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Micropropagation of *Plumbago zeylanica* L.: An Important Medicinal Plant of India

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ABSTRACT

Background and Aim: *In-vitro effrct of various growth regulators on propagation of Plumbago Zeylanica L., a medicinally herbs of India was observed in this study. C. Paniculatus has been used in the various ancient traditional system.*

Materials and Method: *Healthy nodal segments of the herb was used as explants with basic MS medium for shoot initiation and multiplication containing various combinations of different growth regulators. MS full and $^{1/2}$MS were used for rooting of plantlets with 25 to 200 mg/l Activated Charcoal (AC).*

Result: *Maximum mean number of initiated plantlets 1.7 ± 0.48 with mean length 2.7 ± 0.82 were found in MS medium treated with lowest concentration of BA (0.44 µM), while maximum mean number of multiplied plantlets 19.4 ± 0.96 with mean length 6 ± 0.94 were found in MS medium treated with 4.44 µM. Maximum mean number of roods 12 ± 1.56 with Mean length 8.92 ± 0.75 was observed on $^{1/2}$MS medium with 125 mg/l activated charcoal. The rooted plantlets were successfully hardened in 1:1:1 ratio of sand: soil: vermicompost and successfully established in soil.*

Key words: *Plumbago Zeylanica L., Micropropagation, Medicinal Plants, Plant Tissue Culture*.

INTRODUCTION

Plumbago zeylanica, commonly known as Ceylon leadwort, doctorbush or wild leadwort, is a species of plumbago. *P. zeylanica* is a herb, native to SE Asia and grows wild in India. It has been used by rural and tribal people for hundreds of years as a traditional system of medicine. This medicinal plant has various names in various part of India like 'Safaid-sitarak' in Bengali, 'Ceylon leadwort, The Ceylon Leadwort, White Plumbago, White leadwort, Wild Plumbago, Lead wort' in English, 'Chitrak, Chita' in Hindi.

P. zeylanica has clinically significant for various diseases, especially inflammation, leprosy, scabies, ringworm, dermatitis, ulcers, haemorrhoids, and hookworm[28]. The roots are reported abortifacient, while powdered bark, root or leaves are used to treat gonorrhoea, syphilis, tuberculosis, rheumatic pain, swellings, and wound healing[27].

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In Ayurvedic and Unani system of medicines, the plant has significant anticancer[19,30], antitumor[31], anti-inflammatory[20], antioxidant[24,25], antimycobacterial[18], and antimicrobial activities[1,23,29]. This plant have potent mosquito larvicidal activity[15,21].

In this plant, various biologically active compounds like naphthoquinones, binaphthoquinones[4,8,11], coumarins[22], meroterpenes[17], triterpenoids[5,10], anthraquinones[12], steroids[9], naphthoquinone and tdifuranonaphthoquinones[13,14,16] were reported.

The plant is Extreme and destructivally collected from the wild and over exploited by pharmaceutical industries for the procurement of naturally occurring secondary metabolite (plumbagin).

Only few works are done on cultivation, breeding and improvement of *P. zeylanica*. The objective of this study was to develop an improved regeneration protocol from nodal explants of *P. zeylanica* by manipulating concentrations and combinations of phytohormones.

MATERIAL AND METHODS

Collection of Explant

Branches of *Plumbago Zeylanica* L., collected from healthy growing plants from medicinal garden of Prof. T.S. Murthi Science and Technology Station Obedullaganj, Raisen(M.P.)

Sterilization of Explant

Nodal explants were cut and washed in running tap water to remove the superficial dust particles and mud adhering to its surface. Explants were washed with sevelon (3-4 drops/100ml) in a vial by gentle agitating conditions. The explants were thoroughly rinsed with distilled water for several times. Again these explants were dipped in to the 1% fungicide (Bavistin) treatment was given for 15 minutes and then washed with distilled water. For surface sterilization, Explants were transferred to sterile empty flasks under aseptic conditions and given a quick dip in 70% alcohol and subsequently they were washed in distilled water. After that, the explants were surface sterilized with different concentration of sterile ($HgCl_2$) for different duration as per the treatment to find out the best treatment for sterilization of explants. To remove the traces of sterile explants were washed in sterilized distilled water at least 5-6 times. The procedure was carried out in the inoculation chamber under laminar air flow hood.

Preparation of MS Medeium

Culture media was prepared as per described method of Murashig and Skoog and different growth regulator was added as per requirement. For the initiation of ex-plant various concentration of BA (0.44 to 2.22 µM) alone and with IA (0.57 to 1.71µM) and NA (0.54 to 1.61µM) were used, while concentration of BA (0.44 to 2.22 µM) alone and IA (0.57 to 1.71 µM) and NA (0.54 to 1.61 µM) with BA (0.44 to 2.22 µM) were used for multiplication. MS full and $^{1/2}$MS were used for rooting of plantlets with 25 to 200 mg/l Activated Charcoal (AC) combination adding 30 g/l sucrose and 5.7% agar. The hormones used for experiment were taken from stock solutions, which were previously prepared and kept under cold condition in refrigerator. The pH of the medium was adjusted to 5.7 with 0.1 NaOH before autoclaving at 15 lbs and 121°C for 18 min.

Aseptic Inoculation of Explant

Nodal segments about 0.5-0.8 cm were prepared aseptically and were implanted vertically on Surface disinfected nodal explants were inoculated onto full strength MS medium fortified with specific concentrations of growth regulators. The cultures were incubated at a constant temperature of 26±2°C with 16±1 h photoperiod (3000 lux).

RESULT AND DISCUSSION

Surface Sterilization and Induction of Axillary Shoots

Treatment of explants with 0.1% $HgCl_2$ for 3 minutes resulted 100% contamination-free viable cultures. Final observation after 3-4

weeks showed that MS media supplemented with 0.44 µM mg/l of BA proved to be most capability in shoot induction. On this medium an average of 1.7 ± 0.48 shoots with mean shoot length 2.7 ± 0.82 cm were obtained (table 1, figure 1A and 2).

Shoot Multiplication

Shoot multiplication is depending on different concentration of plant growth regulators. Sometimes BA increasing is best for shoot or just opposite. Activated auxiliary shoots from the nodal explants and transfer to fresh medium containing BA alone and combination of BA with NA and IA to establish a stock of shoots used for *in vitro* multiplication. When we look Results in the present study showed the essential of plant growth regulators for *in vitro* multiplication, as the shoots cultured on basal medium did not multiply and become dead. BA at a concentration of 4.44 µM just gave an average of 19.4 ± 0.96 shoots with mean shoot length 6 ± 0.94 cm after 3-4 weeks of culture (table 2, figure 1B and 3). Increasing the concentration of BA, a decrease in shoot multiplication rate was observed. However, comparative number, length and health of shoots on media with BA + IA/NA were not good. An improved protocol was developed by Ceasar *et al*[2]., for the micropropagation of *P. zeylanica* L. from nodal explants. The best response of shoot induction was observed on M) basal medium supplemented with 1.0 mg L-1 thidiazuron (TDZ) and 1.0 mg L-1 Kinetin (KN). Gbadamosi and Egunyomi[7], obtained the highest multiplication rate of the explants using MS medium supplemented with naphthalene acetic acid (NAA) (0.01 - 0.05 mg/l) and benzyl amino purine (BAP) (2.0 - 4.5 mg/l). Sivanesan and Jeong[26], found a maximum number of shoots (38 ± 1.3 per explant) when cultured on MS liquid medium supplemented with 1.0 mg•L-1 BAP, 0.5 mg•L-1 IBA and 2.0 mg•L-1 adenine sulfate. Dohare *et al*[6]., recorded, the excellent shoot formation in the combination of 1mg/l BAP+ 1mg/l NAA and maximum shoot length was recorded (5.38±0.99 cm.). Chandravanshi *et al*[3]., found Maximum plantlets (15.8±1.81) in MS medium treated with 13.3µM N6-benzyl amino purine and 135.74µM Adenine Sulphate at multiplication stage.

In vitro Rooting

After 30 Days of growth, rooting growth is rarely increase day by day in best culture. The multiple-shoot clumps produced on this medium were transferred to solidified MS growth-regulator-free medium for shoot elongation and rooting. On opposite, shoots were also observed for rooting on full or half-strength MS medium with Activated Charcol ensuing excellent response for root induction. Maximum rooting was recorded in medium containing 100 mg/l Activated Charcol Supplemented with ½ MS medium (figure 1C). On this medium an average of 12 ± 1.56 roots with average root length 8.92 ± 0.75 cm was observed after 3-4 weeks (table 3, figure3). Ceasar *et al*[2]., found best roots on half strength MS basal medium containing 1.0 mg L-1 indole-3-butyricacid (IBA). Sivanesan and Jeong[26], observed best rooting on half-strength MS medium containing 0.5 mg•L-1 NAA and 3% (w/v) sucrose. Dohare *et al*[6]., observed that the regenerated shoots were rooted on half strength MS medium supplemented with 1 IBA (0.2-1.0) mg/l and IAA (0.2-1.0) mg/l. But the maximum number roots (7.00±0.98) were produced when the medium supplemented with IAA similarly maximum root length (6.99±0.89cm) contributed by IBA at 1.0 mg/l on MS medium. Chandravanshi *et al*[3]., recorded Highest rooting average (16.10±1.10) on 1/2MS medium with 135.74µM AS.

Sivanesan and Jeong[26], induced The highest percentage of callus when stem explants cultured on MS medium supplemented with 2.0 mg•L-1 BAP and 1.5 mg•L-1 IAA. The greatest percentage of shoot induction (100%) with a mean of 34.2 shoots obtained from callus was cultured on MS medium supplemented with 0.75 mg•L-1 BAP, 1.0 mg•L-1 IAA, NAA and adenine sulfate each.

***Table 1: Effect of plant growth regulators on *in vitro* axillary shoot induction in *P. Zeylanica* L.**

S.No.	MS + PGR (µM)			Observations after 25 days	
	BAP	IAA	NAA	Mean shoot number	Mean shoot length (cm)
Control	0	0	0	0.2 ± 0.42	0.5 ± 1.08
1	0.44 µM	0	0	1.7 ± 0.48	2.7 ± 0.82
2	0.88 µM	0	0	1.4 ± 0.69	1.9 ± 1.10
3	1.33 µM	0	0	1 ± 0.82	1.7 ± 1.567
4	1.77 µM	0	0	0.7 ± 0.82	1.3 ± 1.41
5	2.22 µM	0	0	0.4 ± 0.69	1 ± 1.63
6	0.44 µM	0.57 µM	0	0.8 ± 1.03	1.4 ± 1.83
7	0.88 µM	1.14 µM	0	0.9 ± 0.99	1.6 ± 1.77
8	1.33 µM	1.71 µM	0	0.8 ± 0.91	1 ± 1.15
9	0.44 µM	0	0.54 µM	1 ± 0.94	1.3 ± 1.41
10	0.88 µM	0	1.07 µM	0.7 ± 0.82	1.2 ± 1.39
11	1.33 µM	0	1.61 µM	0.9 ± 0.87	1 ± 0.94

***Table 2: Effect of plant growth regulators on in vitro axillary shoot multiplication in *P. Zeylanica* L.**

S.No.	MS + PGR (µM)			Observations after 25 days	
	BAP	IAA	NAA	Mean shoot number	Mean shoot length (cm)
Control	0	0	0	4.5 ± 0.70711	3.1 ± 0.73
1	0.44 µM	0	0	13.8 ± 1.0328	3.3 ± 0.67
2	0.88 µM	0	0	19.4 ± 0.966092	6 ± 0.94
3	1.33 µM	0	0	17.4 ± 1.429841	5.3 ± 0.82
4	1.77 µM	0	0	14 ± 1.88562	5.2 ± 0.67
5	2.22 µM	0	0	13.9 ± 1.85293	4.1 ± 0.87
6	0.44 µM	0.57 µM	0	16.5 ± 2.54951	5.1 ± 1.19
7	0.88 µM	1.14 µM	0	12.8 ± 1.398412	5.3 ± 1.15
8	1.33 µM	1.71 µM	0	11.2 ± 1.47573	3.3 ± 0.94
9	0.44 µM	0	0.54 µM	13.4 ± 1.42984	4 ± 1.63
10	0.88 µM	0	1.07 µM	12.1 ± 1.96921	3.2 ± 1.22
11	1.33 µM	0	1.61 µM	11.9 ± 1.59513	4 ± 1.41

***Table 3: Effect of auxins on in vitro rooting in *P. Zeylanica* L.**

S. No.	Medium	Activated charcoal (mg/l)	No. of roots/ Plantlets (Mean ± SE)	Root length (Mean ± SE)
1.	Control	0	5.2 ±0.9189366	5.76 ±0.5146736
2.	MS Half	0	5.7 ±0.8232726	6.26 ±0.6686637
3.	MS Half	0	6.5 ±1.3540064	6.52 ±0.8804039
4.	MS Half	25	6.7 ±1.2516656	6.6 ±0.843274
5.	MS Half	50	10.2 ±1.9321836	6.76 ±1.176813
6.	MS Half	100	11.8 ±1.8135294	8.92 ±0.755424
7.	MS Half	125	12 ±1.56347192	6.26 ±1.78835741
8.	MS Half	150	9.2 ±2.2509257	5.78 ±0.7814516
9.	MS Half	200	7.6 ±2.5905812	6.07 ±1.2419071

***Note: Each treatment consisted of 10 replications. Data (Mean ±SE) were recorded after 20 days of culture.**

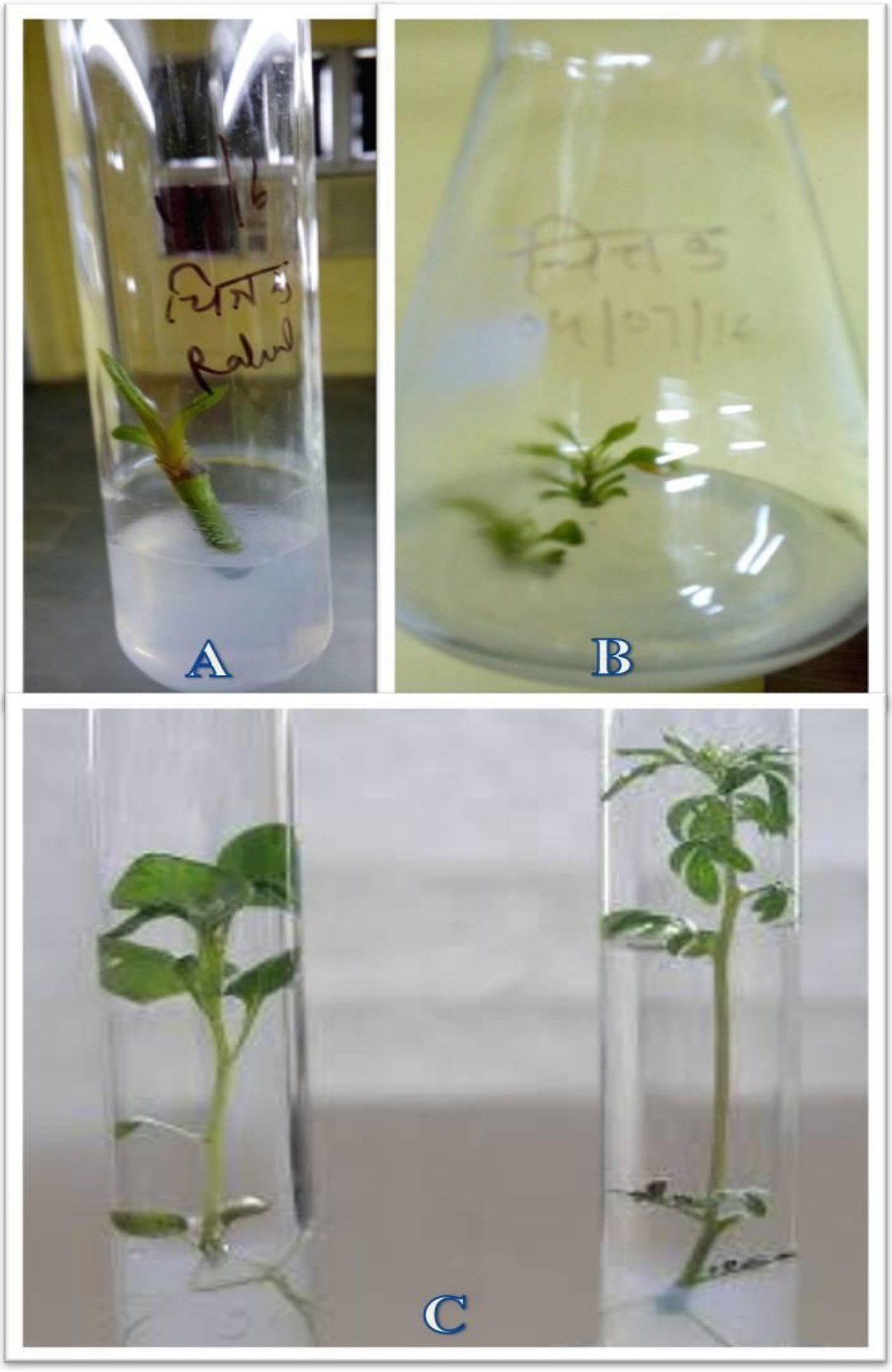

Fig. 1: Micropropagation of *P. Zeylanica L.*(A) Initiation (B) Multiplication (C) Rooting

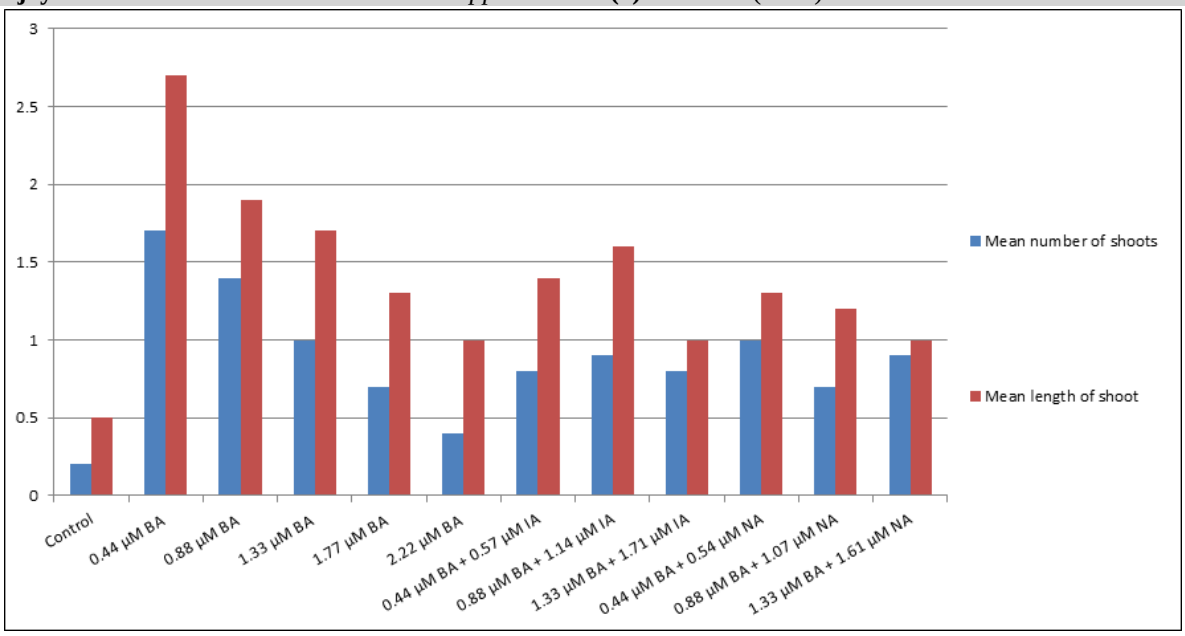

Fig. 2: Effect of plant growth regulators on *in vitro* axillary shoot induction in *P. Zeylanica* L.

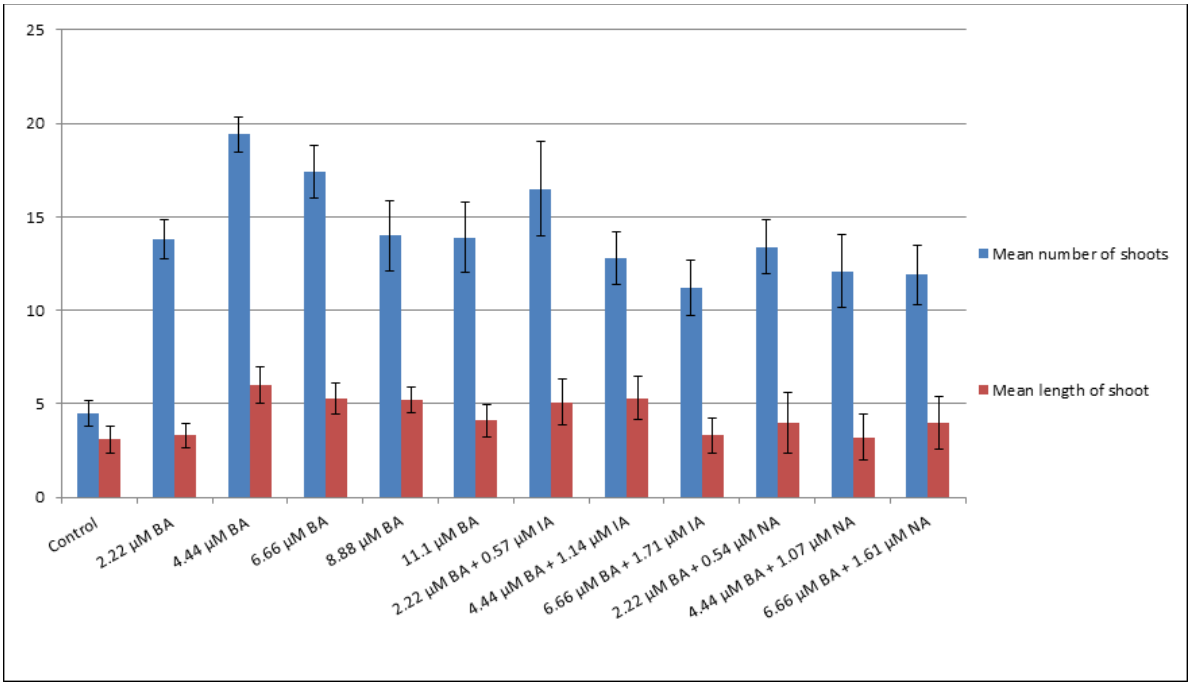

Fig. 3: Effect of plant growth regulators on in vitro axillary shoot multiplication in *P. Zeylanica* L.

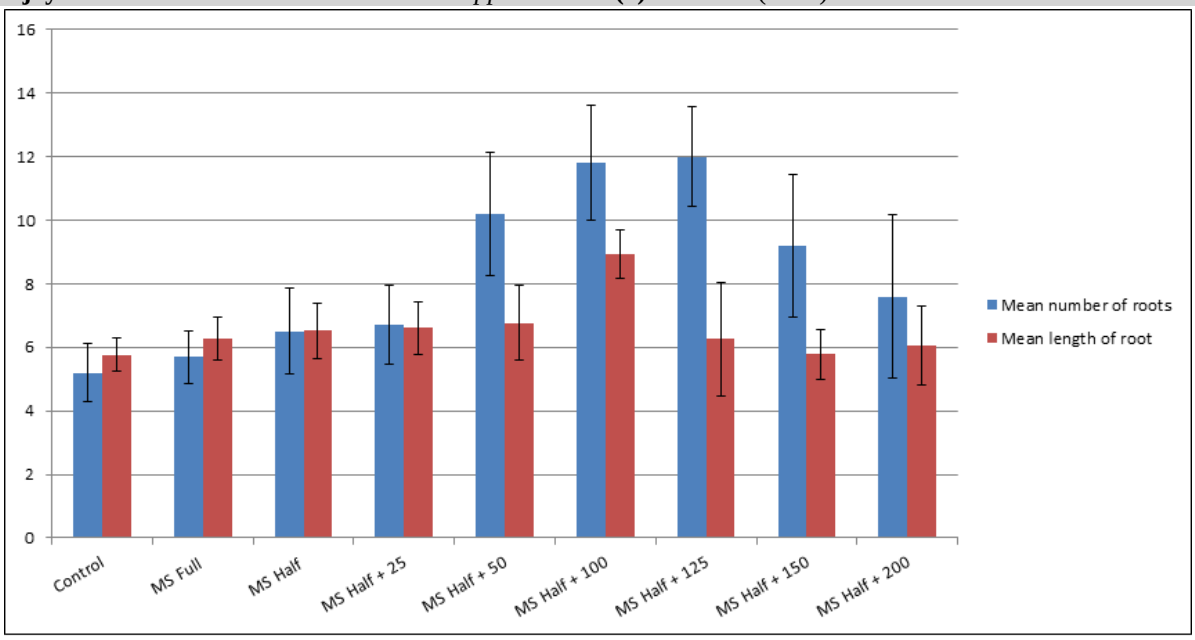

Fig. 4: Table 3: Effect of auxins on in vitro rooting in *P. Zeylanica* L.

CONCLUSION

P. Zeylanica L. has always been a topic of interest of researchers. From tissue culture point of view several studies have been performed to propagate the plant *in vitro*. The highlights of the study are Use of low concentration of plant growth regulators and minimization of time required for field transfer of tissue culture raised plantlets. Free plants produced open the scope for utilization of plant material for antimicrobial testing and suitable pharmaceutical preparations. Apart from this in vitro propagation of *P. Zeylanica* L showed a highest rate of multiplication which cannot be seen in naturally found species of *Celastrus Paniculatus.* The Malkangani research will give a new insight of research in medicinal components of plants through various advance techniques.

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