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COMPARATIVE STUDY OF *COLEUS FORSKOHLII* CULTIVATION THROUGH MICROPROPAGATION AND CONVENTIONAL FARMING METHODS

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1

ABSTRACT

A trial was carried out to evaluate the productivity of Coleus forskohlii Briq cultivated through conventional farming and micropropagation method. Survival percentages of plant were 92% for conventional propagation and 84% for micropropagation. The survival rate of micropropagated plants were maximum of 76% in vermicompost and minimum of 47% in the soil. The average shoots length, number of leaves and leaf area was greater in the conventionally propagated plants compared to the micropropagated plants.

Keywords:- *conventional farming, micropropagation, etc.*

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INTRODUCTION

Coleus forskohlii Briq is a member of the mint family, Lamiaceae. It grows in the subtropical temperate climates of India, Nepal, Sri Lanka and Thailand. It is indigenous to India and is recorded in Ayurvedic *Materia Medica* under the Sanskrit name 'Makandi' and 'Mayani'. The taxonomic position of *Coleus forskohlii* Briq is as follows:

Kingdom –	Plantae
Division –	Magnolophyta
Class -	Magnoliopsida
Order -	Lamiales
Family -	Lamiaceae
Genus -	*Coleus*
Family -	*forskohlii*

The genus *Coleus* was first described by Loureiro in 1790 and the generic name was derived from the Greek word 'COLEOS' meaning sheath. All the species of *Coleus* have four didynamous, dedinate stamens, and the filaments of the stamens unite at their base to form a sheath around the style. The species name was given to commemorate the Finnish botanist, Forskel. The genus *Coleus* consists of 150 species and the following species viz., C. amboinicus, C. *forskohlii, C.spicatus* and C. *malabaricus* occur naturally. *Coleus forskohlii* is a perennial herb that grows upto 1-2 feet and its leaves are teardrop shaped, shimmering green framing a bright purple centre. The color of leaf varies depending on the amount of shade. A cluster of stalked pale purple or blue flowers branches off a single stem. It bears fasciculate tuberous roots. The root stock is typically golden brown, thick, fibrous and radially spreading. The roots are harvested in fall, when the forskolin content is highest and the root color is brightest.

The existence of traditional medicine depends on plant diversity and the related knowledge of their use as herbal medicine. India is one of the twelve mega diversity hot spot regions of the world and one fifth of all plants found in India are used for medicinal purpose. Nearly 25,000 effective plant based medicines are used in folk medicine by rural communities in India. Both plant species and traditional knowledge are important to the herbal medicine trade and the pharmaceutical industry, while plants provide raw materials and the traditional knowledge prerequisite information. Medicinal plants are important for pharmacological research and drug development, not only when constituents are used directly as therapeutic agents but also as starting material for the synthesis of drugs or as models for pharmacologically active compounds. The world market for plant derived chemicals viz., pharmaceuticals, fragrances, flavors and color ingredients exceeds several billion dollars per years. Classical example of phytochemicals in biology and medicine include taxol, vincristine, vinblastine, colchicine as well as Chinese antimalarial artimisinin and the Indian Ayurvedic drug forskolin. The therapeutic properties of the forskolin, the main diterpene constituent of this plant contributed to the emergence of *Coleus forskohlii* as a taxon of importance in modern medicine. Forskolin is used for the treatment of eczema, asthma, psoriasis, cardiovascular disorders and hypertension, where decreased intracellular cAMP level is believed to be a major factor in the development of the disease process. The presence of yellowish to reddish brown cytoplasmic vesicles in cork cells of *Coleus forskohlii* seeds is unique character of this plant and these vesicles store secondary metabolites including forskolin. Forskolin ($C_{22}H_{34}O_7$, MW 410.5) is an off white crystalline solid with a melting point of 228 – 230^{0}C and UV absorption maxima at 210 nm and 305 nm. Medical research has shown that it can be used for the treatment of allergies, respiratory problems, cardiovascular diseases, glaucoma, psoriasis, hypothyroidism and weight loss. Recently, it was reported as a natural remedy for urinary tract infections (UTI) by enhancing the ability of antibiotics to kill the bacteria that causes 90% of infections in the bladder.

Composition of Coleus

1. The key components of coleus are volatile oils, diterpenes and colenols, but the most important is forskolin.
2. The diterpene forskolin is the primary constituent of coleus which is derived from the root portion of the plant. There are approximately 20 constituents in different parts of the coleus plant, but forskolin and colenols are found in the root part of the plant.
3. The leaf extract of coleus have significantly high amount of polyphenols, flavonols and flavones and high antioxidant activity.

Coleus is normally vegetatively propagated. Contamination of seed material by pathogens (bacteria, virus and fungi) causes severe reduction in the yield. That is why, despite tremendous efforts little success had been achieved in conventional seed plant coleus production scheme. In this event plant biotechnology offers a great potential to complement conventional breeding methodology for coleus improvement and production via plant tissue culture technique. But, lack of budget, limited resource allocation and relatively high recurrent cost (chemical expenses) this technology has been envisaged as a major obstacle in benefitting from this technology in developing countries, particularly in India. *In vitro* propagation by nodal cutting has become an established method of rapid multiplication in many plants viz., potato, blueberry, coleus etc. Micropropagation of coleus has great advantage in cultivation for increased yield and uniformity in germplasm. Hence, in the present investigation a comparison in cultivation methods of coleus was carried out to observe the change on productivity and yield.

Table 1: The composition and percentage of nutrients in fertilizer and amount of fertilizer

Nutrient	Fertilizer	Composition of nutrients	% nutrient in fertilizer	Amount of fertilizer applied
N	Urea	NH_2	46%	17.85 Kg
P	SSP	P_2O_5	16%	22.45 Kg
K	MoP	K_2O	14%	31.98 Kg

MATERIAL AND METHODS

Murashige and Skoog medium (MS, 1962) with sucrose 3% (w/v) and agar 8% (w/v) were used as basal medium throughout the experiment. Growth hormones, 6 benzylaminopurine (BAP) and kinetin (Kn) were added to the basal medium. Sprouts from sterilized coleus seeds (grown in plant growth chamber) were aseptically cultured in culture tubes containing 5 ml medium. After inoculation cultures were maintained in the growth room under 16 h photoperiod, 2000 – 3000 lux light intensity and 8 h dark period at 23- 25^{0}C. Newly formed shoots measuring 3 to 4 cm in length were excised individually from the clump and transferred to MS basal media supplemented with indole butyric acid (IBA, 5µM) for the rooting. Plants with newly formed roots were carefully planted in polythene bags containing soil mixture having vermicompost, perlite, farm yard manure and soil in varying ratio. After 2 weeks plants were transferred to pots. Field trial was conducted in Kuberpur farm, Agra where average maximum temperature was 26^{0}C and minimum was 8^{0}C during the examination period. The sterilized seed stem terminal cuttings of coleus were purchased. These stem terminal cuttings were almost 3 cm in diameter. An experiment in a randomized complete block design (RCBD) with three replications was established. Each gross plot had eight furrows spaced at 45 cm x 15 cm. plant to plant spacing was 15 cm giving a plant population of 210 plants per block. Urea, single super phosphate and Murate potash were used as fertilizer source of nitrogen, phosphorus and potassium respectively and were applied at standard recommended rates as: N- 180 kg, P- 80 kg/ha and K- 100 kg/ha. There after irrigation was carried out according to crop water requirements. A comparative observation of the following parameters was done on plants grown by conventional methods and plants developed by micropropagation. The observations were taken for plant growth responses namely: germination, biomass and yield performance.

RESULT AND DISCUSSION

The present investigation was planned to compare the yield performance of *Coleus forskohlii* Briq. grown by conventional method of propagation and micropropagation. The results obtained are described below:

Germination:

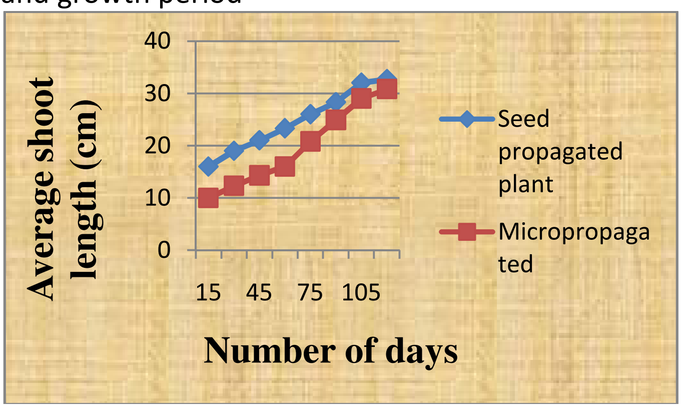
In the field, the germination was considered when radicals came out from soil and attained a length of 2 cm. in the *in vitro* propagation the survival rate of micropropagated plants was taken as criterion for germination at hardening stage. Here, 86.23% germination was attained in the field. Pot experiment showed less germination than field experiment. In pots the best results i.e. 78% was obtained in the mixture of vermicompost, perlite, FYM and soil in 1:1:1:1 ratio. Seed treatment improved the germination ability in the field. Shoot culture initiation and shoot proliferation study was also carried out before hardening. For shoot culture initiation MS media supplemented with 2µM BAP was used. Shoot cultures inoculated on MS supplemented with Kn showed increase in the multiplication. This is in conformity with the findings of Lane (1979), Bhojwani (1980), Garland and Stolz (1981), Bhojwani and Rajdan (1992), Hussain et al. (1990). According to their study in micropropagation, for shot proliferation growth regulators, especially cytokinins are one of the most important factors affecting the response. A range of cytokinins (Kn, BA, 2-ip and zeatin) has been used in micropropagation work.

Table 2: characteristics of plants produced from seed stem terminal cuttings and micropropagation data were recorded after 15 days

1Days after germination	Seed propagated		Micropropagated	
	Shoot length	No. of leaves	Shoot length	No. of leaves
15	16.0±0.46	09.0±0.46	10.0±0.46	6.0±0.46
30	19.0±0.93	12.0±0.46	12.3±0.82	8.67±0.26
45	21.0±0.93	13.3±0.70	14.3±1.18	11.3±0.26
60	23.3±0.26	19.7±0.26	16.0±1.27	14.3±0.26
75	26.0±0.46	21.7±0.26	20.8±1.36	15.7±0.53
90	28.3±0.26	18.3±0.53	24.9±0.53	16.2±0.53
105	32.0±0.81	12.5±0.93	29.0±1.23	9.5± 0.46
120	32.7±0.54	7.33±0.17	30.8±0.71	4.67±0.33

#values are mean ± standard error.

Figure 1: Showing relation between shoot length and growth period



Biomass:

Shoot Length

Data presented in the table 2 indicates that the average shoot length was more in seed propagated plant. It was 32.7 ± 0.54 cm. the average shoot length of micropropagated plants were 30.8 ± 0.71 cm. in the present experiment plant height increased in response to the fertilizer treatment and the nitrogen fertilizers increased coleus plant height more in the fields. Tiwari et al. (1970) also reported that nitrogen increases plant height in zea mays.

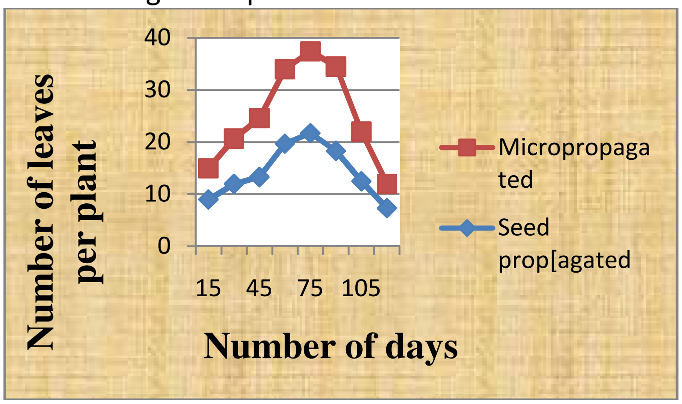
Number of leaves

Data presented in the table 2 showed that the number of leaves increased with days in both seeds propagated and micropropagated plants. After 87 days of showed that the number of leaves increased with days in both seed propagated and micropropagated plants. After 87 days of germination leaves started yellowing and falling. The counting was lowest in number in both seed propagated and micropropagated plants after 120 days. There was a drastic increase in number of leaves up to 72 days of germination then after it showed a decline. In this connection, Moorby and Morris (1967) reported that nitrogen fertilizer plays a significant role in the production of stem and axillary branches, which resulted in greater number f leaves in field. In Zea mays L. Tiwari (1970) reported that nitrogen increases the number of effective leaves at starting stage.

Leaf area

The leaf area also increased with increase in the age of plants in both the methods. The maximum leaf area 14.12 cm^2 was found at 73 days of plant growth in field. In micropropagated plants it was 12.01 cm^2. In the present study, N, P and K were used as fertilizers in the field. Nitrogen fertilizer increased the leaf area which increases the amount of solar radiation intercepted and consequently increases days of flowering and days of physiological maturity, plant height and dry matter production of different plant parts (Krishnippa, 1989).

Figure 2: Showing relationship between number of leaves and growth period



Yield performance

To check the yield performance, the seeds were divided into three grades, these were less than 25 g, between 25 to 75 g and greater than 75 g. table 3 shows that in total the number of seeds was higher in micropropagated plants. The total weight of seeds was 0.36 kg per plant more in seed propagated plants in comparison to micropropagated plants. The total weight of seeds was 1.353 kg per plant in seed propagated plant and 0.982 kg per plant in micropropagated plants. Nitrogen phosphorus fertilizers improved total seed yield of coleus. The fertilizer increases the total leaf area which in turn increased the amount of solar radiation intercepted and more photo assimilates might have been produced and assimilated to seeds. In this regard, Millard and Marshall (1986), Yibekal (1988) reported that yield improvement as a result of nitrogen fertilization could be attributed to increased solar interception. This is in conformity with the findings of Lauer (1986) and Ojala et al. (1990) who observed that high N levels promoted excessive vegetative growth. Coleus seed yield is directly dependent on the supply of N, P may substantially delay leaf senescence leading to enhanced leaf area duration and increased seed yield (Mackerron and Heilbronn, 1985). It has been also reported that the potassium application significantly increased the seed yield of coleus by increasing the size of seeds (Verma and Grewal, 1977).

CONCLUSION

On the basis of above comparison between seed propagation and micropropagation in the present study, it can be concluded that all the morphological and physiological parameters along with the yield attributes were better in seed propagated plants than the micropropagated one.

Table 3: Yield performance of seed seeds as compared to Micropropagated seeds (Seed number and weight/plant)

Grade	Seed propagated		Micropropagated	
	No. of seeds	Wt. of seeds	No. of seeds	Wt. of seeds
< 25	2	0.06	12	0.24
25-75	3	0.25	5	0.39
> 75	7	0.98	1	0.26
Total	12	1.29	18	0.89

The values shown are mean.

Figure 3 A

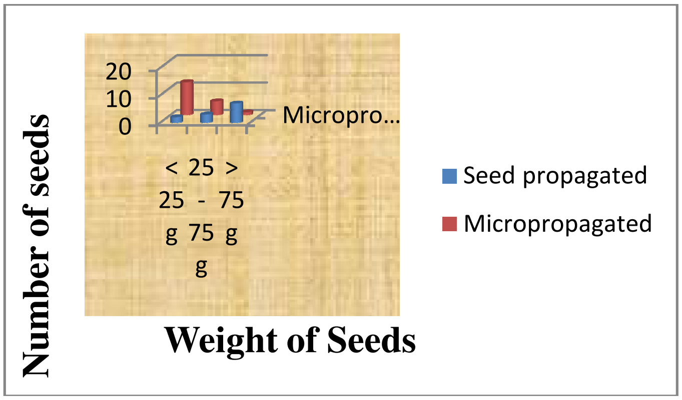


Figure 3 B

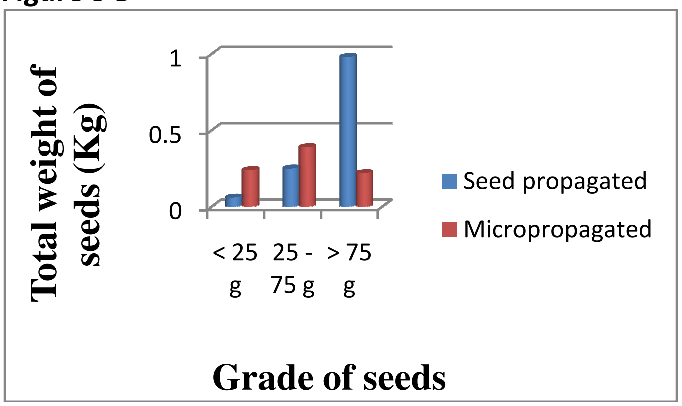


Figure 3: showing the comparative response of seed yield (per plant) under various weight and grades (A) weight of seeds (B) grade of seeds.

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