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Effect of 2-4D on Callus Induction and Effect of BAP,Kinetin, IAA on Shoot multiplication, Elongation and RootInitiation of An Important Medicinal Plant Coleus Forskohlii and Influence of magnetic field

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Abstract:-We have developed an efficient and large scale in vitro clonal callus induction and embriod formation. We have also developed an efficient and large scale in vitro clonal propagation of valuable medicinal plant by enhanced shoot crown explants proliferation. Crown explants from Coleus forskohlii initially treated with Bavistin 0.1% and 0.1% Endosulphan and 0.05 % Fluconazole were cultured in solidified modified murashige and skoog (MS) media, each with different hormonal combination for the establishment of cultures, multiplication and rooting of plants. Several plant growth regulators BAP (6 benzyl aminopurine), kinetine (Kn), IAA (indole acetic acid) alone or in combination were tested for their capacity to induce shoot from shoot crown explants. For establishment of the shoot crown explants kinetin and BAP was necessary. We got huge clusters of Coleus forskohlii with modified MS media supplemented

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with BAP ,Kn , IAA at pH 5.7. These clusters of plantlets were obtained after 4 weeks of growth in the media. High multiplication efficiency, phenotypic stability and yield ensure the efficacy of the protocol developed for the production of medicinal herb Coleus forskohlii.2-4 D(2 mg/l) is responsible for callus induction and , callus were transferred to MS medium with lower concentrations of 2mg BAP and 1mg indole acetic acid (IAA)for embroid formation.. The objective of the study was to investigate shoot multiplication on MS (Murashige and Skoog) media supplemented with 6 Benzyl Amiopurine(BAP) Kinetin(Kn and IAA) showed that which the number of bud formation in shoot cultures of Coleus forskohlii during the initiation stage increased proportionately with the concentrations used However, the highest concentration of BAP (2mg/l) and 2 mg/l Kn simultaneously increased the formation of shoots.After the first apical bud appeared, explants were transferred to MS medium with lower concentrations of 2mg BAP and 1mg indole acetic acid (IAA). Proliferation media supplemented with media supplemented with BAP and IAA showed enhanced shoot initiation and multiplication and media supplemented with Kinetin(2mg/l) and (2mg/l) BAP showed enhanced multiplication and elongation Under the electric field we observed better result.

Key words: Coleus forskohill, Medicinal herb, Shoot culture, Callus culture, Micropropagation, Plant Regeneration.

INRODUCTION

Plant tissue culture is one area of biotechnology that had a dramatic impact on agriculture[1-39]. Plant tissue culture is the only way to increase the number of plant within a short time period. Theoretically, a single cell or piece of plant tissue can produce an infinite number of new plants. The main industrial goal of plant tissue culture is to produce a large number of plants in a month instead of years. Medicinal plant *Coleus forskohlii* (Sanskrit name *Makandií*) is endogenous medicinal plant to India and distributed in Shimla eastwards to Nepal, in the hills of Bihar, Gujarat and peninsular India. It is common on dry, barren hills and is cultivated in Maharashtra, Gujarat and other parts of India. Fasciculate roots of *Coleus forskohlii* is used as tonic and constitute important ingredient for asthma, intestinal colic, uterine cramps, as well as painful urination. Chemically, it is a plant rich in alkaloids, which are considered to have a high probability of influence on the biological systems. The production of high quality planting material propagated from vegetative parts has created global trading area, benefited growers,

farmers, nursery owners and improved rural employment. However, there are still major opportunities to produce and distribute high quality medicinal plant *Coleus forskohlii*. The main advantage of tissue culture technology lies in the production of high quality planting material that can be multiplied round the season basis under disease-free conditions. This species perpetuates both vegetatively by tubers and sexually through seeds. It has a sluggish perpetuation through seed on account of poor seed germination (16%). Further, seed-raised progenies display an appreciable spectrum of variation due to its preferential out-crossing nature. As the tubers constitute the commercial product, there is always scarcity of the propagating material. In this situation, plant tissue culture offers an effective means for rapid multiplication of this species through meristems and tip culture. In conventional cultivation, plantation is possible only once a year (June-July). Micropropagation of medicinal plants through tissue culture technology has been described previously but most of them have reported on micropropagation by means of stimulating auxiliary shoot from shoot tip as explants. Clonal improvement of plant and conservation of genetic resources has been described by many research workers. Micropropagation offers an opportunity to achieve plant stock that is fee of disease and pest. Genetic integrity of plants is maintained through proper protocol for commercial propagation of *Coleus forskohlii.* In vitro plants are usually susceptible to genetic alteration due to culture stress. Propagation of medicinal plants through tissue culture technology has been described but most of them have reported on micropropagation by means of stimulating auxiliary shoot from shoot tip as a explant. *Coleus* is an annual herb with tubers, crown leaf and flower as different parts. *Forskohlii* has very good Ayurvedic medicinal use. *Coleus forskohlii* is a traditional medicinal plant and has worldwide acceptability. It is a rich source of alkaloids, vitamins, minerals, proteins, carbohydrates, steroids, and polysaccharides. A number of health tonics (sexual tonics) are prepared from *Coleus*. *Coleus* species have been used as an herbal medicine to treat various disorders of the cardiovascular, respiratory, gastrointestinal, and central nervous systems.

Forskolin is a diterpene that acts directly on adenylate cyclase. Adenylate cyclase is an enzyme that activates cyclic adenosine monophosphate, or cyclic AMP (cAMP) in the cell. Cyclic AMP promotes the breakdown of stored fats in animal and human

fat cells. It regulates the body's thermogenic response to food, increases the body's basal metabolic rate, and increases utilization of body fat. It may also release fatty acids from adipose tissue, which results in increased thermogenesis, loss of body fat, and theoretically increased lean

body mass. Forskolin increases cAMP a c c umu l a t i o n , and therefore stimulates lipolysis. So, with high concentrations of forskolin, cAMP and lipolysis increase. Enhanced lipolysis increases fat degradation and fat usage as a fuel in the body. This may promote fat and weight loss. It is thought that supplementing with forskolin may enhance fat loss without loss of muscle mass. A previous proof-of-concept preliminary study was conducted to test the effects of CF on body composition. The study used a population of six overweight, but o t h e r w i s e healthy, women (BMI > 2 5) who ingested forskolin twice daily for eight weeks. Each subject maintained her previous daily physical exercise and eating habits. The results of the study showed a significant decrease in the mean values for body weight and fat content using bioelectrical impedance (BIA) methodology. Lean body mass significantly increased compared to baseline. No side effects were reported. This preliminary study showed that, given 25 mg of forskolin twice a day, overall body weight could improve by increasing lean body mass and by decreasing weight from body fat. The present communication reports an efficient regeneration system via multiple shoot bud induction from shoot crown explants in *Coleus forskohlii* and standardization of optimum protocol for rapid
mass scale propagation.

Material and Methods

Plant material: Dormant roots of *Coleus forskohlii* were collected from Asansol, West Bengal, India. Explant was prepared from selected sprouted roots crown shoot buds. Root with sprouted crown shoot buds were sprayed with gentamycin (500 mg/l), 0.5% Fluconazole, 0.1% Bavistin and 0.1% Endosulphan at interval of 24 hours for seven days before the explant were used for tissue culture. Appropriate thin slices of shoot crown (5-6 mm) possessing one juvenile adventitious shoot buds were thoroughly washed under tap water and rinsed four times in distilled water and after that the explants were submerged in distilled water which contained 0.05% Fluconazole, 0.1% Bavistin and 0.1% Endosulphan for four hours. This was followed by surface sterilization with 1% sodium hypochlorite and 1-2 drops tween 20 for 15 minutes. After four washing in sterile distilled water explants were trimmed from the sides
and used as explants.

Culture medium and culture condition: Crown shoot buds were placed on semisolid modified MS medium

supplemented with different concentration 2-4 D for callus induction . Callus are transferred on semisolid modified MS medium supplemented with different combinations BAP(0.5, 1.5, 2.0, 4.0, 8.0, 12.0 mg/l) and 1mg/l IAA for embroid formation.Shoots are produced with the same concentration. shoot were placed on semisolid modified ms medium supplemented with different combinations of Kn(0.5, 1.5, 2.0, 4.0, 8.0, 12.0 mg/l) and BAP(1.5, 2.0, 3.0, 4.0 mg/l) or BAP or Kn with IAA,IBA and Thiamine HCl for shear proliferation. Multiplication was observed in modified MS medium supplemented with different combination of hormones. MS medium was supplemented with

100 mg Inositol, Thiamine HCl(0.5mg/l), , 30gm/l sucrose with different combinations plant(Kn, IAA, BAP, IBA regulators. pH of the medium was adjusted from 5.7 with 0.1 N KOH or 0.1 N HCl prior to adding 8.5gm/l agar (qualigens). 50 ml of molten medium was dispersed into culture bottle, closed by cap, wrapped with one layer transparent cling film and sterilized at 15 psi pressure for 15 minutes at 1210 C. Cultures were incubated for 16 hours under fluorescent light (2000-3000) lux at 24 +_ 20 C.

Induction of rooting and acclimatization: Rooting was initiated in the half strength modified ms basal medium containing 30 gm/l sucrose (w/v) supplemented with different concentrations of IAA or IBA and/or Thiamine HCl. All the cultures were incubated for 16 hours at 24 + 20 C under fluorescent light (3000-4000) lux. Number of roots per shoot were assessed after four weeks. About 2-4 cm long rooted plants were thoroughly washed to remove the adhering media and also treated with 0.1% Bavistin and 0.1% m-45 solution. Treated plantlets were planted in earthen or plastic pots containing a mixture of loam soil, sand and varmicompost (1:1:1 v/v). The plantlets were hardened in presence of 90-95% relative humidity. The plants were irrigated at 2-4 days interval and were supplied with 1/8 strength organic solution containing 0.1% bavistin and 0.1% M-45. Plants were transferred after 4 weeks into open field. Survival rate was evaluated after 4 weeks of plantation. Each experiment was repeated four times

with 5 cultures per treatment.

RESULTS AND DISCUSSION

The rapid production of pathogen free plants is one of the fundamental goal of the plant tissue culture.

Fungal contamination was serious problem during the establishment of *Coleus forskohlii* explant on medium Pretreatment is required before the explant was used for plant tissue culture. Selected

field grown plants were sprayed with gentamycin (500 mg/l), 0.5% fluconazole, 0.1% bavistin, 0.1% endosulphan at interval of 24 hours for 7 days before the explant were used for tissue culture. Pretreatment was very much useful in reducing the rate of contaminations. About 70% crown buds from sprouted *coleus* and 40% from the field grown plants were free of contamination and retained their regeneration capacity. Crown shoot buds were placed on semisolid modified MS medium supplemented with different concentration 2-4 D for callus induction . Callus are transferred on semisolid modified MS medium supplemented with different combinations BAP(0.5, 1.5, 2.0, 4.0, 8.0, 12.0 mg/l) and 1mg/l

IAA for embroid formation.Shoots are produced with the same concentration. **2-4 D(** 2 mg/l) is responsible for callus induction and , callus were transferred to MS medium with lower concentrations of 2mg/l BAP and 1mg/l indole acetic acid (IAA)for embroid formation.. shoot were placed on semisolid modified ms medium supplemented with different combinations of Kn(0.5, 1.5, 2.0, 4.0, 8.0, 12.0 mg/l) and BAP(1.5, 2.0, 3.0, 4.0 mg/l or BAP or Kn with IAA,IBA and Thiamine HCl for shear proliferation. There was no sign up growth when crown shoot buds were cultured in modified ms media without cytokinine or auxin. In the present study high concentration of cytokinine reduce the number of shoots as well as shoot length. The shoot crown bud of *coleus forskohlii* were cultured on modified ms medium and supplemented with various concentration of Kn, BAP, individually and in different combination of plant regulatory hormone for shoot regeneration. MS media was found to support a greater response for shoot Regeneration than B5 medium. MS medium was found to be more effective than other medium for medicinal plants[24,25,26,27]. Various morphogenic responses were obtained with different combination and concentration of either Kn, BAP or with IAA, IBA [table 1]. Experimental observations revealed that higher concentration of cytokinine reduced the number of micropropagated shoots[14] and some research worker reported cytokinine was very critical for shoot elongation of many other plant species[28,29,30]. It was also revealed that BAP alone or in combination with other cytokinine induced shoot organogenesis from shoot tips[31]. In our plant species greater numbers of morphologically distinct multiple shoots were developed in MS medium containing BAP combined with Kn, but some research workers reported that BAP and kinetin combination developed fewer multiple shoots in some species[32]. The minimum number of roots were produced at the base of shoot at low concentration of Kn or BAP or combination of Kn and BAP. Rooting is completely arrested at kinetin concentration more than 6.0 mg/l/. The

number of maximum shoots was obtained in ms medium supplemented with Kn (2.0 mg/l) and BAP (2.0 mg/l), but shoots were varies when the concentration of kinetin was raised [table 2]. The shoot length was strongly affected by high concentration of cytokinine. Experimental observation support that MS medium 1. containing low concentration of auxin along with cytokinine increases the rate of shoot multiplication[6,30,33,34,35,44,45, 46,47,48]. More than hundred shoots/culture was produced at a combination of Kn (2 mg/l) and BAP (2mg/l) and 54 shoots/ culture was produced at a combination of Kn (3.0 mg/l),BAP(2mg/land IAA (2.0 mg/l) [table 1]. The shoot length was strongly affected by high concentration of Cytokinines.

Excised shoots were rooted on 1/2 strength modified MS medium[36,37,38] with different type of auxin. Four week old healthy excised shoots on transfer to 1/2 strength ms medium containing 2% sucrose (w/v) supplemented with 3.0 mg/l IAA formed vigorous fibrous and thick strong roots during the culture of 4 week[table 2]. IBA was most effective in inducing rooting of several other plants[17,39,40,41] effect of IAA and/or combination of IAA and Thiamine HCl was observed in our plant species. Maximum roots / shoots was observed in 1/2 strength ms medium containing 3.0 mg/l IAA[table 2]. Higher concentration of IBA or IAA or combination of IAA and Thiamine HCl was not favourable. Four week old rooted tissue culture plants were planted in earthen or plastic pots. The survival rate was 95%. The acclimatized plants showed normal growth . In conclusion, our protocol has established a reproducible, quick and large scale micropropagation method for *coleus forskohlii*.Noumber of roots and shoots is increased under the electric is observed[42 43].

TABLE 1: INFLUENCE OF CYTOKININE OR CYTOKININE COMBINED WITH AUXIN ADDED TO THE MODIFIED MS MEDIUM ON SHOOT BUD INDUCTION FROM SHOOT CROWN EXPLANT IN *COLEUS FORSKOHLII A*FTER 4 WEEKS OF CULTURE.

BAP (mg/l)	Kin (mg/l	IAA (mg/l)	IBA (mg/	THIAMIN HCL (mg/l	Percentage Explants producing shoots	Mean no of shoot per explant
0	0	0	0	0	0	0
0	0.5	0.1	0	0.5	30	0.49
0	4.0	2.0	0	0.5	50	10.11

2.0	0	1.0	0	0.5	87	15.2
3.0	0	2.0	0	0.5	75	21.12
2.0	2.o	1.0	0	0.5	95	100.11
2.0	3.0	2.0	0	0.5	54	42.20
4.0	8.0	4.0	0	0.5	45	0.18
8.0	4.0	4.0	0	0.5	36	4.75
8.0	8.0	4.0	0	0.5	2	0.20
8.0	12	4.0	0	0.5	82	0.00

TABLE 2: INFLUENCE OF CYTOKININE OR CYTOKININE COMBINED WITH AUXIN ADDED TO THE MODIFIED MS MEDIUM ON SHOOT BUD INDUCTION FROM SHOOT CROWN EXPLANT IN *COLEUS FORSKOHLII* AND INFLUENCE OF MAGNETIC FIELD INTENSITY *A*FTER 4 WEEKS OF CULTURE.

BAP (mg/l)	Kin (mg/l	IAA (mg/l)	IBA (mg/	THIAMIN HCL (mg/l	Percentage Explants producing shoots	Mean no of shoot per explant
0	0	0	0	0	0.11	0.1
0	0.5	0.1	0	0.5	33	0.55
0	4.0	2.0	0	0.5	50.09	11.0
2.0	0	1.0	0	0.5	95.7	16.83
3.0	0	2.0	0	0.5	82.5	23.23
2.0	2.o	1.0	0	0.5	105.27	110.12
2.0	3.0	2.0	0	0.5	59.4	46.42
4.0	8.0	4.0	0	0.5	49.5	0.198
8.0	4.0	4.0	0	0.5	39.6	4.49
8.0	8.0	4.0	0	0.5	2.2	0.33
8.0	12	4.0	0	0.5	90.2	0.099

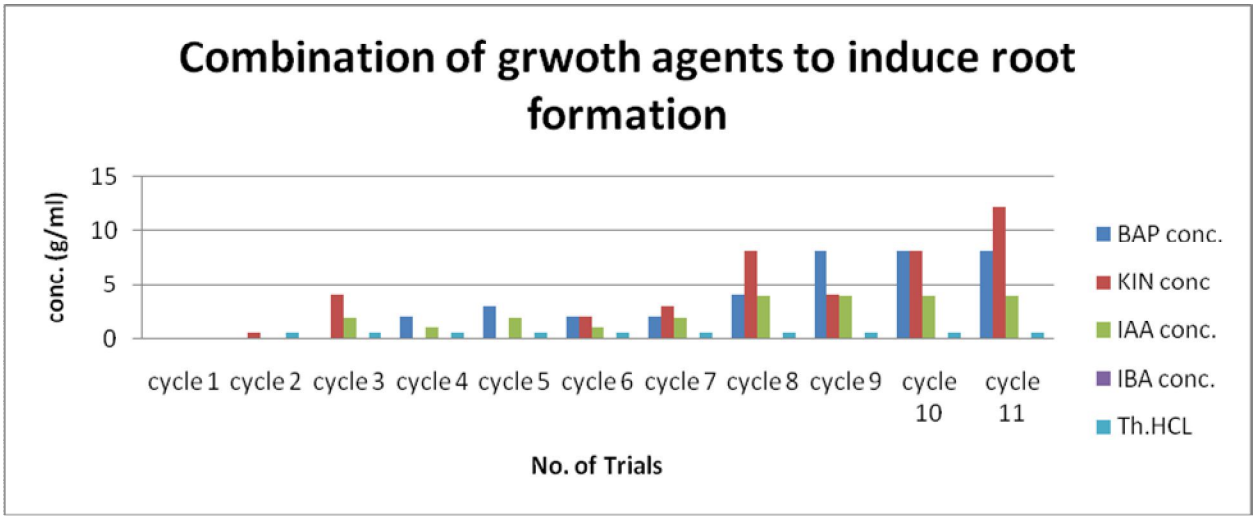
TABLE 3: INFLUENCE OF DIFFERENT CONCENTRATION OF AUXIN (IAA,IBA,NAA) ADDED TO THE ½ STRENGTH MODIFIED MS MEDIUM ON ROOTING OF IN-VITRO FORMED SHOOTS OF *Coleus forskohlii* AFTER 4 WEEKS OF CULTURE.

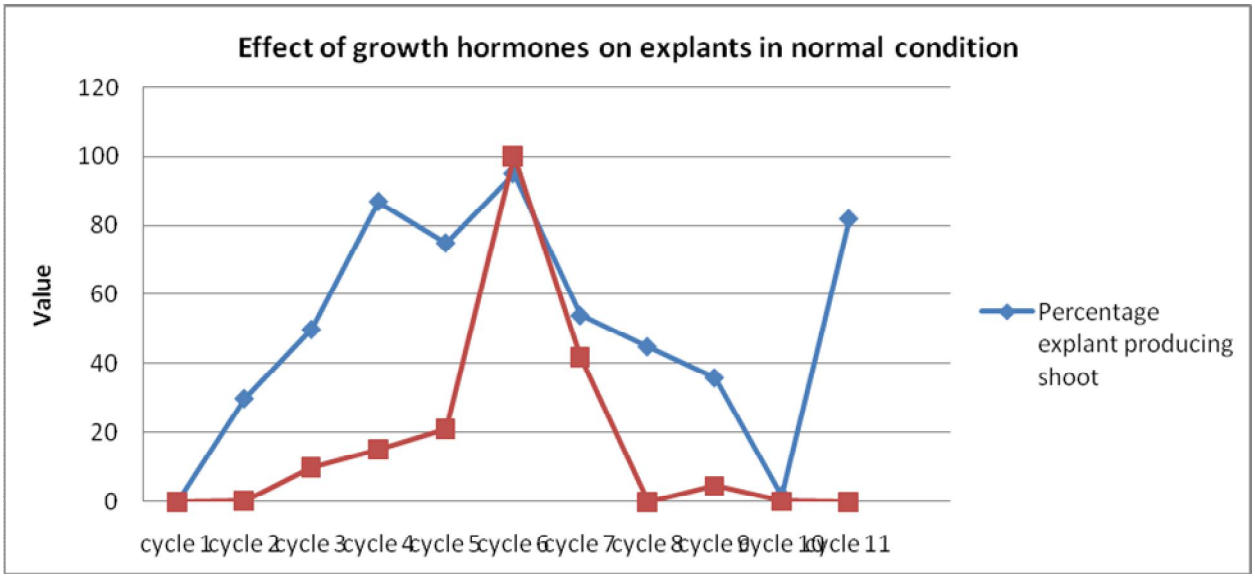
IBA (mg/l)	IAA (mg/l)	Thiamin HCL(mg/	No of rc s/per cultu
0	0	0	0
0	0.1	0.5	7
0	0.2	0.5	9
0	0.5	0.5	11
0	1.0	0.5	65
0	2.0	0.5	75
0.5	2.0	0.5	85
1.0	2.0	0.5	23

TABLE 4: INFLUENCE OF DIFFERENT CONCENTRATION OF AUXIN (IAA,IBA,NAA) ADDED TO THE ½ STRENGTH MODIFIED MS MEDIUM ON ROOTING OF IN-VITRO FORMED SHOOTS OF *Coleus forskohlii* AND INFLUENCE OF MAGNETIC FIELD AFTER 4 WEEKS OF CULTURE.

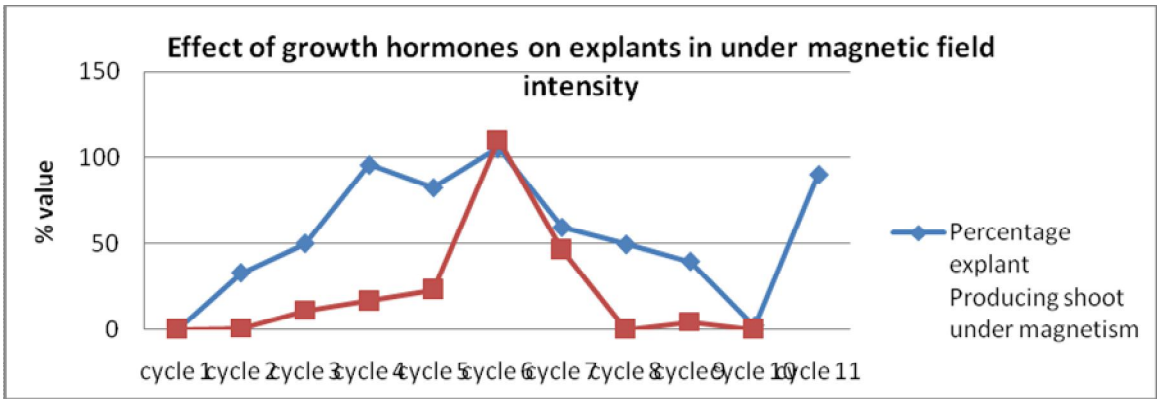
IBA (mg/l)	IAA (mg/l)	Thiamin HCL(mg/	No of root s/p culture
0	0	0	0
0	0.1	0.5	9

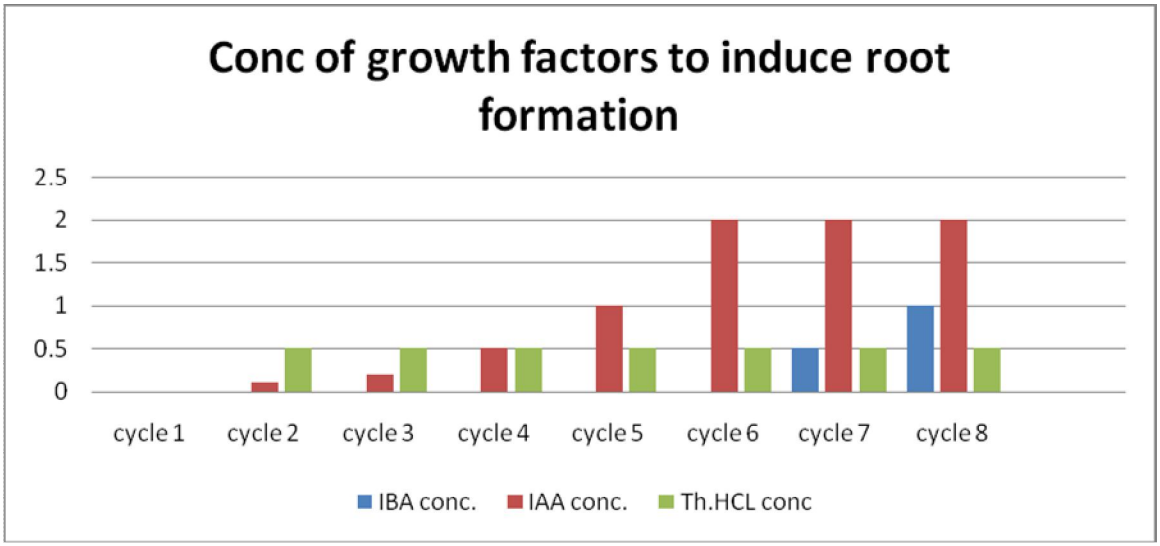
0	0.2	0.5	12
0	0.5	0.5	14
0	1.0	0.5	68
0	2.0	0.5	77
0.5	2.0	0.5	88
1.0	2.0	0.5	26

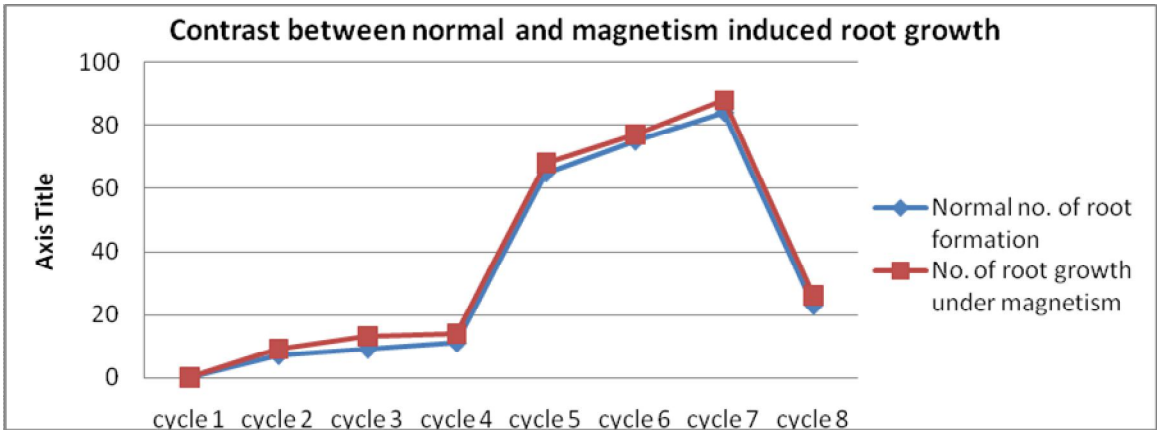




. The exposure to the magnetic field improved the process kinetics. No of shoots and roots Biomass and ethanol yields of fermentations inoculated with treated inoculum were higher than those in the control fermentation, which was inoculated with an untreated inoculums[42,43].







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