 application has a considerable effect on stem height or plant height.

Khan *et al*. (2015) studied the effects of plant growth regulators on growth and essential oil content in Palmarosa (*Cymbopogon martinii* Roxb.) at department of Bioengineering, Integral University, Lucknow. They observed that GA_3 significantly improved plant growth in *C. martinii.* Plant height, leaf area, tillers number and herbage yield showed increasing trend at 100 ppm concentration of GA_3. Similarly, chlorophyll content, protein content, NR activity and oil content also increased due to GA_3 treatment as compared to untreated plants and the increase was maximum at 100 ppm concentration. Effect of IAA and KN was similar

to GA_3, but maximum effect was observed at 50 ppm concentration. Geraniol content of the essential oil of *C. martinii* increased due to GA_3 and KN treatment, while there was reduction in geranyl acetate content.

MATERIALS

AND

METHODS

III. MATERIALS AND METHODS

The details of the materials used and experimental methods followed during the course of the present investigation are discussed in this section.

3.1 Experimental site and location

The present investigation entitled "Effect of PGR on Growth and Quality of Madhunashini–(*Gymnema sylvestre* R. Br.)" was conducted during July-2014 to Dec-2014, at the Model Nursery on Medicinal and Aromatic Plants, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, (AES III South Gujarat Heavy Rainfall Zone), which is situated 12 kms away from the West coast of the Arabian sea at an altitude of about 12 m above MSL, at 20°- 58' N latitude and 72°- 54' E longitude.

3.2 Climate and weather

The climate of experimental area is typically tropical, characterized by fairly hot summer, moderate cold winter and humid warm monsoon. In general, monsoon commences during the second week of June and ends by the second fortnight of September. Most of the precipitation is received from South West monsoon, concentrated during the months of June, July and August. The average / mean minimum and maximum temperature during the course of experiment varied from 13.2 ^{0}C to 36.4 ^{0}C and the maximum average morning RH recorded was 97 mm and minimum was 70 mm. Maximum average evening RH reported was 86 mm and minimum was 31 mm. Total rainfall during rainy season accounted to 1490 mm and Avg. 32.39 mm with total 46 rainy days. The

data of the meteorological observations recorded during the course of investigation, are presented in Annexure 1.

3.3 Soil characteristics

The soil of good quality red earth was used along with the FYM and Vermicompost in the ratio of 2:1:1 having better water holding capacity with fairly good drainage and reasonably suitable for Madhunashini propagation at Model Nursery on Medicinal and Aromatic plants, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari.

For the soil analysis, samples were taken from 9 treatments. Sample 1st taken from treatment no. 1, 2, 3; likewise sample 2nd and 3rd taken from treatment no. 4, 5, 6 and 7, 8, 9, respectively. The initial (chemical) properties of soil are presented in Table 3.1.

Table No. 3.1: Initial chemical properties of soil

Sr. No. Particulars		**Initial value**			**Method employed**	**References**
		S_1	**S_2**	**S_3**		
1.	Soil pH (1:2.5 soil: water ratio)	7.62	7.56	7.55	Potentiometric	Jackson (1973)
2.	Electrical Conductivity (1:2.5 soil: water ratio) dsm^{-1} at 20^0C	0.14 dS/m	0.21 dS/m	0.28 dS/m	Conductometric	Jackson (1973)
3.	Soil Organic carbon (%)	0.85	0.99	1.17	Walkley and Black rapid titration method	Jackson (1973)
3.A.	Soil Organic Matter (%)	1.47	1.70	2.01		
4.	Available N (kg/ha)	306.15	404.91	325.90	Alkaline permanganate oxidation	Subbiah and Asija (1956)
5.	Available P (kg/ha)	97.0	56.0	73.0	Spectrophoto-metric	Olsen *et al.* (1954)
6.	Available K (kg/ha)	782.20	1160.54	1073.18	Flamephotometric	Jackson (1973)

3.4 Experimental details

In the present experiment, two forms of PGR (auxins) *viz.*, IBA and IAA along with the control were applied in different concentrations to study their effects on growth and rooting of *Gymnema sylvestre* R.Br.

3.5 Source of cuttings and their preparation

The cuttings were procured from the Model Nursery on Medicinal and Aromatic Plants, ASPEE college of Horticulture and Forestry, Navsari Agricultural University, Navsari. The apical and semi hardwood portions of the cuttings with a length of 15-20 cm without any branches were selected with uniform thickness and 2-3 leaves per cutting with half leaves. It helps to reduce the transpiration rate.

3.6 Preparation of rooting media

Good quality Red earth was used along with the FYM and Vermicompost in the ratio of 2:1:1 and it was filled into 7' × 9' (15 x 20 cms) sized perforated polythene bags. Before planting the cuttings, media was drenched with Carbendazim (0.2%) as a prophylactic measures against fungal diseases.

3.7 Design and layout of experiment

The experiment was laid out in CRD. There were 10 treatments including control in which two PGR's IBA and IAA were used in different concentrations with three repetitions and thirty cuttings per repetition.

3.8 Treatment details:

3.8.1 Total number of treatments: 10

3.8.2 Number of Repetitions: 3

3.8.3 Experimental design: Completely Randomized Design

3.8.4 Month of planting of cuttings: July, 2014

3.8.5 Concentrations of plant growth regulators used in research work:

A. IBA concentrations

1) 500 ppm
2) 1000 ppm
3) 1500 ppm

B. IAA concentrations

4) 500 ppm
5) 1000 ppm
6) 1500 ppm

C. IBA+IAA concentrations

7) 250 ppm +250 ppm
8) 500 ppm +500 ppm
9) 750 ppm +750 ppm
10) Control

Table No. 3.2: Details of various treatment combinations applied

Treatments	Plant Growth Regulators concentration (ppm)
T_1	IBA 500
T_2	IBA 1000
T_3	IBA 1500
T_4	IAA 500
T_5	IAA 1000
T_6	IAA 1500
T_7	IBA 250 + IAA 250
T_8	IBA 500 + IAA 500
T_9	IBA 750 + IAA 750
T_{10}	Control

3.9 Preparation of plant growth regulator solution

The 1000 ml stock solutions of 5000 ppm IBA and IAA were prepared by dissolving 5 gm of IBA and IAA with addition of small quantity of NaOH pellets. Than as per the requirements of concentrations of auxins in ppm i.e IBA and IAA; solution was prepared from stock by adding water to make final volume up to 1000 ml of each PGR. e.g for the preparations of 500 ppm, take 100 ml solution and make final volume 1000 ml by adding water. Likewise all concentrations of PGR were made for experiment.

3.10 Application of treatments to cuttings and planting

The basal end of the prepared cuttings were dipped to a depth of $^{1}/_{3}$ end portion into the prepared solution for 20 to 25 minutes. The untreated cuttings, which represent the control, were similarly allowed to stand in distilled water for the same duration.

3.11 Planting of cuttings

The treated cuttings were planted in the polythene bags in net house complex. The planting of cuttings was done in slightly slanting/oblique position. Overview of experiment laid at Model Nursery on Medicinal and Aromatic Plants, NAU, Navsari (Plate 2).

3.12 Observations Recorded

The shoot and root characters were recorded periodically as per the objectives of experiment, as given below:

3.12.1 Shoot characters:

1. **Days required for sprouting (50 %)**

Days taken by cuttings to sprout in each treatment were recorded and mean number of days taken for sprouting were also worked out.

2. **Plant height (cm) at 30, 60, 90 and 120 DAP**

The plant height was measured in centimeters with the help of a scale and meter tap, after 30, 60, 90 and 120 days interval.

Plate No. 2. Overview of Experiment Laid at Model Nursery on Medicinal and Aromatic Plants, NAU, Navsari.

3. Number of branches / plant at 30, 60, 90 and 120 DAP

The data on number of branches / cutting as influenced by the application of different PGR and the number of branches / plant was measured at every 30, 60, 90 and 120 days interval.

4. Number of leaves / plant at 30, 60, 90 and 120 DAP

The data on number of leaves / cutting influenced by application of various concentrations of growth regulator along with the number of leaves per plant was recorded at every 30, 60, 90 and 120 days interval.

5. Length of shoots (cm) at 30, 60, 90 and 120 DAP

The length of shoot was measured in centimetres with the help of a scale and meter tap.

6. Leaf area (cm^2)

Leaf area was measured with the help of image analysis software image J (Abramoff *et al.* 2004).

7. Leaf morphology (Shape of the margin and apex)

Shape of leaf is elliptic-ovate; Apex is acute with rounded base and entire margin as well as smooth texture. There is variation in leaf size (length and width) amongst the treatments of various concentrations of IBA and IAA applied to that of control. No major deviations/changes have been recorded as far as leaf shape (margin) and apex is concerned.

3.12.2 Root characters

1. Length of the main root (cm)

Length of main root was measured in centimetres with the help of scale.

2. Root girth of main root (mm)

Root diameter was measured with the help of vernier calliper in mm scale and then recorded observation multiply with JI (pie) value i.e 3.14 or 22/7 to obtain root girth.

3.13 Statistical analysis and interpretation of data

The experimental data were subjected to the statistical analysis by using variance technique as described by (Panse and Sukhatme, 1967). The method of analysis of variance for CRD (Completely Randomized Design) was used. The treatment differences were tested by 'F' test of significance based on null hypothesis. The appropriate standard error (S.Em.±) was calculated in each treatment and critical difference (CD) at 5 % level of probability was worked out to compare the treatment means, where the treatment effects were significant.


EXPERIMENTAL
RESULTS

IV. EXPERIMENTAL RESULTS

The results of the experiment entitled, "Effect of PGR on Growth and Quality of Madhunashini-(*Gymnema sylvestre* R.Br.)" are discussed in this chapter. The data collected on various parameters taken for study were subjected to further statistical analysis. The ANOVA for experimental design was also carried out for all the characters under study.

1. Days required for sprouting (50 %):

The data on days taken to 50 % sprouting are summarized in Table 4.1 and graphically presented in Fig. 4.1. All the plant growth regulators treatment applied proved significantly superior over the control.

From the table and graph it can be concluded that, among the different treatment concentrations, treatment (T_5) [IAA-1000 ppm] took minimum days required for sprouting 19.67 days, which was at par with treatment (T_4) [IAA-500 ppm] 20.67 days.

Similarly, amongst all the various concentrations of IBA, IAA and combination of IBA+IAA, treatment (T_7) [IBA-250 ppm + IAA-250 ppm], (T_3) [IBA-1500 ppm] and (T_8) [IBA-500 ppm + IAA-500 ppm] resulted early sprouting in cuttings (22.33 days, 23.33 days and 23.67 days, respectively). This was followed by treatment (T_1 and T_6) [IBA-500 ppm] and [IAA-1500 ppm] 24.33 days, (T_2) [IBA-1000 ppm] 25.33 days, (T_9) [IBA-750 ppm + IAA-750 ppm]

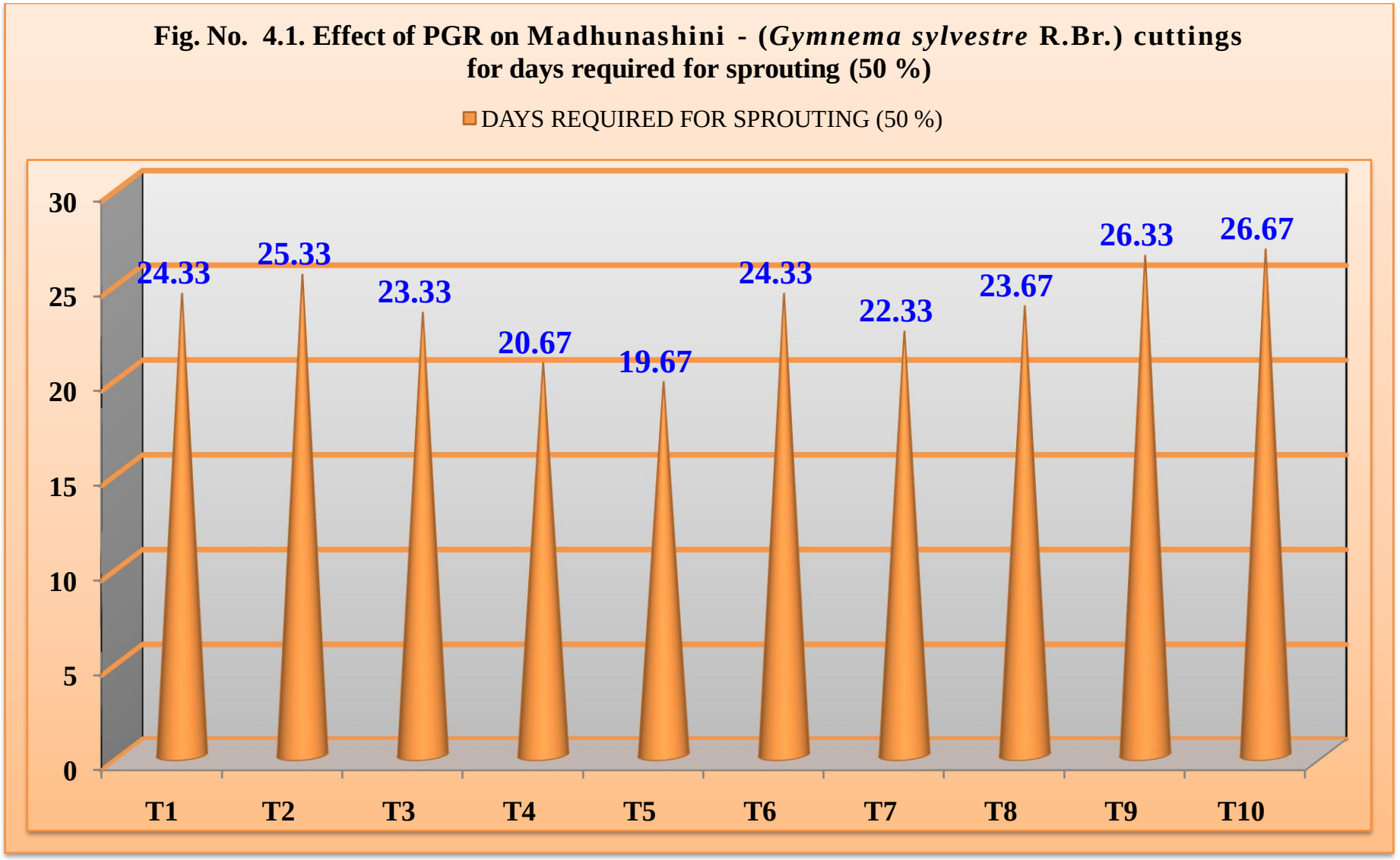
26.33 days. Overall trend was (T_5>T_4>T_7>T_3>T_8>T_1andT_6>T_2>T_9).

In comparison with applied treatment concentrations, control i.e. untreated stem cuttings (T_{10}) treatment showed maximum days for sprouting (26.67 days).

Table 4.1: Effect of PGR on number of days required for 50 % sprouting in Madhunashini-(*Gymnema sylvestre* R.Br.) cuttings.

Treatments	Number of days required for 50 % sprouting
T_1 : IBA 500 ppm	24.33
T_2 : IBA 1000 ppm	25.33
T_3 : IBA 1500 ppm	23.33
T_4 : IAA 500 ppm	20.67
T_5 : IAA 1000 ppm	**19.67**
T_6 : IAA 1500 ppm	24.33
T_7 : IBA 250 ppm + IAA 250 ppm	22.33
T_8 : IBA 500 ppm + IAA 500 ppm	23.67
T_9 : IBA 750 ppm + IAA 750 ppm	26.33
T_{10}: Control	26.67
S. Em. ±	0.380
C.D. at 5 %	1.12
C.V. %	2.78

Fig. No. 4.1. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for days required for sprouting (50 %)



ON X AXIS – TREATMENTS AND ON Y AXIS – NO. OF DAYS TAKEN FOR SPROUTING (50 %)

Plate No. 3. Effect of PGR on cuttings of Madhunashini - (*Gymnema sylvestre* R.Br.) after 30 DAP.

T_1: IBA 500 ppm

T_2: IBA 1000 ppm

T_3: IBA 1500 ppm

T_4: IAA 500 ppm

T_5: IAA 1000 ppm

T_6: IAA 1500 ppm

T_7: IBA 250 ppm + IAA 250 ppm

T_8: IBA 500 ppm + IAA 500 ppm

T_9: IBA 750 ppm + IAA 750 ppm

T_{10}: Control

Plate No. 4. Effect of PGR on cuttings of Madhunashini - (*Gymnema sylvestre* R.Br.) after 60 DAP.

T_1: IBA 500 ppm

T_2: IBA 1000 ppm

T_3: IBA 1500 ppm

T_4: IAA 500 ppm

T_5: IAA 1000 ppm

T_6: IAA 1500 ppm

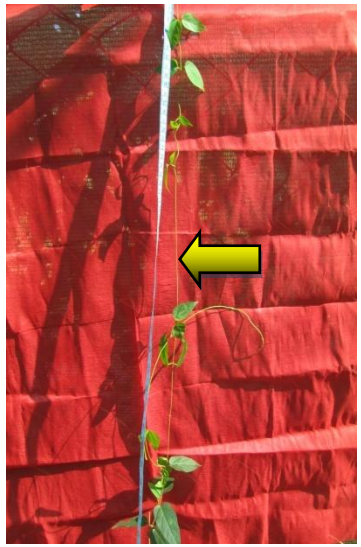
T_7: IBA 250 ppm + IAA 250 ppm

T_8: IBA 500 ppm + IAA 500 ppm

T_9: IBA 750 ppm + IAA 750 ppm

T_{10}: Control

Plate No. 5. Effect of PGR on cuttings of Madhunashini - (*Gymnema sylvestre* R.Br.) after 90 DAP.



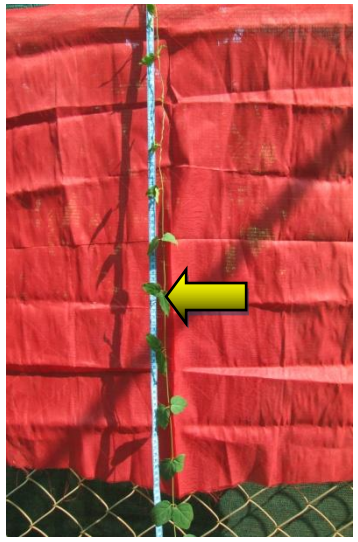
T_1: IBA 500 ppm

T_2: IBA 1000 ppm

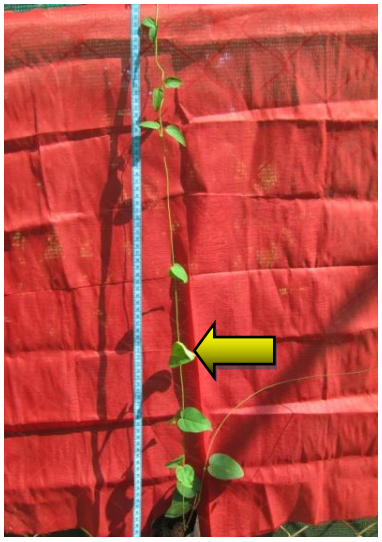
T_3: IBA 1500 ppm

T_4: IAA 500 ppm

T_5: IAA 1000 ppm



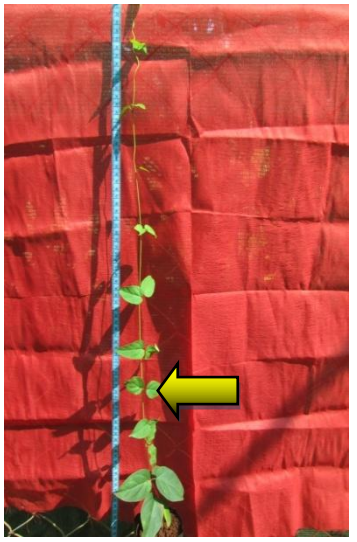
T_6: IAA 1500 ppm



T_7: IBA 250 ppm + IAA 250 ppm

T_8: IBA 500 ppm + IAA 500 ppm

T_9: IBA 750 ppm + IAA 750 ppm



T_{10}: Control

With respect to the parameter on number of days required for sprouting (50 %), best treatment is T_5 as already depicted in Table 4.1.

Overall, effect of PGR on cuttings of Madhunashini – (*Gymnema sylvestre* R. Br.) is photographically depicted in (Plates 3, 4 and 5).

2. Plant height (cm) at 30, 60, 90 and 120 DAP

The data on plant height observed at 30, 60, 90 and 120 DAP are summarized in Table 4.2 and graphically presented in Fig. 4.2. All the plant growth regulators treatment applied proved significantly superior over control.

Amongst various concentrations of IBA, IAA and their combinations applied, maximum plant height was recorded in treatment (T_5) [IAA-1000 ppm] at 30, 60, 90 and 120 DAP with a values of 8.67 cm, 47.83 cm, 111.07 cm and 117.91 cm, respectively. At 30 DAP, treatment (T_5) [IAA-1000 ppm] was at par with the treatment (T_4) [IAA-500 ppm], (T_2) [IBA-1000 ppm] and (T_1) [IBA-500 ppm] with a values of 8.32 cm, 8.12 cm and 8.03 cm, respectively and at 90 DAP, treatment (T_5) [IAA-1000 ppm] was at par with the treatment (T_4) [IAA-500 ppm] with a value of 105.45 cm. Whereas, at 60 DAP and 120 DAP treatment (T_5) [IAA-1000 ppm] was followed by treatment (T_4) [IAA-500 ppm] with values of 43.60 cm and 107.23 cm, respectively. Overall trend for the parameter after 30, 60, 90 and 120 DAP is (T_5>T_4).

Minimum plant height was recorded in the treatment (T_{10})-[Control] after 30, 60, 90 and 120 DAP with values of

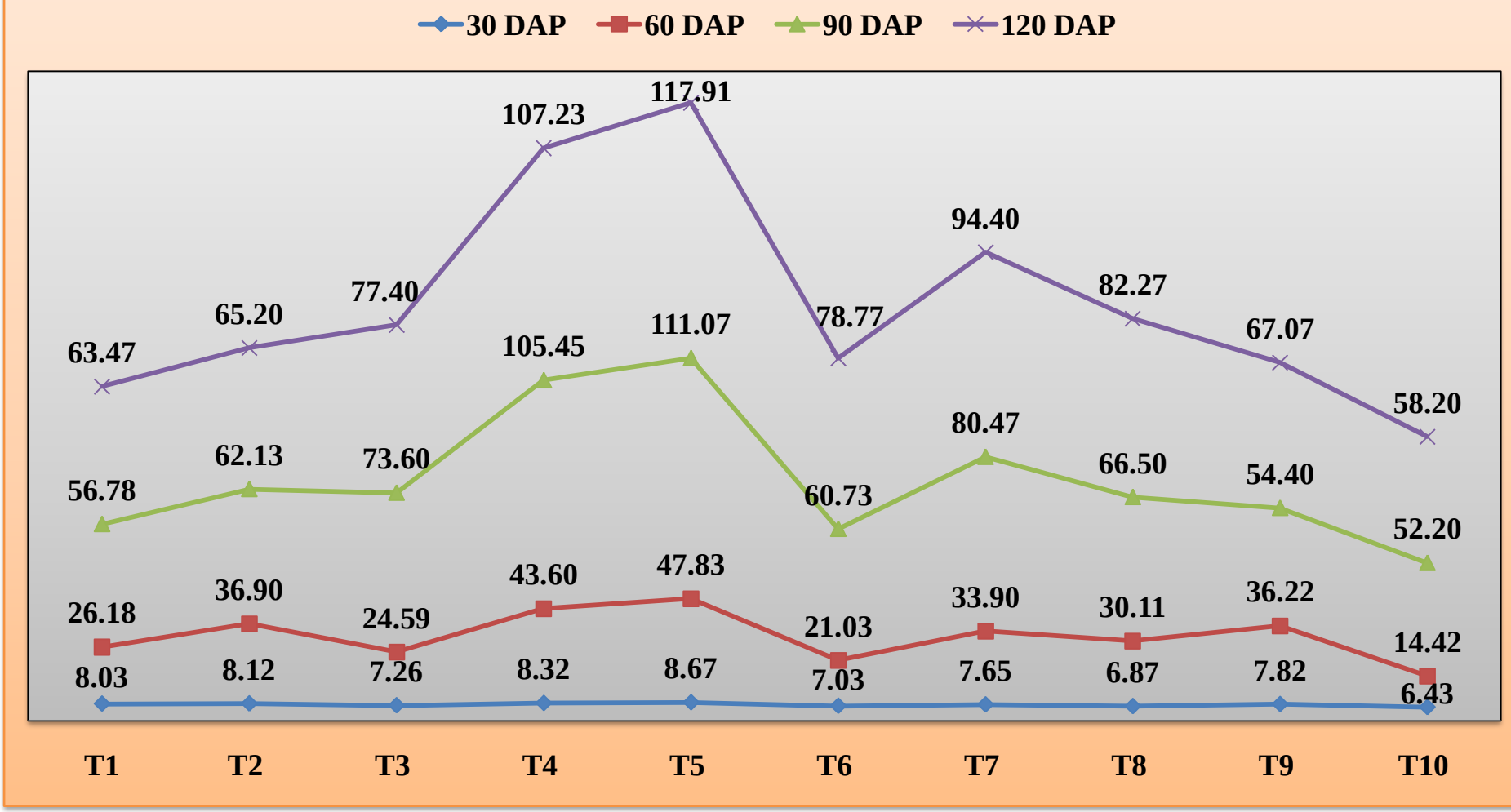
6.43 cm, 14.42 cm, 52.20 cm and 58.20 cm, respectively (Plate 6 and 7).

Table 4.2: Effect of PGR on plant height in Madhunashini- (*Gymnema sylvestre* R.Br.) cuttings at 30, 60, 90 and 120 DAP.

Treatments	Plant height (cm)			
	30 DAP	60 DAP	90 DAP	120 DAP
T_1 : IBA 500 ppm	8.03	26.18	56.78	63.47
T_2 : IBA 1000 ppm	8.12	36.90	62.13	65.20
T_3 : IBA 1500 ppm	7.26	24.59	73.60	77.40
T_4 : IAA 500 ppm	8.32	43.60	105.45	107.23
T_5 : IAA 1000 ppm	**8.67**	**47.83**	**111.07**	**117.91**
T_6 : IAA 1500 ppm	7.03	21.03	60.73	78.77
T_7 : IBA 250 ppm + IAA 250 ppm	7.65	33.90	80.47	94.40
T_8 : IBA 500 ppm + IAA 500 ppm	6.87	30.11	66.50	82.27
T_9 : IBA 750 ppm + IAA 750 ppm	7.82	36.22	54.40	67.07
T_{10}: Control	6.43	14.42	52.20	58.20
S. Em. ±	0.233	0.997	2.308	2.440
C.D. at 5 %	0.68	2.94	6.81	7.19
C.V. %	5.29	5.48	5.53	5.20

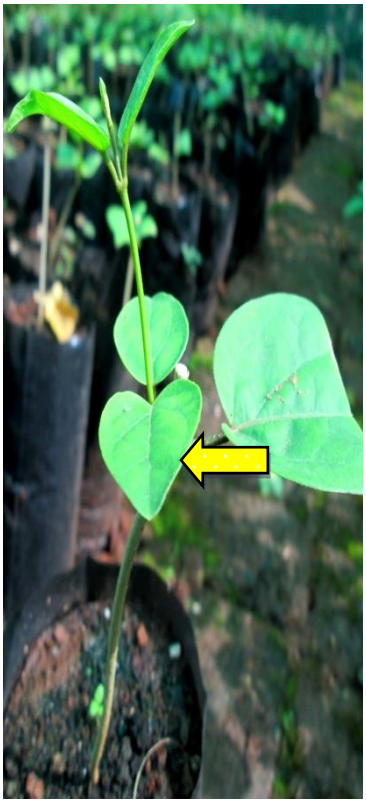
With respect to the parameter on effect of PGR on plant height, best treatment is T_5 as highlighted in the above given Table 4.2.

Fig. No. 4.2. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for plant height (cm) at 30, 60, 90 and 120 DAP



ON X AXIS – TREATMENTS AND ON Y AXIS – PLANT HEIGHT IN cms

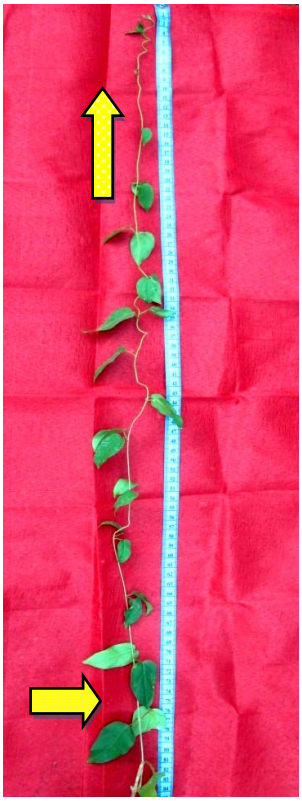
Plate No. 6. Comparison of (T_5) IAA-1000 ppm and Control in *Gymnema Sylvestre* R.Br. after 30, 60, 90 and 120 DAP



30 DAP

60 DAP

90 DAP



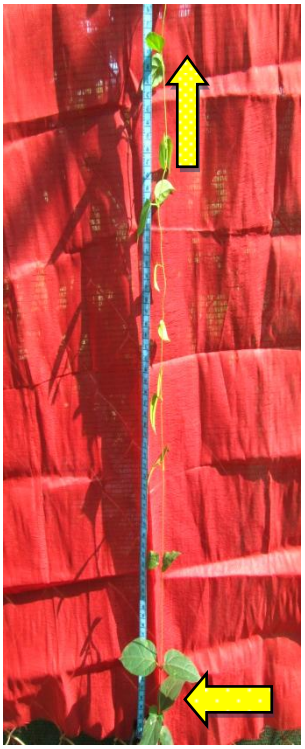
120 DAP

T_{10} - CONTROL

30 DAP

60 DAP

90 DAP



120 DAP

Plate No. 7. Comparison of (T_4) IAA 500 ppm and Control in *Gymnema Sylvestre* R.Br. after 30, 60, 90 and 120 DAP

30 DAP

60 DAP

90 DAP

120 DAP

T_{10} - CONTROL

30 DAP

60 DAP

90 DAP

120 DAP

3. Number of branches / plant at 30, 60, 90 and 120 DAP.

Number of sprouted branches from the cuttings treated with different concentrations of (IBA, IAA and combinations of IBA and IAA) was more than those under control, as can be seen from the data presented in Table 4.3 and Figure 4.3.

The results revealed that mean value of different concentrations of auxin treatments varied from that of control, treatment (T_{10}).

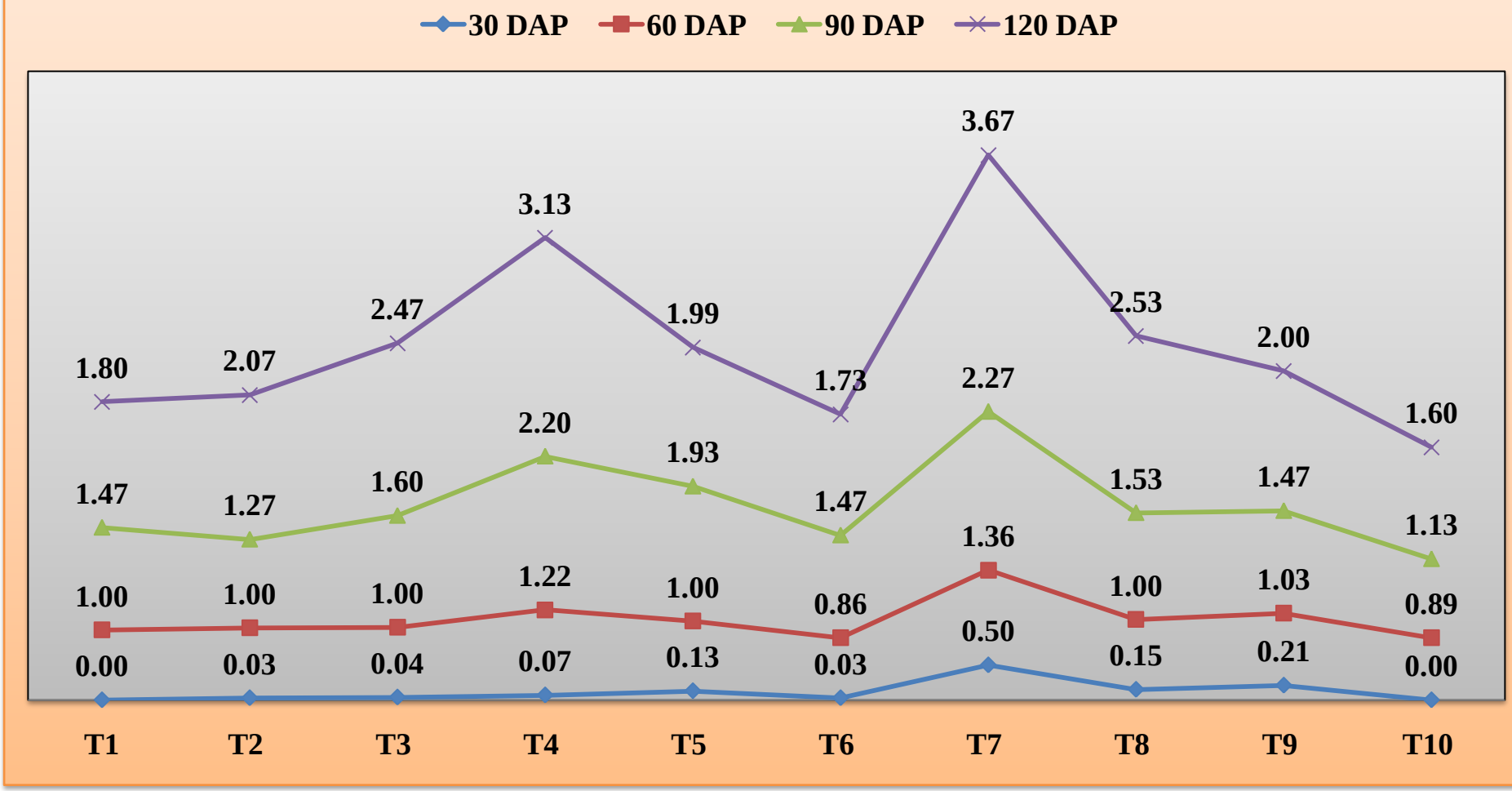
Among all the different concentrations of IBA, IAA and its combinations applied, at 30 and 60 DAP number of branches / plant was non-significant with values of 0.50 and 1.36, respectively in the treatment (T_7) [IBA-250 ppm + IAA-250 ppm]; whereas, at 90 and 120 DAP maximum number of branches / plant was recorded in the treatment (T_7) [IBA-250 ppm + IAA-250 ppm] with a values of 2.27 and 3.67, respectively. At 90 DAP, treatment (T_7) [IBA-250 ppm + IAA-250 ppm] was at par with treatment (T_4) [IAA-500 ppm] and (T_5) [IAA-1000 ppm] with number of branches 2.20 and 1.93 respectively. Whereas, at 120 DAP, treatment (T_7) [IBA-250 ppm + IAA-250 ppm] was at par with treatment (T_4) [IAA-500 ppm] with 3.13 number of branches / plant. Overall trend at 90 DAP and 120 DAP is (T_7>T_4).

Minimum number of branches / plant at 30, 60, 90 and 120 DAP was recorded in treatment (T_{10}) [Control] with a values of 0.00, 0.89, 1.13 and 1.60 (Plate 8).

Table 4.3: Effect of PGR on number of branches / plant in Madhunashini-(*Gymnema sylvestre* R.Br.) cuttings at 30, 60, 90 and 120 DAP.

Treatments	Number of branches / plant			
	30 DAP	**60 DAP**	**90 DAP**	**120 DAP**
T_1 : IBA 500 ppm	0.00 (0.70)*	1.00 (1.22)	1.47 (1.39)	1.80 (1.51)
T_2 : IBA 1000 ppm	0.03 (0.72)	1.00 (1.21)	1.27 (1.32)	2.07 (1.60)
T_3 : IBA 1500 ppm	0.04 (0.73)	1.00 (1.22)	1.60 (1.44)	2.47 (1.72)
T_4 : IAA 500 ppm	0.07 (0.75)	1.22 (1.30)	2.20 (1.64)	3.13 (1.90)
T_5 : IAA 1000 ppm	0.13 (0.79)	1.00 (1.22)	1.93 (1.55)	1.99 (1.57)
T_6 : IAA 1500 ppm	0.03 (0.72)	0.86 (1.16)	1.47 (1.39)	1.73 (1.49)
T_7 : IBA 250 ppm + IAA 250 ppm	**0.50 (0.83)**	**1.36 (1.35)**	**2.27 (1.66)**	**3.67 (2.04)**
T_8 : IBA 500 ppm + IAA 500 ppm	0.15 (0.80)	1.00 (1.22)	1.53 (1.42)	2.53 (1.73)
T_9 : IBA 750 ppm + IAA 750 ppm	0.21 (0.76)	1.03 (1.23)	1.47 (1.40)	2.00 (1.58)
T_{10}: Control	0.00 (0.70)	0.89 (1.17)	1.13 (1.27)	1.60 (1.44)
S. Em. ±	0.04	0.05	0.051	0.060
C.D. at 5 %	NS	NS	0.15	0.17
C.V. %	9.91	8.01	6.16	6.34

Fig. No. 4.3. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for no. of branches / plant at 30, 60, 90 and 120 DAP



ON X AXIS – TREATMENTS AND ON Y AXIS – NO. OF BRANCHES / PLANT

Plate No. 8. Comparison of (T_7) IBA 250 ppm + IAA 250 ppm and Control in *Gymnema Sylvestre* R.Br. after 30, 60, 90 and 120 DAP

30 DAP	60 DAP	90 DAP	120 DAP

T_{10} - CONTROL

30 DAP	60 DAP	90 DAP	120 DAP

With respect to the parameter on effect of PGR on number of branches / plant, best treatment is T_7 as highlighted in above Table 4.3.

***** Figure in the parenthesis are arcsine transformed value and those of outside are original value.

4. Number of leaves / branch at 30, 60, 90 and 120 DAP

The data on number of leaves per branch as affected by various concentrations of IBA, IAA and it's combinations applied are presented in Table 4.4 and graphically illustrated in Fig. 4.4.

The maximum number of leaves / plant at 30, 60, 90 and 120 DAP was significantly recorded in treatment (T_7) [IBA-250 ppm + IAA-250 ppm] with values of 2.67, 12.67, 30.27 and 38.87, respectively. At 30 DAP, treatment (T_7) [IBA-250 ppm + IAA-250 ppm] was at par with the treatment (T_4) [IAA-500 ppm], (T_5) [IAA-1000 ppm], (T_3) [IBA-1500 ppm], (T_8) [IBA- 500 ppm + IAA-500 ppm], (T_9) [IBA-750 ppm + IAA-750 ppm], (T_2) [IBA-1000 ppm] and (T_1) [IBA-500 ppm] with number of leaves / plant accounting to values of 1.75, 1.71, 1.68, 1.66, 1.65, 1.63 and 1.60, respectively.

Whereas at 60 DAP, treatment (T_7) [IBA-250 ppm + IAA-250 ppm] was at par with treatment (T_4) [IAA-500 ppm], (T_9) [IBA-750 ppm + IAA-750 ppm], (T_5) [IAA-1000 ppm], (T_8) [IBA- 500 ppm + IAA-500 ppm] and (T_6) [IAA-1500

ppm] with a values of 12.61, 10.69, 10.47, 9.22 and 9.00, respectively.

At 90 DAP, treatment (T_7) [IBA-250 ppm + IAA-250 ppm] was at par with treatment (T_4) [IAA-500 ppm] and (T_5) [IAA-1000 ppm] with a values of 27.27 and 26.67, respectively. While in the case of 120 DAP, treatment (T_7) [IBA-250 ppm + IAA-250 ppm] was at par with treatment (T_4) [IAA-500 ppm], (T_8) [IBA- 500 ppm + IAA-500 ppm] and (T_5) [IAA-1000 ppm] with a values of 31.53, 31.33 and 31.19, respectively. Overall trend at 30, 60, 90 and 120 DAP is (T_7>T_4).

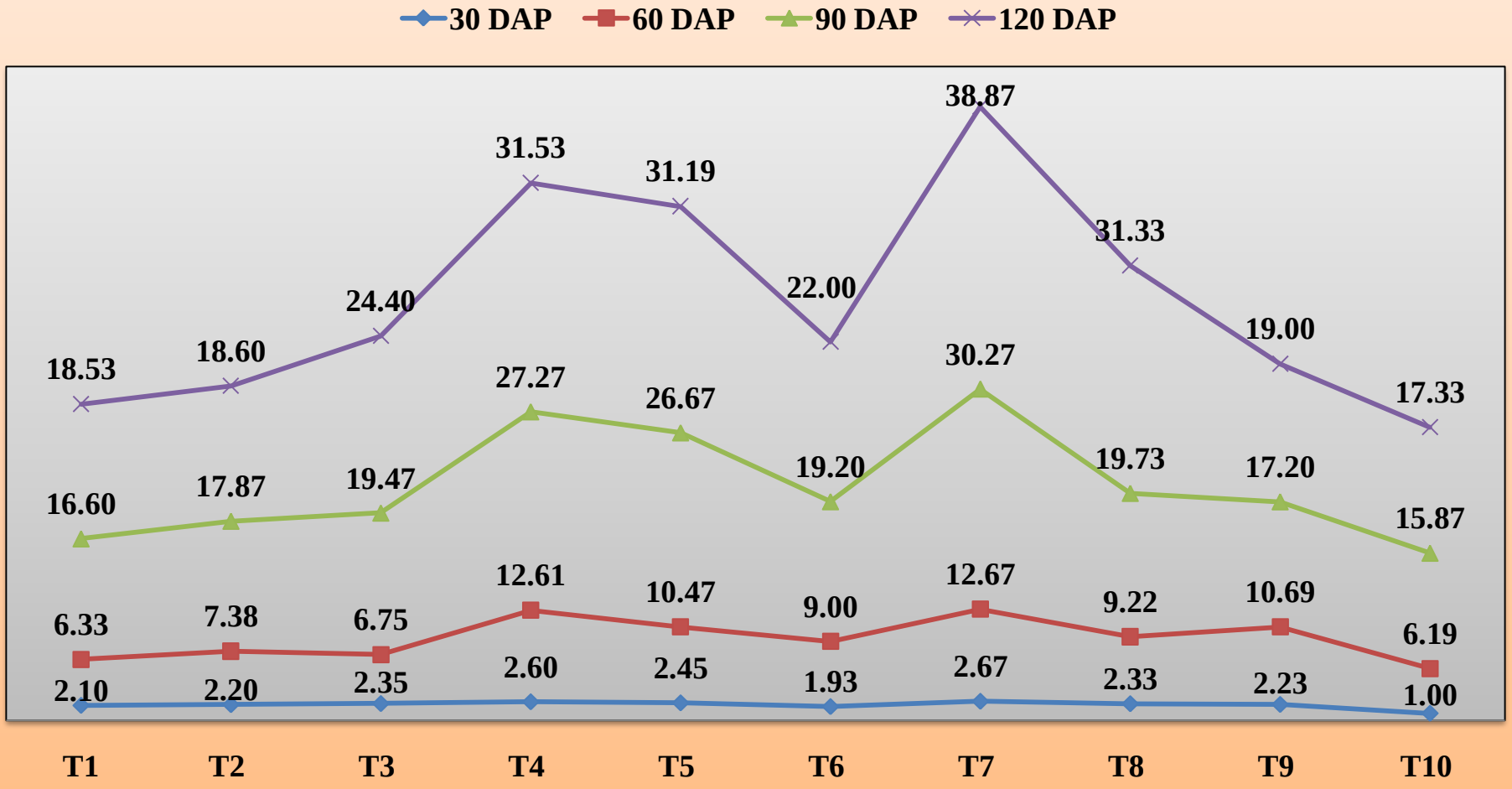
Minimum number of leaves / plant at 30, 60, 90 and 120 DAP was recorded in treatment (T_{10}) [Control] with a values of 1.00, 6.19, 15.87 and 17.33, respectively (Plate 8 and 9).

Table 4.4: Effect of PGR on number of leaves / branch in Madhunashini-(*Gymnema sylvestre* R.Br.) cuttings at 30, 60, 90 and 120 DAP.

Treatments	Number of leaves / branch			
	30 DAP	**60 DAP**	**90 DAP**	**120 DAP**
T_1 : IBA 500 ppm	2.10 (1.60)*	6.33 (2.61)	16.60 (4.12)	18.53 (4.33)
T_2 : IBA 1000 ppm	2.20 (1.63)	7.38 (2.78)	17.87 (4.28)	18.60 (4.35)
T_3 : IBA 1500 ppm	2.35 (1.68)	6.75 (2.68)	19.47 (4.45)	24.40 (4.98)
T_4 : IAA 500 ppm	2.60 (1.75)	12.61 (3.61)	27.27 (5.26)	31.53 (5.65)
T_5 : IAA 1000 ppm	2.45 (1.71)	10.47 (3.22)	26.67 (5.20)	31.19 (5.61)
T_6 : IAA 1500 ppm	1.93 (1.55)	9.00 (3.07)	19.20 (4.40)	22.00 (4.73)
T_7 : IBA 250 ppm + IAA 250 ppm	**2.67 (1.77)**	**12.67 (3.62)**	**30.27 (5.54)**	**38.87 (6.27)**
T_8 : IBA 500 ppm + IAA 500 ppm	2.33 (1.66)	9.22 (3.10)	19.73 (4.49)	31.33 (5.62)
T_9 : IBA 750 ppm + IAA 750 ppm	2.23 (1.65)	10.69 (3.31)	17.20 (4.20)	19.00 (4.40)
T_{10}: Control	1.00 (1.22)	6.19 (2.58)	15.87 (4.04)	17.33 (4.20)
S. Em. ±	0.084	0.234	0.187	0.257
C.D. at 5 %	0.24	0.69	0.55	0.75
C.V. %	8.97	8.64	7.05	8.89

With respect to the parameter on effect of PGR on number of leaves / branch, best treatment is T_7 as highlighted in above Table 4.4.

Fig. No. 4.4. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for no. of leaves / branch at 30, 60, 90 and 120 DAP



ON X AXIS – TREATMENTS AND ON Y AXIS – NO. OF LEAVES / BRANCH

Plate No. 9. Comparison of (T_8) IBA 500 ppm + IAA 500 ppm and Control in *Gymnema Sylvestre* R.Br. after 30, 60, 90 and 120 DAP

30 DAP

60 DAP

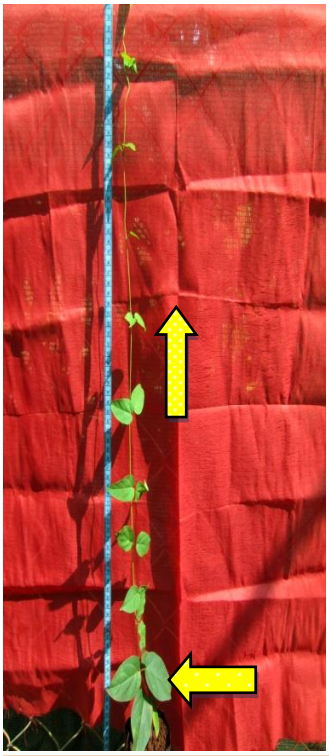
90 DAP

120 DAP

T_{10} - CONTROL

30 DAP

60 DAP



90 DAP

120 DAP

***** Figure in the parenthesis are arcsine transformed value and those of outside are original value.

5. Shoot length (cm) at 30, 60, 90 and 120 DAP

Data / observations pertaining to length of shoots (cm) in Madhunashini cuttings treated with different concentrations of IBA, IAA and combinations of IBA and IAA are presented in Table 4.5 and graphically depicted in Fig. 4.5.

Among all the treatments applied, maximum length of shoots was reported at 30, 60, 90 and 120 DAP in the treatment (T_5) [IAA-1000 ppm] with a values of 5.72 cm, 45.00 cm, 102.80 cm and 110.61 cm. At 30 DAP, treatment (T_5) [IAA-1000 ppm] was at par with treatment (T_4) [IAA-500 ppm] and (T_7) [IBA-250 ppm + IAA-250 ppm] with a values of 5.62 cm and 5.34 cm, respectively. Whereas at 60 DAP, treatment (T_5) [IAA-1000 ppm] was followed by treatment (T_4) [IAA-500 ppm] i.e 39.05 cm. While in case of 90 and 120 DAP, treatment (T_5) [IAA-1000 ppm] was at par with treatment (T_4) [IAA-500 ppm] with values of 101.07 cm and 105.17 cm, respectively. Overall trend for 30 DAP goes as (T_5>T_4> T_7), for 60, 90 and 120 DAP, it is (T_5>T_4).

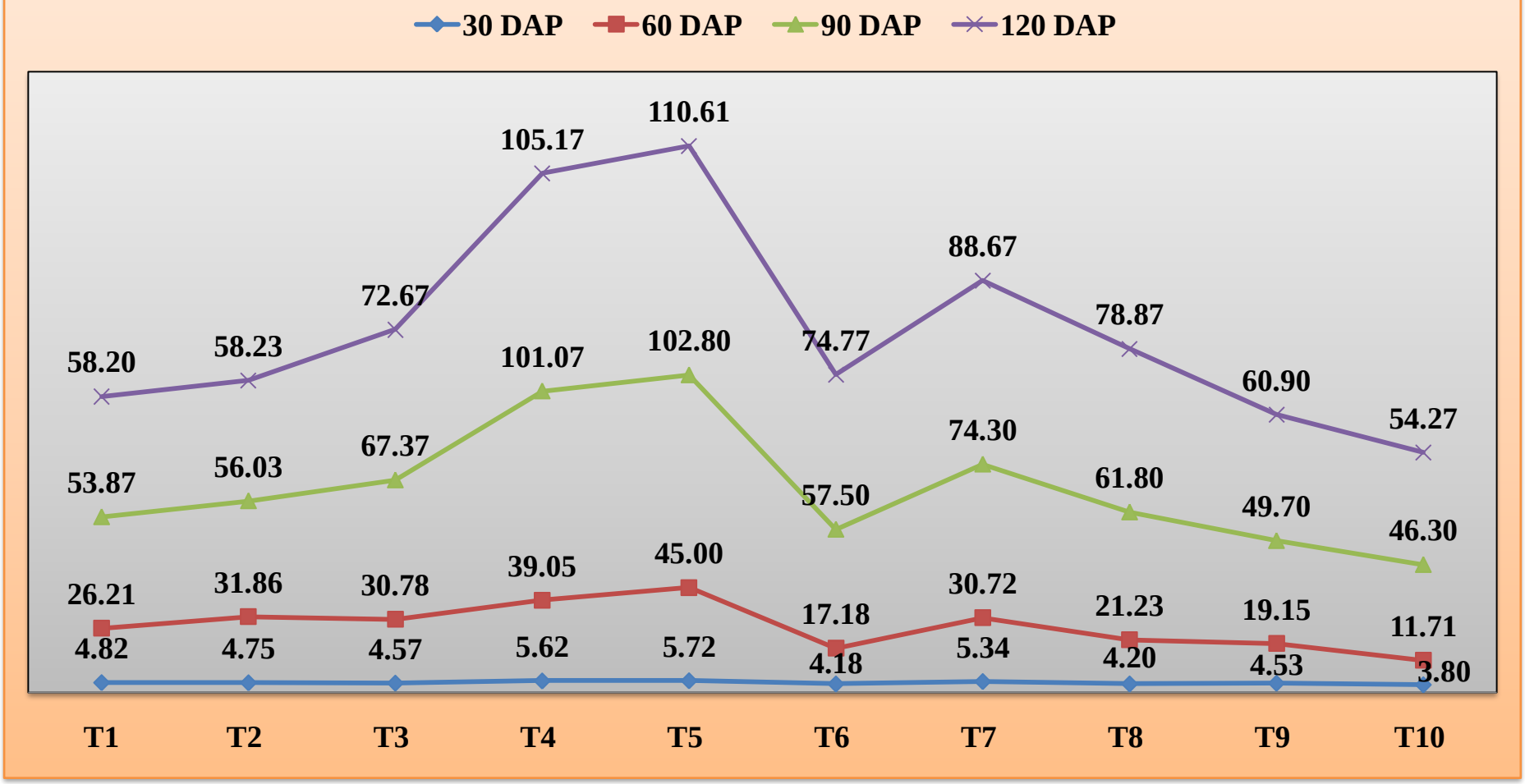
Minimum shoot length was recorded at 30, 60, 90 and 120 DAP in the treatment (T_{10}) [control] or untreated cuttings of *G. sylvestre*, with a values of 3.80 cm, 11.71 cm, 46.30 cm and 54.27 cm, respectively (Plate 6 and 7).

Table 4.5: Effect of PGR on shoot length in Madhunashini- (*Gymnema sylvestre* R.Br.) cuttings at 30, 60, 90 and 120 DAP.

Treatments	Shoot Length (cm)			
	30 DAP	60 DAP	90 DAP	120 DAP
T_1 : IBA 500 ppm	4.82	26.21	53.87	58.20
T_2 : IBA 1000 ppm	4.75	31.86	56.03	58.23
T_3 : IBA 1500 ppm	4.57	30.78	67.37	72.67
T_4 : IAA 500 ppm	5.62	39.05	101.07	105.17
T_5 : IAA 1000 ppm	**5.72**	**45.00**	**102.80**	**110.61**
T_6 : IAA 1500 ppm	4.18	17.18	57.50	74.77
T_7 : IBA 250 ppm + IAA 250 ppm	5.34	30.72	74.30	88.67
T_8 : IBA 500 ppm + IAA 500 ppm	4.20	21.23	61.80	78.87
T_9 : IBA 750 ppm + IAA 750 ppm	4.53	19.15	49.70	60.90
T_{10}: Control	3.80	11.71	46.30	54.27
S. Em. ±	0.142	0.642	1.762	2.488
C.D. at 5 %	0.42	1.89	5.19	7.34
C.V. %	5.19	4.07	4.55	5.65

In context to the parameter on effect of PGR on length of the shoot, best treatment is T_5 as highlighted in above Table 4.5.

Fig. No. 4.5. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for shoot length (cm) at 30, 60, 90 and 120 DAP



ON X AXIS – TREATMENTS AND ON Y AXIS – SHOOT LENGHT IN cm

6. Leaf area (cm^2) (Final- At the end)

The data on leaf area as affected by different concentrations of IBA, IAA and combination of IBA and IAA are shown in Table 4.6 and graphically illustrated in Fig. 4.6.

The data revealed that the different concentrations of PGRs, i.e IBA, IAA and its combinations significantly increased the leaf area as compared to control.

The maximum leaf area during study conducted was reported by the treatment (T_4) [IAA-500 ppm] i.e 24.09 cm^2, which was significantly followed by treatment (T_6) [IAA-1500 ppm], (T_3) [IBA-1500 ppm], (T_7) [IBA-250 ppm + IAA-250 ppm], (T_5) [IAA-1000 ppm] and (T_9) [IBA-750 ppm + IAA-750 ppm], with a values of (22.36 cm^2, 22.24 cm^2, 21.71 cm^2, 21.51 cm^2 and 19.95 cm^2, respectively). Overall trend was (T_4>T_6>T_3>T_7>T_5>T_9).

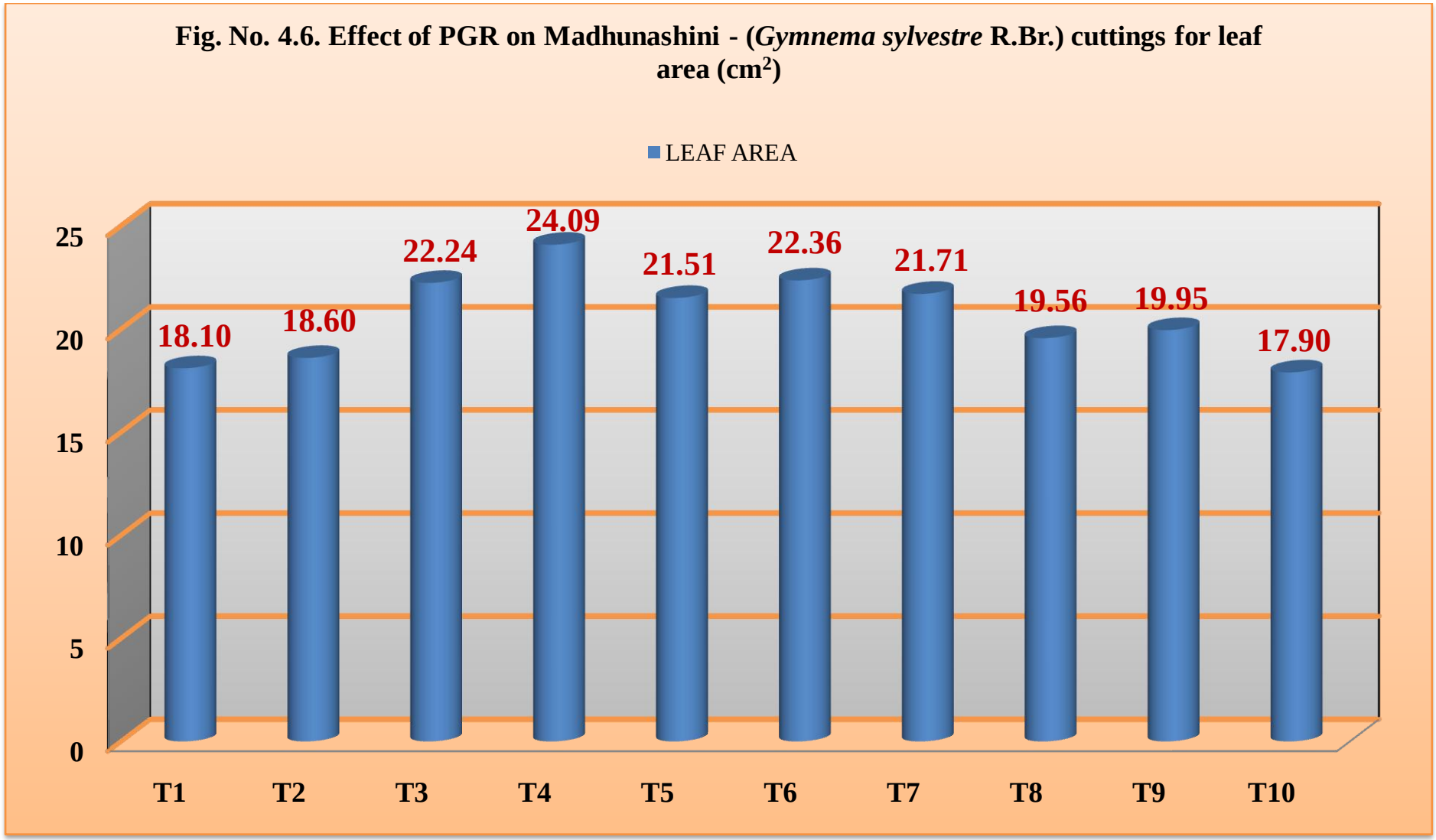
Minimum leaf area was recorded in the treatment (T_{10}) i.e Control with a value of 17.90 cm^2, conferring that various concentrations of auxin i.e IBA, IAA and combinations of IBA and IAA together has significant effect on the leaf area in *G. sylvestre* (Plate 10).

Table 4.6: Effect of PGR on leaf area in Madhunashini-(*Gymnema sylvestre* R.Br.) cuttings.

Treatments	Leaf Area (cm^2)
T_1 : IBA 500 ppm	18.10
T_2 : IBA 1000 ppm	18.60
T_3 : IBA 1500 ppm	22.24
T_4 : IAA 500 ppm	**24.09**
T_5 : IAA 1000 ppm	21.51
T_6 : IAA 1500 ppm	22.36
T_7 : IBA 250 ppm + IAA 250 ppm	21.71
T_8 : IBA 500 ppm + IAA 500 ppm	19.56
T_9 : IBA 750 ppm + IAA 750 ppm	19.95
T_{10}: Control	17.90
S. Em. ±	0.565
C.D. at 5 %	1.68
C.V. %	4.75

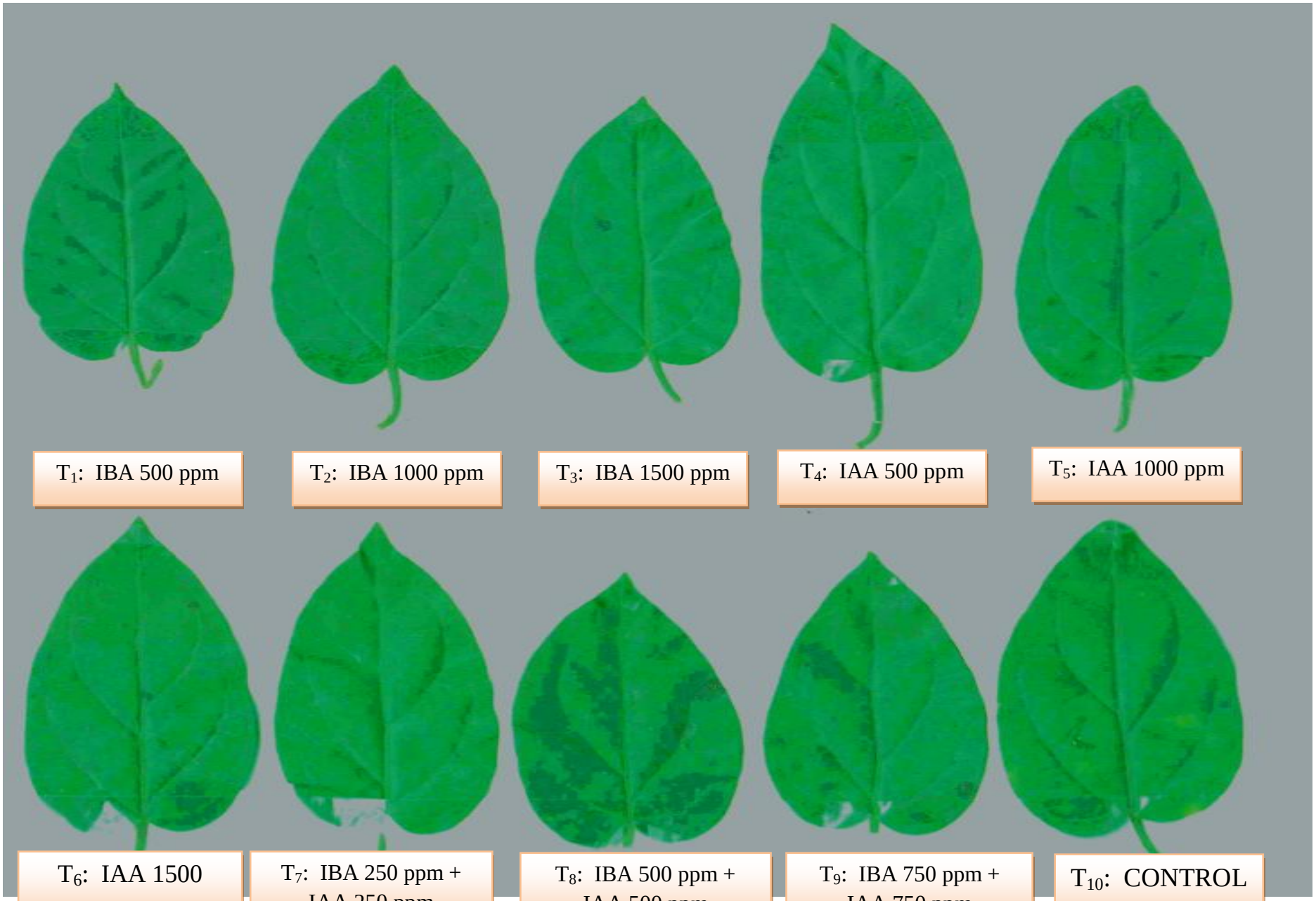
With respect to the parameter on effect of PGR on the leaf area of the plant selected, best treatment is T_4 as highlighted in above Table 4.6.

Fig. No. 4.6. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for leaf area (cm^2)



ON X AXIS – TREATMENTS AND ON Y AXIS – LEAF AREA (cm^2)

Plate No. 10. Effect of PGR on leaf area of Madhunashini – (*Gymnema sylvestre* R.Br.)



7. Leaf morphology

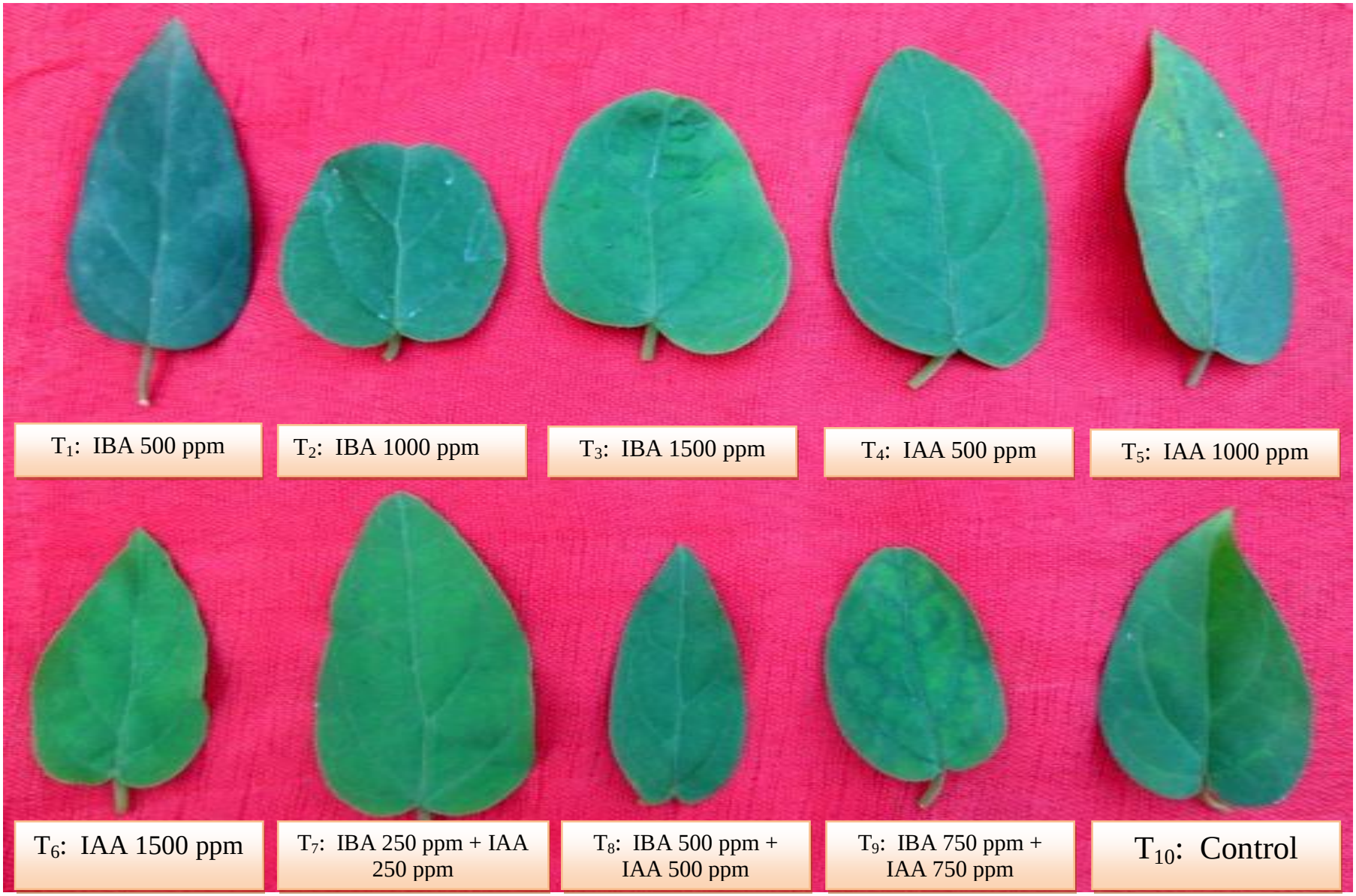
For the leaf morphology of *Gymnema sylvestre* R. Br., there were no significant variations observed, except for some minor differences in leaf base and leaf apex.

Overall, the leaves were broader than longer with shape of the lamina ranging from elliptic to obovate to ovate.

Treatments T_1 (IBA-500 ppm), T_5 (IAA-1000 ppm), T_6 (IAA-1500 ppm), T_8 (IBA-500 ppm + IAA-500 ppm) along with control i.e treatment T_{10} exhibited perfectly acute apex with equal leaf base and entire margin.

Whereas in treatments T_2 (IBA-1000 ppm), T_3 (IBA-1500 ppm), T_4 (IAA-500 ppm), T_7 (IBA-250 ppm + IAA-250 ppm) and T_9 (IBA-750 ppm + IAA-750 ppm) leaves were much more elliptic with rounded / obtuse leaf apex and leaf base was rounded with slight inward incision or basal notch (Plate 11).

Plate No. 11. Effect of PGR on Leaf Morphology of Madhunashini – (*Gymnema sylvestre* R.Br.)



8. Length of the main root (cm) (Final- At the end)

The data on the length of the main root (cm) as affected by different concentrations of auxin applied are presented in Table 4.7 and graphically depicted in Fig. 4.7.

Length of main root differed significantly due to different levels of the IBA, IAA and combinations of IBA and IAA in comparison with untreated cuttings of *G. sylvestre*.

Amongst various concentrations of IBA, IAA and their combinations used, treatment (T_8) [IBA-500 ppm + IAA-500 ppm] gave maximum root length with a value of 25.69 cm, which was at par with (T_7) [IBA-250 ppm + IAA-250 ppm], (T_5) [IAA-1000 ppm],(T_9) [IBA-750 ppm + IAA-750 ppm], (T_4) [IAA-500 ppm] and (T_2) [IBA-1000 ppm] with a value of 25.31 cm, 24.96 cm, 24.26 cm, 23.45 cm and 23.44 cm, respectively). Overall trend was (T_8>T_7>T_5>T_9>T_4>T_2).

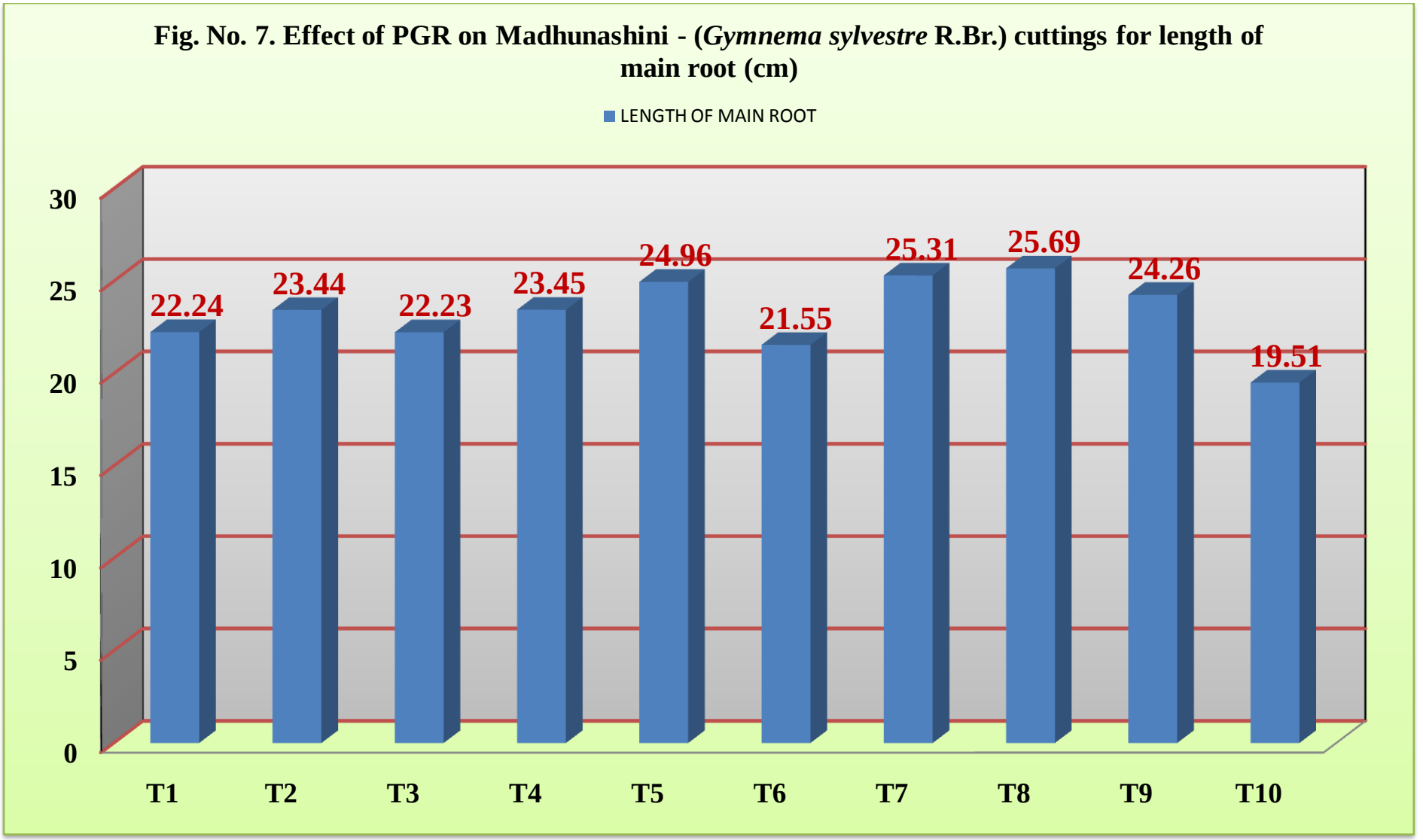
Whereas, minimum root length was recorded in treatment (T_{10}) [control] with a value of 19.51 cm (Plate12).

Table 4.7: Effect of PGR on length of the main root in Madhunashini-(*Gymnema sylvestre* R.Br.) cuttings.

Treatments	Length of Main the Root (cm)
T_1 : IBA 500 ppm	22.24
T_2 : IBA 1000 ppm	23.44
T_3 : IBA 1500 ppm	22.23
T_4 : IAA 500 ppm	23.45
T_5 : IAA 1000 ppm	24.96
T_6 : IAA 1500 ppm	21.55
T_7 : IBA 250 ppm + IAA 250 ppm	25.31
T_8 : IBA 500 ppm + IAA 500 ppm	25.69
T_9 : IBA 750 ppm + IAA 750 ppm	24.26
T_{10}: Control	19.51
S. Em. ±	0.764
C.D. at 5 %	2.25
C.V. %	5.68

With respect to the parameter on effect of PGR on length of the main root, best treatment is T_8 as highlighted in above Table 4.7.

Fig. No. 7. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for length of main root (cm)

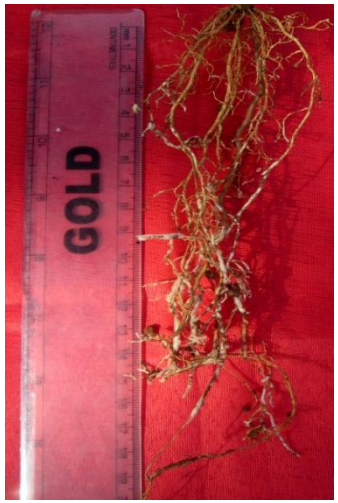


ON X AXIS – TREATMENTS AND ON Y AXIS – LENGHT OF THE MAIN ROOT (cms)

Plate No. 12. Effect of PGR on Main Root Length of Madhunashini - (*Gymnema Sylvestre* R.Br.)

T_1: IBA 500 ppm

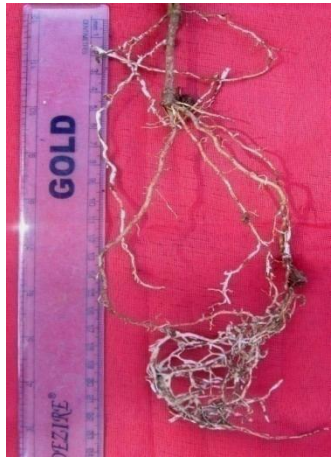
T_2: IBA 1000 ppm



T_3: IBA 1500 ppm

T_4: IAA 500 ppm

T_5: IAA 1000 ppm



T_6: IAA 1500 ppm

T_7: IBA 250 ppm + IAA 250 ppm

T_8: IBA 500 ppm + IAA 500 ppm

T_9: IBA 750 ppm + IAA 750 ppm

T_{10}: Control

9. Main root girth (mm) (Final- At the end)

The mean data on the whole root girth is presented in Table 4.8 and graphically represented in Fig. 4.8.

The present data on root girth reveals that the concentrations of auxin (IBA, IAA and its combinations) used, significantly influenced the mean girth of the main root.

The highest root girth during study conducted was recorded in the treatment (T_4) [IAA-500 ppm] i.e. 7.69 mm, which was at par with the treatment (T_7) [IBA-250 ppm + IAA-250 ppm] with the value of 7.36 mm. Overall trend was ($T_4 > T_7$).

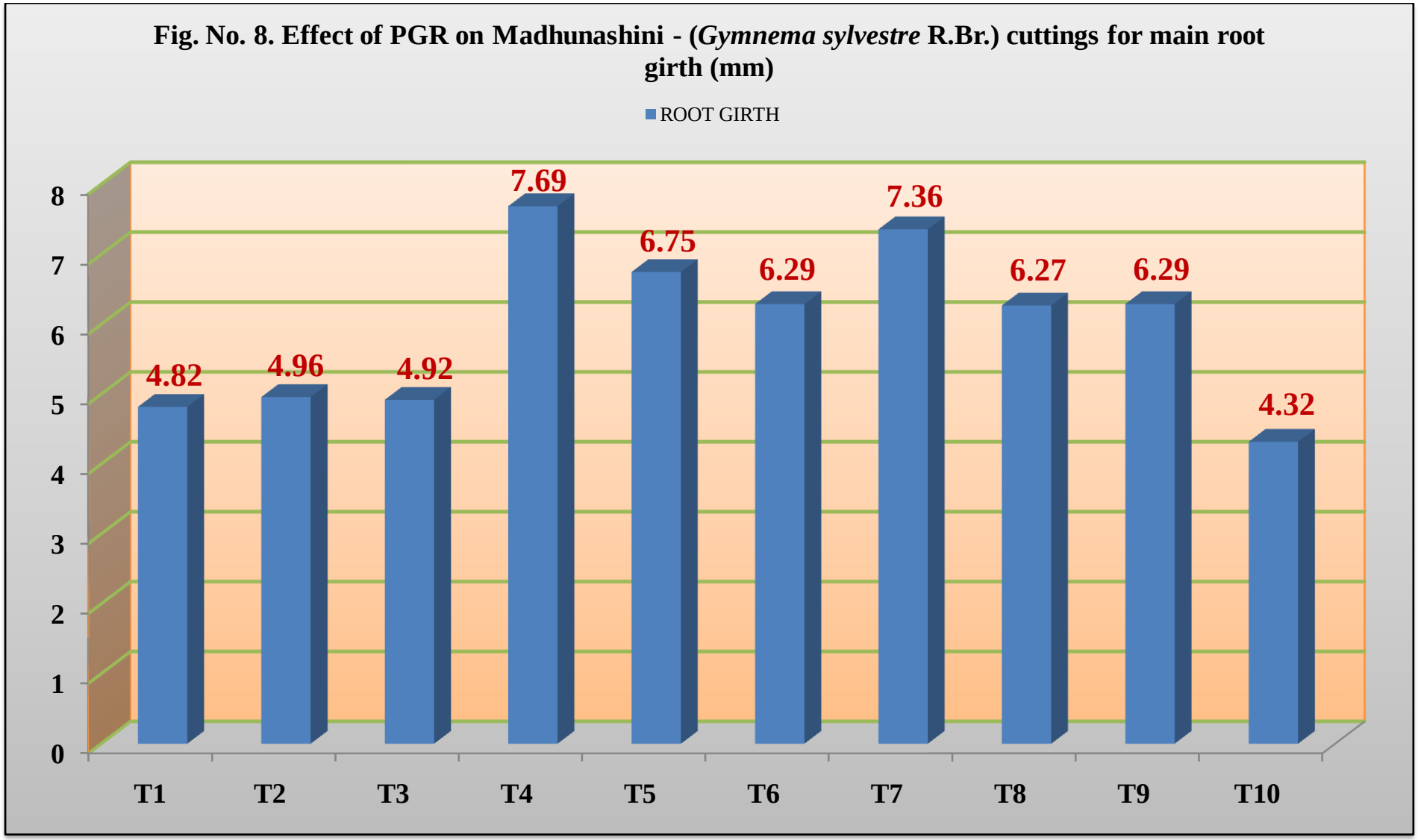
Whereas, lowest root girth was observed in the treatment (T_{10}) [control], i.e 4.32 mm (Plate 13).

Table 4.8: Effect of PGR on main root girth in Madhunashini-(*Gymnema sylvestre* R.Br.) cuttings.

Treatments	Girth of Main Root (mm)
T_1 : IBA 500 ppm	4.82
T_2 : IBA 1000 ppm	4.96
T_3 : IBA 1500 ppm	4.92
T_4 : IAA 500 ppm	**7.69**
T_5 : IAA 1000 ppm	6.75
T_6 : IAA 1500 ppm	6.29
T_7 : IBA 250 ppm + IAA 250 ppm	7.36
T_8 : IBA 500 ppm + IAA 500 ppm	6.27
T_9 : IBA 750 ppm + IAA 750 ppm	6.29
T_{10}: Control	4.32
S. Em. ±	0.158
C.D. at 5 %	0.46
C.V. %	4.60

In context to the parameter on effect of PGR on girth of the main root, best treatment is T_4 as highlighted in above Table 4.8.

Fig. No. 8. Effect of PGR on Madhunashini - (*Gymnema sylvestre* R.Br.) cuttings for main root girth (mm)



ON X AXIS – TREATMENTS AND ON Y AXIS – MAIN ROOT GIRTH (mm)

Plate No. 13. Effect of PGR on Main Root Girth of Madhunashini – (*Gymnema sylvestre* R.Br.)

T_1: IBA 500 ppm

T_2: IBA 1000 ppm

T_3: IBA 1500 ppm

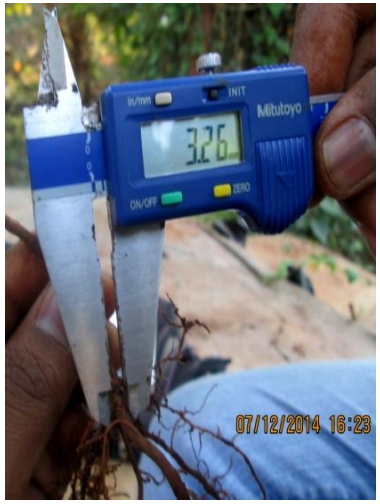
T_4: IAA 500 ppm

T_5: IAA 1000 ppm

T_6: IAA 1500 ppm

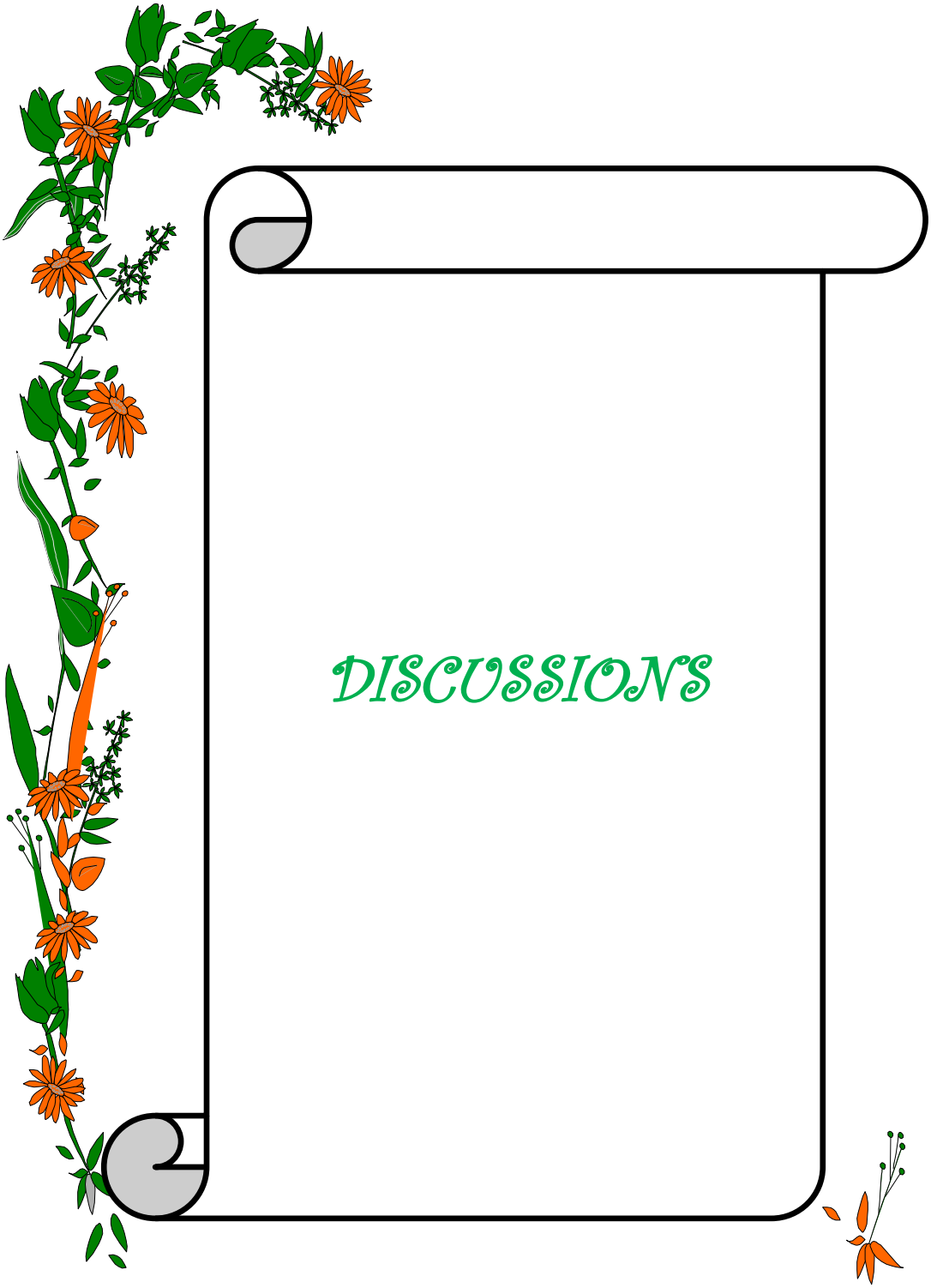
T_7: IBA 250 ppm + IAA 250 ppm

T_8: IBA 500 ppm + IAA 500 ppm



T_9: IBA 750 ppm + IAA 750 ppm

T_{10}: Control


DISCUSSIONS

V. DISCUSSIONS

The results of the experiment entitled, "Effect of PGR on Growth and Quality of Madhunashini - (*Gymnema sylvestre* R.Br.)" are discussed in this chapter. In this chapter thorough and sincere attempt has been made to discuss results critically and supported with relevant references based on experimental evidences for the objectives set-in at initiation of the study.

5.1. Effect of different PGR's on growth and quality of *Gymnema sylvestre* R. Br.

5.2. Effect of different PGR's on rooting of *Gymnema sylvestre* R. Br.

5.1. Effect of different PGR's on growth and quality of *Gymnema sylvestre* R. Br.

Our results showed that the auxins have positive effect on sprouting behaviour of *G. sylvestre*. Auxins are well known PGR; it inhibits the apical growth of plants and initiates the early sprouting in plant. Results revealed that minimum days required for sprouting was reported in treatment T_5 [IAA-1000 ppm]. Here IAA performed better in comparison to IBA when applied individually and was also superior as compare to IAA+IBA used in combination. Similar results have been reported by, Sehrawat *et al*. (2001) in *Rauvolfia serpentina* L., Rani *et al*. (2012) in *G. Sylvestre* R. Br. and Bisht *et al*. (2014) in *Acorus calamus* L.

G. sylvestre cuttings showed better performance for plant height and shoot length when treated with different auxin concentrations. Highest plant height and shoot length was recorded in the treatment T_5 [IAA-1000 ppm]. Auxins and especially IAA and IBA have potential to enhance rapid cell division and elongation; hence it may have increased the inter-nodal length of plants which ultimately increased the plant

height as well as shoot length. In, the present study, application of IAA alone gave better results than the IBA and their combinations. These results are line with earlier findings by Sehrawat *et al*. (2001) in *Rauvolfia serpentina* L., Bhandari *et.al.* (2009) in *Verbascum thapsus* L., and Minakshi and Lingakumar (2011) in *Mentha arvensis* L.

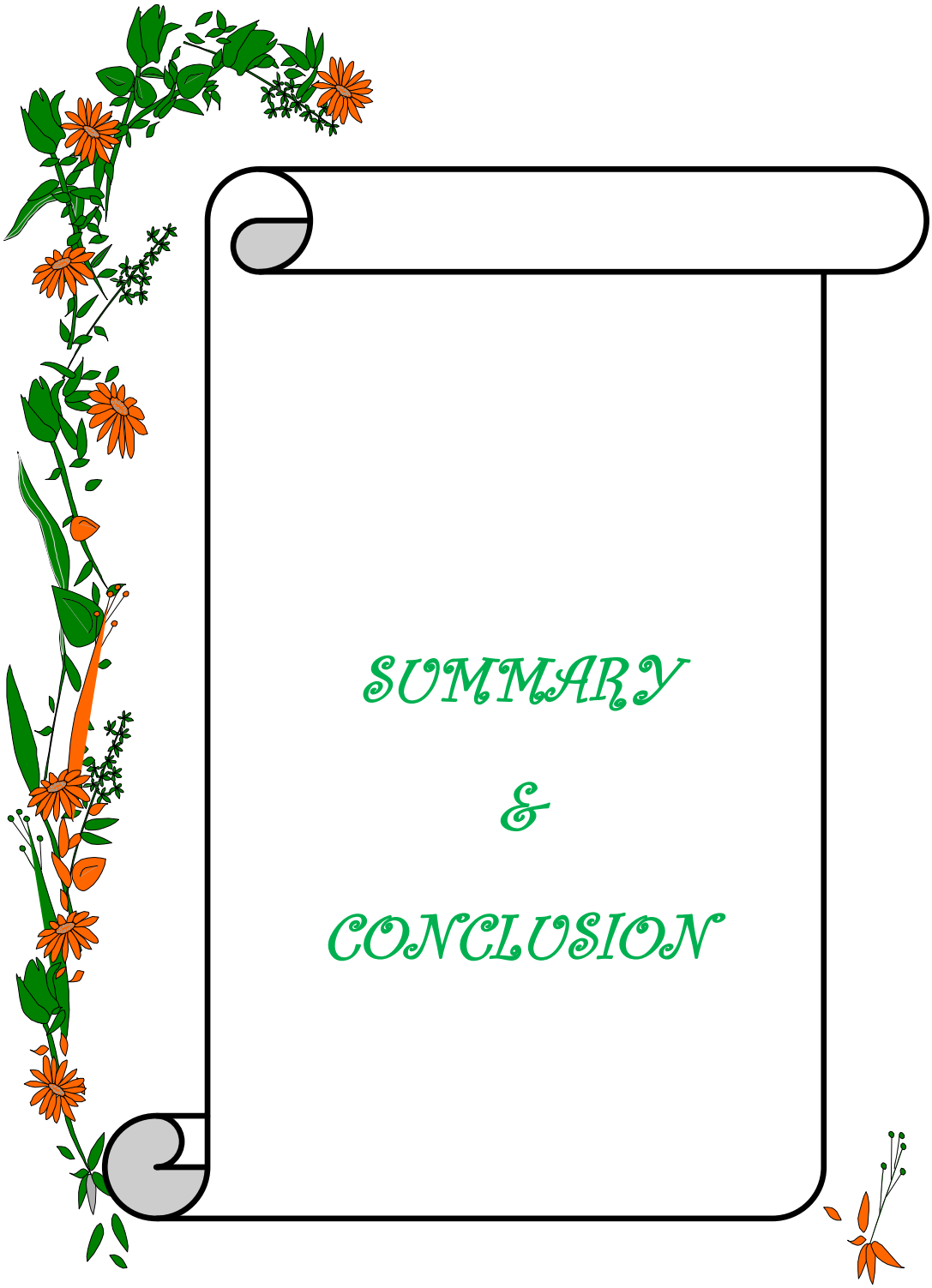
Auxins (IAA and IBA) also showed significant effect on number of branches / plant and number of leaves / branch. Findings revealed that the combinations of IBA + IAA i.e treatment T_7 [IBA 250 ppm + IAA 250 ppm] gave maximum number of branches and leaves. However, it was at par with 500 ppm and 1000 ppm IAA, i.e treatment T_4 and T_5. Auxins have important physiological property to regulate shoot apical dominance in plants and it stimulates the lateral buds so ultimately it increases the number of branches / plant and number leaves / branch. These observations are similar with the earlier obtained results by Bhandari *et.al.* (2009) in *Verbascum thapsus* L. and Minakshi and Lingakumar (2011) in *Mentha arvensis* L.

For leaf area, 500 ppm IAA i.e treatment T_4 showed better result. Overall, IAA, when applied solely performed better than IBA and combinations of them. This result is similar with the earlier findings by Bhandari *et.al.* (2009) in *Verbascum thapsus* L., Minakshi and Lingakumar (2011) in *Mentha arvensis* L. and Khan *et al*. (2015) in *Cymbopogon martini* Roxb.

5.2. Effect of different PGR's on rooting of *Gymnema sylvestre* R. Br.

Our results showed that both the auxins used i.e IAA and IBA have positive effect on rooting behavior of *G. sylvestre*. Highest root length was recorded in treatment T_8 [IBA 500 ppm + IAA 500 ppm]. During the present study, application of combination of IBA + IAA gave better response over the IBA and IAA when used alone. With respect to

the maximum root girth, treatment T_4 [IAA 500 ppm] was superior over other treatments applied. Among all auxins applied, IAA performed well over the IBA and combinations of them. Auxins have potential to increases the radicle growth in plants and also promote root initiation. It induces, both growth of the pre-existing roots and adventitious root formation, i.e, branching of the roots. Hence, it may increase the uptake of essential nutrients and moisture from soil. Also auxins have marked effect on apical as well as horizontal growth of plants. Hence, it may have resulted in increase in root girth. These findings co-relates with earlier findings by Deshpande (2005) in *Rauvolfia tetraphylla* L., Ramulu *et al*. (2005) in *Hemidesmus indicus* R. Br., Dhuria (2008) in *Rauvolfia serpentina* L., Bhandari *et.al.* (2009) in *Verbascum thapsus* L., Alagesaboopathi (2011) in *Andrographis lineata* Nees., Minakshi and Lingakumar (2011) in *Mentha arvensis* L., Lal and Jha (2012) and Rani *et al*. (2012) in *G. Sylvestre* R. Br. and Bisht *et al*. (2014) in *Acorus calamus* L.



SUMMARY

&

CONCLUSION

VI. SUMMARY AND CONCLUSION

The present investigation entitled, "Effect of PGR on Growth and Quality of Madhunashini - (*Gymnema sylvestre* R.Br.)" was carried out at the Model Nursery on Medicinal and Aromatic Plants, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari during the period of July-2014 to December-2014.

Influence of two auxins i.e IAA and IBA, either alone (500 ppm, 1000 ppm and 1500 ppm) or in combination (250 ppm IBA + 250 ppm IAA; 500 ppm IBA + 500 ppm IAA and 750 ppm IBA + 750 ppm IAA) were investigated for enhancing rooting success and growth of *G. sylvestre* cuttings. The experiment was laid out in CRD. Overall, there were ten treatments with three repetitions and in each repetition consisted thirty cuttings. The planting medium comprised of Red earth soil, FYM and Vermicompost in the ratio of 2:1:1.

Observations on shoot and root characters *viz*., Days required for 50% sprouting, plant height, no. of branches / plant, no. of leaves / branch, shoot length, leaf area, initial and final leaf morphology, length of main root, main root girth were recorded and subjected to ANOVA analysis and means were compared with critical difference at 0.05 level.

The observations recorded are documented and statistically analyzed along with photographic evidences in previous chapters and are summarized as below:

6.1) Present study showed that the *G. sylvestre* cuttings were highly responsive to auxin treatments for growth, quality and rooting parameters as compared to untreated cuttings.

6.2) Earliest sprouting was recorded in treatment (T_5) [IAA-1000 ppm] with a value of 19.67 days, which was 7 days earlier than treatment (T_{10}) [control].

6.3) Maximum plant height was recorded at 30, 60, 90 and 120 DAP, in treatment (T_5) [IAA-1000 ppm] with values of (8.67 cm, 47.83 cm, 111.07 cm and 117.91 cm, respectively), which was (1.34, 3.31, 2.12 and 2.02, respectively) times more than treatment (T_{10}) [Control].

6.4) Maximum number of branches / plant was observed at 30, 60, 90 and 120 DAP in treatment (T_7) [IBA-250 ppm + IAA-250 ppm] with a values of (0.50, 1.36, 2.27 and 3.67, respectively, which was (1.52, 2.00 and 2.12) times higher than the treatment (T_{10}) [Control] at 60, 90 and 120 DAP, respectively.

6.5) Maximum number of leaves / branch observed at 30, 60, 90 and 120 DAP in treatment (T_7) [IBA-250 ppm + IAA-250 ppm] with a values of (2.67, 12.67, 30.27 and 38.87, respectively), which was (1.00, 2.04, 1.90 and 2.24, respectively) times higher than the treatment (T_{10}) [Control].

6.6) Lengthiest shoot was recorded at 30, 60, 90 and 120 DAP in treatment (T_5) [IAA-1000 ppm] i.e (5.72 cm, 45.00 cm, 102.80 cm and 110.61 cm, respectively), which was (1.50, 3.84, 2.22 and 2.03, respectively) times more than the treatment (T_{10}) [Control].

6.7) Maximum leaf area was obtained in the treatment (T_4) [IAA-500 ppm] with a value of 24.09 cm^2, which was 1.34 times higher than treatment (T_{10}) [Control].

6.8) Looking into the leaf morphology, overall the leaves are broader than longer with shape of the lamina ranging from elliptic to obovate to ovate.

6.9) Longest root was recorded in treatment (T_8) [IBA-500 ppm + IAA-500 ppm] with the value of 25.69 cm, which was 1.31 times longer than treatment (T_{10}) [control].

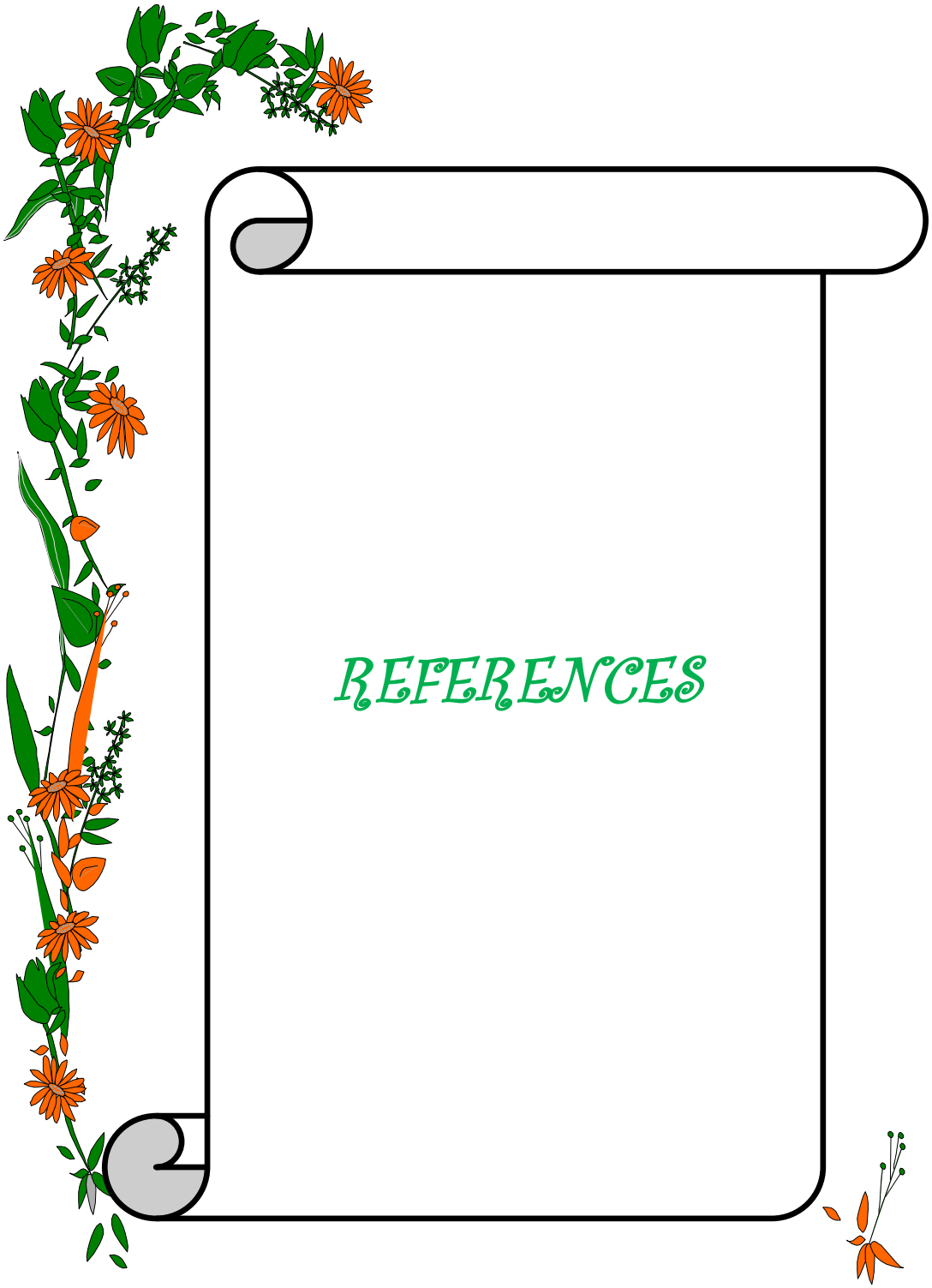
6.10) Thickest root was observed in treatment (T_4) [IAA-500 ppm] i.e. (7.69 mm), which was 1.78 times thicker than treatment (T_{10}) [control].

CONCLUSION:

On the basis of result obtained in present study, it can be concluded that auxin treatments positively influences rooting, establishment and growth of *G. sylvestre* cuttings. Among different auxins and their various concentrations applied, 1000 ppm IAA i.e. treatment T_5 has better influence on plant growth and it was at par with other treatments T_4, T_7 and T_8 found best for rooting and leaves character. Hence, it can be conclude that the IAA-1000 ppm can be used for large scale propagation of *G. sylvestre* through cutting.

FUTURE THRUST:

Future research can be directed toward influence of auxin treatments on overall production of Gymnemic acid in rooted cutting. For further success in enhancement of rooting, influence of different potting mixture can also be studied.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VII. REFERENCES

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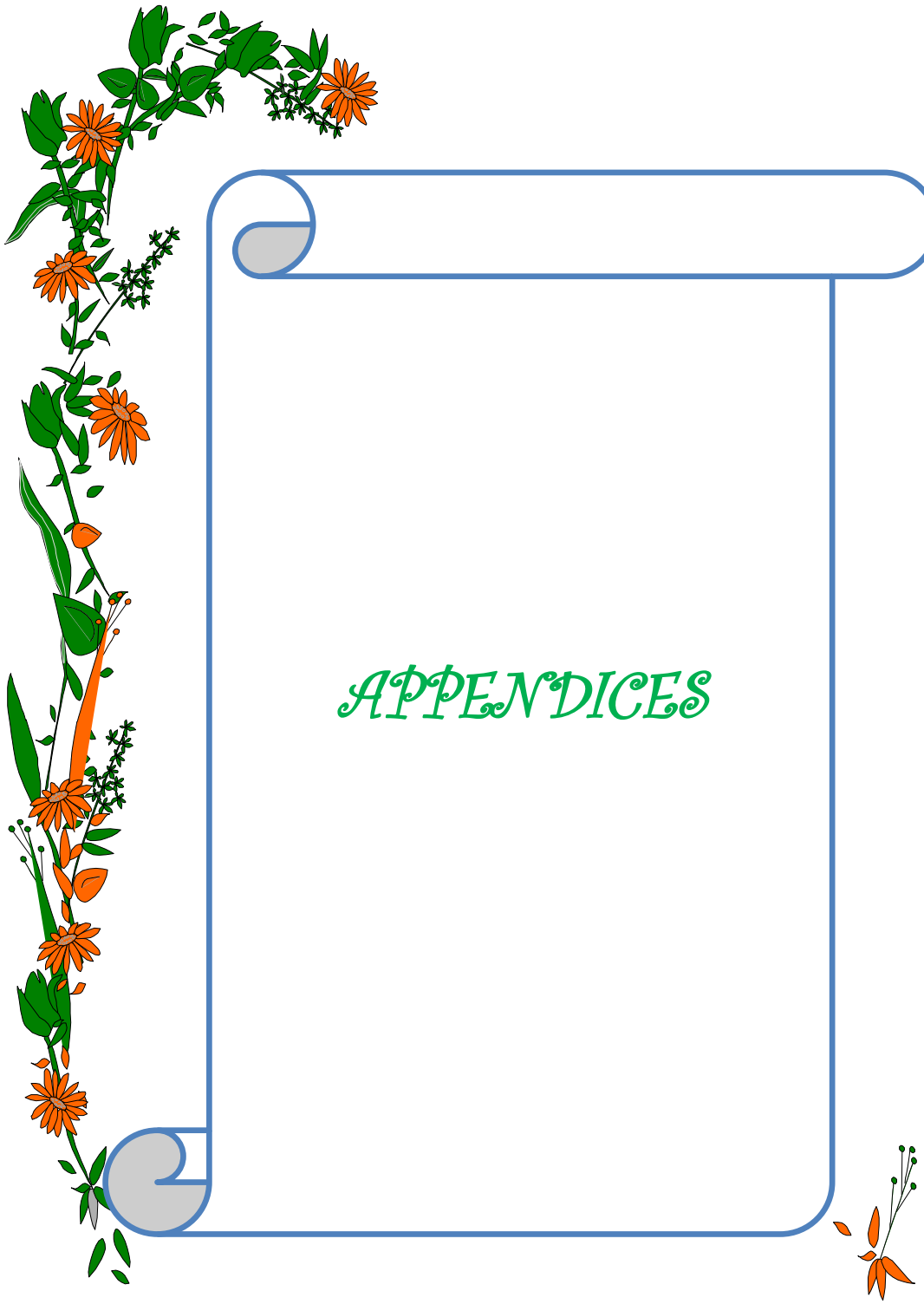

APPENDICES

Annexure. 1: Weekly meteorological data on weather parameters during the experimental period (July-December 2014).

Week No.	Date	Temp.(°C)		R.H. (mm)		Wind Km/h	Sunshine Hours	Rainfall (mm)	Rainy Days	Evaporation (mm)
		Max.	Min.	Morning	Evening					
27	2-8	33.5	26.5	84	67	9.8	7.4	021.0	2	6.0
28	9-15	31.0	25.1	92	75	7.6	4.3	040.5	2	5.3
29	16-22	29.7	26.1	91	84	9.8	1.2	221.5	4	3.7
30	23-29	29.7	24.9	90	86	9.9	1.6	282.0	5	3.1
31	30-5	28.6	25.4	93	86	8.8	1.8	268.5	6	2.6
32	6-12	29.7	24.8	93	79	8.4	3.9	064.5	7	4.1
33	13-19	30.0	24.9	89	77	8.5	2.8	040.0	5	3.4
34	20-26	32.2	25.6	94	78	2.6	3.9	024.0	2	3.5
35	27-2	30.0	24.3	97	84	3.1	0.7	120.0	5	2.9
36	3-9	29.7	25.1	93	83	9.4	3.5	061.0	5	3.0
37	10-16	29.7	24.2	96	84	4.1	2.7	279.0	3	2.6
38	17-23	32.0	24.2	91	64	3.6	8.5	001.0	0	3.9
39	24-30	34.3	24.6	90	68	2.7	6.4	000.0	0	4.2
40	1-7	36.4	24.4	83	41	3.4	8.1	000.0	0	4.5

41	8-14	36.3	22.5	83	42	3.4	8.6	000.0	0	4.5
42	15-21	36.3	22.5	85	39	2.9	9.8	000.0	0	4.6
43	22-28	35.1	21.7	76	43	3.1	6.8	000.0	0	4.7
44	29-4	35.3	19.4	89	33	2.3	9.0	000.0	0	4.5
45	5-11	34.4	18.8	80	38	2.6	8.6	000.0	0	4.5
46	12-18	32.6	22.7	85	58	2.7	5.4	067.0	2	3.4
47	19-25	33.2	18.9	82	38	2.3	8.6	000.0	0	3.8
48	26-2	33.0	15.5	83	31	1.9	8.8	000.0	0	3.3
49	3-9	32.6	15.73	70	29	3.0	8.8	000.0	0	3.7
50	10-16	30.2	14.0	82	48	2.9	6.6	000.0	0	3.1
51	17-23	29.3	13.2	70	48	4.9	7.6	000.0	0	3.2

CERTIFICATE

This is to certify that I have no objection for supplying to copy or any art of this thesis to any scientist at a time through reprographic process if necessary for rendering reference service in a library or documentation centre.

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