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CONSERVATION OF *PSORALEA CORYLIFOLIA* AN ENDANGERED IMPORTANT MEDICINAL PLANT THROUGH TISSUE CULTURE AND COMPARATIVE STUDY OF ORGANIC FARMING

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ABSTRACT
Psoralea corylifolia L. commonly known as 'Babachi' is an important endangered medicinal plant (Indian bread root) belongs to family Fabaceae (Leguminosae). Due to its versatile properties the plant is in tremendous demand but due to its overexploitation and poor seed germination ability it has been depleted in its natural environment. The plant is propagated by seed germination but because of hard seed coat it shows low rate of seed germination. An alternative method for its conservation and efficient rapid mass propagation is thus need of the hour. Therefore, the present work was aimed to develop an efficient *in vitro* propagation protocol for its rapid and large scale production. Different combinations of growth regulators in MS media were used to promote its shoot and root formation. Maximum (80.0%) shoot induction of *Psoralea corylifolia* through mature meristem was observed on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA. While as induced shoots shows highest (85.0%) and profused shoot multiplication rate on medium supplemented with 1.5 mg/l BAP with 0.5 mg/l NAA. Regenerated and multiplied shoots shows maximum (75.5%) root induction on MS medium supplemented with 1.0 mg/l IBA within 20 days. Hence the present study will help to propagate the medicinal plant for industrial herbal extraction of valuable pharmaceutical substances to be used in different diseases. The present research also focused on the methods and benefits of organic farming of the medicinal plant prevalent nowadays, although more better and profitable techniques can be introduced by carrying out further research in this regard. Hence it Concludes that application of organic and biofertilizers acts as substitute of inorganic fertilizers. in order to grow the medicinal and aromatic plants, should not be considered as a simple objective and short term benefits, but as a mean to improve the environmental conditions and human health. Therefore the present investigation was undertaken with the aim to prescribe the panacea by developing an effective method for rapid and large scale multiplication with protocol for effective organic farming to boost the vigour and other quantitative traits of the plant.

KEYWORDS: Psoralea corylifolia, in vitro propagation, endangered, conservation, growth regulators and organic farming.

INTRODUCTION
Psoralea corylifolia L. commonly known as 'Babachi' belongs to family Fabaceae (Leguminosae). It is an erect annual herb, 30-180 cm high, found almost throughout India. The plant is propagated by seed germination but because of hard seed coat it shows low rate of seed germination (**Chand *et al*; 2002**). Hence there is an urgent need for cultivation of this endangered important medicinal plant. Low seed germination percentage, less seed viability, long gestation period and delicate field handling are some factors which discourage commercial cultivation of *Psoralea corylifolia* **(Jeyakumar M. *et al*; 2002).** Due to great medicinal importance of *Psoralea corylifolia*, it is being over exploited and accordingly its population in nature has squeezed and has been listed in the Red Data Book of (IUCN) under the category "endangered". Thus the present study has been designed to develop a reliable and reproducible protocol which could be used for mass multiplication, conservation and to meet the increasing requirement.

The significance of an efficient *in vitro* protocol would be to obtain maximum number of plantlets in minimum period of time with proper rooting along with acclimatization in the field. The different regeneration systems which have been developed need to be field tested and the field data is collected so, that the complete

technology packages could be ready for commercialization and transfer to the user agencies.

Organic farming is a technique, which involves cultivation of plants in natural ways. It is a production system which avoids or excludes the use of synthetic preparations, artificial fertilizers, pesticides, growth accelerators and fodder additives. This process involves the use of biological materials, avoiding synthetic substances to maintain soil fertility and ecological balance thereby minimizing pollution and wastage. "Pesticides that kill insects also kill a tiny part of the living element in us" **(Patakh *et al;* 2007**). Organic fertilizers in comparison of the chemical fertilizers have lower nutrient content and are slow release but they are as affective as chemical fertilizers over long period of use (**Naguib, 2011)**. The medicinal plants are the basic source of raw material for preparation of ayurvedic medicines. By implementing good agro technique and organic farming practices in medicinal plants cultivation, the safety and quality of plant materials and finished products could be assured. Furthermore, the application of vermicompost in the field enhances the quality of soils by increasing microbial activity and microbial biomass which are key components in nutrient cycling, production of plant growth regulators and protecting plants from soil-borne diseases and arthropod pest attack. The present study focused on the methods and benefits of organic farming of the medicinal plants prevalent nowadays, although more better and profitable techniques can be introduced by carrying out further research in this regard. Hence it concludes that application of organic and biofertilizers acts as substitute of inorganic fertilizers. in order to grow the medicinal and aromatic plants, should not be considered as a simple objective and short term benefits ,but as a mean to improve the environmental conditions and human health.

Therefore the present study was undertaken with the aim to prescribe the panacea by developing an effective method for rapid and large scale multiplication together with protocol for effective organic farming to boost the vigour and other quantitative traits of the species.

MATERIALS AND METHODS

Different explants i.e. nodal segments, axillary and apical meristems of 5-10 centimetre length were excised from the mother plants with the help of scissors maintained in nursery and were used for shoot induction experiment of *Psoralaea corylifolia*. The explants were washed under running tap water and then washed thoroughly in sterile double distilled water. These explants were then kept in 1.0 % Bavistin (Carbendazim Powder BASF India Limited) a broad spectrum fungicide for 10 minutes followed by 5.0% (v/v) Teepol (Qualigens Fine Chemicals, India), a liquid detergent for 5 minutes by continuous shaking method. The treated explants were washed in sterile double distilled water for 4 to 5 times to remove the chemical inhibitors. Further surface sterilization treatment was conducted in a laminar air flow chamber. The explants were then surface sterilized by immersing in a freshly prepared solution of 3.0% (w/v) NaOCl (Qualigens Fine Chemicals, India) for 4 to 5 minutes under laminar flow. Finally the explants were washed 5 to 6 times with sterile double distilled water for 5 minutes to remove all traces of sterilizing agents used (**Shahid *et al*; 2007).**

The stock solutions for Murashige and Skoog (MS) medium (macronutrients, micronutrients, iron and organic) were prepared**.** The medium was supplemented with various growth regulators and other growth adjutants as per requirement. The thermostable growth regulators were added to the medium before making up the total final volume with distilled water.

The media was prepared in culture tubes (25×150 mm, Borosil) and prior to autoclaving pH of the media was adjusted to 5.8 using 0.1 N. NaOH (Sodium hydroxide) or 0.1 N. HCl (Hydrogen chloride) with a pH meter (Lab India, India) and was solidified by adding 1% agar (HiMedia Lab. Ltd., India) and sterilized by autoclaving at 15 lb pressure per square inch, 121°C temperature for 15 minutes. The sterilized explants were then inoculated aseptically into the medium and incubated at 25 ± 2°C with relative humidity of 55 ± 5% and exposed to photocycle of 2500 Lux intensity for 16 hrs . The surface sterilized explants were then inoculated on MS media supplemented with various concentrations of BAP, Kn, NAA, IAA and IBA shown in (**Table 1- 3**) either singly or in various combinations for shoot induction, multiplication and root formation. Visual observations like shoot induction, morphology, number of days taken for bud break, percentage of bud break and number, length of shoots regenerated per explants and length of roots per regenerated shoots were recorded after 2 weeks shown in **(Table 1- 3)**. After micropropagation of *Psoralea corylifolia* the well developed rooted shoots/plantlets were taken out from the culture medium flasks and washed thoroughly with running tap water to remove all traces of medium attached to the roots for hardening. Finally the tissue culture raised plantlets were planted in hardened pots containing mixture of soil, sand and farmyard in the ratio of (1:2:1) for acclimatization and were maintained in green house.

Effect of the organic farming on tissue culture raised plants of *Psoralea corylifolia*

After the propagation of *Psoralea corylifolia* through tissue culture technique impact of organic fertilizers on the growth of plants under field condition was studied and were compared with control i.e. without any organic fertilizer. To study the effect of organic farming on tissue culture raised plants of *Psoralea corylifolia* three organic fertilizer combinations were used. i.e. soil, sand and vermiculite (1:2:1), soil, sand and solurite (1:2:1) and soil, sand and dry cow dung powder (1:2:1) and were compared with the effect of control i.e. soil and sand only in the ratio of (1: 2) under green house conditions. The pots were maintained in hardening unit i.e. green

house (micropropagation unit) for observation. **(Table. 4).**

Growth parameters of tissue culture raised plants supplemented with organic fertilizers were compared with the control (without organic fertilizers). Visual plant growth observations like, height of plants and number, length of shoots and length of roots, morphology of leaves, number of days taken for flower formation, number of days required for seed formation, were recorded and finally data obtained was analyzed statiscally.

RESULTS AND DISCUSSION

Establishment of cultures

To standardize the medium and growth regulators for shoot induction of *Psoralea corylifolia*: The shoot induction through nodal explants on MS media supplemented with BAP and Kn along with auxins i.e. IBA IAA and NAA showed a significant variation in terms of bud break, number of shoots induced per explant.

Shoots initiated from mature meristem explants shows good response in every medium tried in the experiment. The axillary buds of the newly sprouted branches, which were nearer to the apical bud, were more responsive. Maximum (80.0%) shoot induction was observed on medium supplemented with 1.0 mg/l BAP when combined with 0.5 mg/l NAA of 5.0±0.8 number of shoot buds were observed having length of 6.8±0.2centimeters within 20 days culture shown in **Table. 1 Figure.1. (a-c).**

However minimum (40.0%) shoot induction was observed on medium containing 0.5 mg/l Kn with 0.5 mg/l IBA with the formation of 2.1± 0.1 number of shoots having length of 3.2±0.1 centimetres within 30 days.

Hence, for maximum (80.0%) bud break and shoot initiation from axillary and apical meristems medium containing 1.0 mg/l BAP with 0.5 mg/l NAA proved the most effective followed by1.0 mg/l BAP (65.5 %) alone. It is observed that addition of Auxin like 0.5 mg/l IBA not shows any effect to promote shoot initiation **(Table. 1).** The shoot clusters obtained were found to retain their vigour and health. The initiated shoots were further transferred to fresh medium for multiplication experiment.

Similarly **Pandey P. *et al;* (2013)** reported that highest shoot regeneration (95%) in *Psoralea corylifolia* were obtained on medium containing 12 µM/l BAP with 10.0 µM/l NAA and 15.0 µM/l Kn. Many other investigation also shows similar findings (**Nirmala Sehrawat *et al;* (2013), Punita Tiwari *et al;* (2012)** in *Psoralea corylifolia* in which 2.5 µM/l BAP was found optimal for shoot induction on 5.0 -10.0 mg/1 BAP with 0.5mg/1 NAA. However according to the literature in the present study shoot induction was observed on media supplemented with lowest concentration of plant growth regulators as well as within minimum time period.

To standardize multiplication of shoots on different media containing different growth hormones of *Psoralea corylifolia*: In order to get profused rapid shoot multiplication MS media supplemented with cytokinin and auxin combinations were also used. Auxins, like IBA, IAA and NAA were added and tested along with varied concentration of BAP and Kn. It was observed that every medium gives response and the cultures showed highest percentage (85.0%) of shoot formation with an average of 12.0±0.8 adventitious shoots, directly from the axillary node, without any callus formation on medium supplemented with 1.5 mg/l BAP with 0.5 mg/l NAA within 20 days. **Figure1 (d-f).** While as Medium supplemented with 1.0 mg/l BAP and 0.5 mg/l IBA also observed an effective medium for shoot multiplication shows 55.0% of shoot multiplication rate in 30 days of about 4.5±0.6 number of shoots having length of 5.2±0.7 centimetres**.** (**Table. 2).**

However, only few shoots has been observed on Kn in the concentration of (0.5mg/l) with IBA and NAA. An average of 50.0- 60.0 % of shoot multiplication was seen which is of 4.3±0.1 in number having length of 5.8±0.9 centimeter over a period of 30 days. However, Kn supplemented medium, with various combination of auxins doesn't shows any significant results as 1.5 mg/l Kn with 0.5 mg/l NAA shows 60.0% of shoot multiplication rate of about 5.0±0.7 number of shoots having length of 7.0±0.2 centimetres within 25 days. While as1.0 mg/l KN with 0.5 mg/l IBA shows only 35.0% of shoot multiplication rate of about 3.5±0.6 number of shoots having length of 4.4±0.1 centimeters**.** Shoots formed were stunted and turned yellow after some days**. (Table. 2).**

Therefore, from all concentrations 1.5 mg/l BAP with 0.5mg/l NAA produced the most desirable results both in terms of multiplication fold and cluster of perfective elongated shoots. Thus, as usual BAP was found to be the most effective indicating, the cytokinin specificity of any nodal explants of *Psoralea corylifolia* for shoot formation. The addition of IBA and NAA with optimal concentration of BAP significantly increase the frequency of shoot multiplication compared to BAP alone. (**Table.2).** The obtained results are parallel with the earlier reports on *Psoralea corylifolia* for highest rate of shoot multiplication (**Jeyakumar M. *et al;* (2002)** and (**Anis M. *et al*; 2005).** In contrast to the synergistic effect of BAP in combination with NAA has been also previously reported in *chrysanthemum* (**Khaleghi *et al;* 2008)** *Alstroemeria* cv. "Fuego, **(Kashif Waseem *et al*; 2011),** Plantain *Musa* spp **(Adane Gebeyehu Demissie, 2013)** and also in *Chrysanthemum morifolium* **(Hoque and Fatema, 1995**), **(Hoque *et al;* 1998), (Nahid *et al*; 2007).**

Rooting of *in vitro* regenerated shoots

The induction of roots has been observed in every medium tried. Following the protocol of **(Anis M. *et al;* 2005)** medium with root inducing growth regulators at the concentration of 0.5 to 1.5 mg/l IAA, 0.5 to 1.5 mg/l NAA and 0.5 to 2.0 mg/l IBA were tested from *in vitro* raised shoots. Maximum (75.5%) root induction was achieved directly from the base of the shoots on medium supplemented with 1.0 mg/l IBA of average length 7.0±0.9 centimeters within 20 days. **Figure.1 (g-h)**. On the contrary, by increasing the concentration of IBA results decrease in root formation, comparatively 60.5 % of root initiation was observed on medium supplemented with 1.5 mg/l of IBA within 25 days having length of 5.0±0.9 centimeters. shown in **(Table. 3.)**.

Minimum (55%) root induction was observed on higher (2.0mg/l) concentrations of IBA. Roots were induced along with extensive callusing at the basal end of shoots which hindered its further growth. The roots formed were very slender and thin in the medium containing 1.0 mg/l NAA and IAA and shows only 45.5 % to 40.0 % of root induction after 25days having length of 3.7±0.6 centimetres, while as 1.0 mg/l IAA and 1.0 mg/l NAA failed to develop any root system. Among the various concentration and combinations full strength of MS basal medium supplemented with 1.0 mg/l IBA shows best root induction protocol for healthy roots within minimum time period shown in **(Table. 3.)**.

Similar results were reported in *Psoralea corylifolia* by **Jeyakumar M. *et al;* (2002), Anis M. *et al;* (2005), Baskaran P. *et al;* (2007 and 2010), Pandey P.*et al;* (2013), Punita Tiwari *et al;* (2012), Nirmala Sehrawat *et al;* (2013).** Superiority of IBA for root induction has been reported earlier in many other plant species such as in *Glinus lotoides* (L.) **(Teshome S. *et al*; 2015)**, *Eclopta alba* L. **(Archana Sharma *et al*; 2013)**, *Chrysanthemum* **Kashif Waseem *et al*; (2011), Hoque and Fatema (1995), Hoque *et al; (*1998), Sarker and Shaheen (2001), Khan *et al;* (1994)** and **Karim *et al;* (2002).**

Identification of suitable hardening medium for better establishment: The *in vitro* raised roots are often thick and lack root hairs as well as good vasculature. These roots frequently die or collapse after the plantlets move from cultures and new functional lateral and adventitious roots are formed during acclimatization. Therefore it is very essential to create good *in vitro* rooting conditions for transplantation. The transfer of plants from the culture flasks to the soil requires a careful, stepwise procedure. After 15 to 20 days of culture on rooting media, the rooted plantlets were transplanted to pots for hardening prior to their final transfer to soil. Rooted plantlets were taken out of the culture bottles with the help of forceps and washed thoroughly with water to remove any remaining of the medium**.** A minimal survival rate of 40-50% was recorded during the months of May, June, July and August. However, the plants taken out after September showed a substantial increase in survival percentage. All hardened plants survived on transfer to pots in greenhouse containing soil, sand and farmyard in ratio of (1:2:1) mixture gave the maximum survival percentage with better plant growth resulting as a suitable medium for hardening. **Figure1.(i).**Similarly, *in vitro* raised rooted shoots of tomato plants were more effectively when transferred to garden soil, farmyard soil and sand 2:1:1 ratio mixture and the plants were successfully acclimatized. **(Sherkar H. D. and Chavan A. M., 2014)**.

To study the impact of the organic farming on tissue culture raised plants of *Psoralea corylifolia*: To study the impact of organic farming on tissue culture raised plants of *Psoralea corylifolia* hardened *in vitro* raised plants of 15 to 20 centimetres height with healthy root system were transferred to the field for cultivation by the application of different organic fertilizers. The optimized physical condition has to be maintained throughout the experiment**.** In the present study three organic fertilizer combinations were used. i.e. soil, sand and vermiculite (1:2:1), soil, sand and solurite (1:2:1) and soil, sand and dry cow dung powder (1:2:1) and were compared with the effect of control i.e. soil and sand only in the ratio of (1: 2) under green house conditions**. (Table. 4.)**. The plants with vigorous growth (85%) were observed in compost containing soil, sand and vermiculite (1:2:1) combination. About 9.0±0.1 number of shoots were achieved having length of about 15.3.±0.2 centimetres within 45 days **Figure.2 (j).** However least growth (40 %) was observed in control condition i.e. soil and sand (1:2) was observed about 3.5±0.6 number of branches emerges obtaining the length of about 5.0 ± 0.1 centimetres after 65 days. **Figure.2 (m). (Table. 4.)**.

While as 70.0 % growth were observed in soil: sand: solurite (1:2:1) combination of about 6.0±0.9 number of branches having length of about 9.5±0.2 centimeters within 50 days. **Figure.2 (k)** following the growth rate of 60.0 % in soil, sand and dry cow dung powder (1:2:1) combination **Figure.2 (l).**

In the present experiment, it was observed that the best growth (85 %) of *in vitro* raised plants of *psoralea corylifolia* were in the compost containing soil, sand and vermiculite (1:2:1) combinations. **Figure.2 (j).** Overall studies showed that *Psoralea corylifolia* can be propagated through *in vitro* and can be cultivated by using organic compost for fulfilling the demand of important medicinal applications. **Table. 4, Figure.2(m).**

Same studies has been previously reported on *Psoralea corylifolia* (**Baskaran P. *et al;* 2008)** were exposed simultaneously, 15.0 roots per shoot as well as hardened in moistened soil mixture and vermiculite (3:1 v/v). The higher survival percentage (100%) was achieved in winter season (Sep -Dec, 25-28^{0}c) and would be useful for mass propagation and germplasm conservation of *Psoralea corylifolia.* The mixture of soil, sand, organic

manure and vermiculite in ratio of (1:1:1:1) were also used in micropropagated plants for hardening **Arya and Arya (1996)**.

The use of vermicompost extract results in the control of some soil borne pathogens on three ornamental plant species significantly reduced sporulation of the pathogen *Phytophthora cryptogea* **(Orlikowski, 1999).**While aqueous extracts of organic matter (vermicompost) were capable of reducing the growth of pathogenic fungi such as *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Corticium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* (**Nakasone *et al;* 1999**).

Similarly in *Gloral* **(Azizi *et al*; 2006)**, in Tea (**Edwards *et al*; 2006)** and in *Thymus Vulgaris* L. **(Ateia *et al*; 2009**) using vermicompost in *Asparagus recemosus* **(Saikia and Upadhyaya, 2011).** The production of natural substances by plants is affected by genotype and environmental conditions. Adopting organic farming in medicinal plants cultivation is the need of the day. Continuous usage of inorganic fertilizer affects soil structure. Hence plant and animal manures, compost and vermicompost can serve as alternative mineral fertilizers for improving soil structure and microbial biomass.

Table. 1. Effect of MS media and growth regulators either alone or in combination on shoot initiation of *Psoralea corylifolia*.

S. No.	MS+Auxin/cytokinin (mg/l).	%age of bud break.	No. of days required.	% age of callus formation.	Mean No. of shoots produced ± SE.	Mean shoot length in cm.±SE.
1	0.5 BAP.	40.3	30	35	2.0± 0.3	3.0±0.9
2	1.0 BAP.	65.5	25	25	4.0±0.21	5.0±0.23
3	0.5 KN.	35.1	35	40	1.7±0.8	2.4±0.1
4	1.0 KN.	50.0	30	55	2.7±0.8	3.5±0.9
5	0.5 BAP+0.5 IBA.	45.0	30	45	2.5±0.1	3.7±0.9
6	0.5 BAP+0.5 NAA.	60.9	25	30	3.0±0.4	4.4±0.5
7	0.5 KN+0.5 IBA.	40.0	30	50	2.1±0.1	3.2±0.1
8	0.5 KN+ 0.5NAA.	42.5	30	40	2.5±0.8	3.4±0.1
9	1.0 BAP+0.5 NAA.	80.0	20	20	5.0±0.8	6.8±0.2
10	1.0KN+0.5 NAA.	50.5	25	40	3.0±0.9	4.0±0.1

Table. 2. Effect of MS media and growth regulators either alone or in combination on shoot multiplication from induced shoots of *Psoralea corylifolia*.

S. No.	MS+Auxin/cytokinin (mg/l).	%age of shoot multiplication.	No of days required.	Mean No. of shoots produced ± SE.	Mean shoot length in cm.±SE.
1	0.5 BAP.	40.0	20	3.0±1.2	3.9±0.02
2	0.5 KN.	30.5	25	1.0±0.52	2.0±0.3
3	1.0 BAP.	50.5	25	4.0±0.6	5.2± 0.01
4	1.0 KN.	35.0	30	2.0±0.1	3.0±0.5
5	1.0 BAP+0.5 IBA.	55.0	30	4.5±0.6	5.2±0.7
6	1.0 KN+0.5 IBA.	45.0	30	3.5±0.6	4.4±0.1
7	1.5 BAP +0.5 NAA.	85.0	20	10.0±0.1	12.0±0.8
8	1.5 KN+0.5 NAA.	60.0	25	5.0±0.7	7.0±0.2
9	2.0 BAP+0.5 NAA.	70.9	25	7.0±0.2	9.5±0.6
10	2.0 KN+0.5 NAA.	50.5	30	4.3±0.1	5.8±0.9

Table 3. Effect of MS media and growth regulators on root induction of *Psoralea corylifolia*:

S. No.	Media composition. (mg/ l).	% age of root induction.	No. of days required.	Mean root length in cm.±SE.
1	MS +0.5 IAA.	45.0	30	3.5±0.7
2	MS +0.5 IBA.	50.0	25	4.0±0.3
3	MS +0.5 NAA.	40.0	20	3.0±0.3
4	MS +1.0 IAA.	40.5	20	3.0±0.9
5	MS +1.0 IBA.	75.0	20	7.0±0.9
6	MS +1.0 NAA.	45.5	25	3.7±0.6
7	MS +1.5 IAA.	55.5	25	4.5±0.2
8	MS +1.5 IBA.	60.5	25	5.0-±0.9
9	MS +1.5 NAA.	50.0	30	3.2 ±0.2
10	MS +2.0 IBA.	55.0	25	4.4±0.8

Table. 4. Effect of different organic fertilizers and control on tissue culture raised plants of *Psoralea corylifolia*:

S. No.	Organic fertilizers.	%age of plant growth.	No. of days required.	No. of branches.	Shoot length cm±SE.
1	Soil+sand(control)	40	65	3.5±0.6	5.0±0.1
2	Soil+sand +vermiculite	85	45	9.0±0.1	15.3±0.2
3	Soil+sand +solurite	70	50	6.0±0.9	10.5±0.2
4	Soil+sand +dry cow dung	60	60	4.0±0.6	7.0±0.8

(Figure. 1) *In vitro* regeneration of *Psoralea corylifolia*, a-c. Shoot induction from nodal explant on MS medium + BAP. (1.0 mg/l)+NAA (0.5mg/l) d-e. Multiplication of regenerated on MS medium + BAP (1.5 mg/l)+NAA (0.5mg/l) g-h. Differentiation of roots from nodal segments on MS medium +IBA (0.5mg/l) i. Hardening of tissue culture raised plantlets of *Psoralea corylifolia* in green house.

Soil+sand + vermiculite after 45 days **Soil + sand + solurite after 50 days.**

Soil+sand +dry cow dung powder after 60 days. **Soil+sand only after 65 days.**

Figure 2(j-m) Tissue culture raised plants of *Psoralea corylifolia* in field supplemented with different organic fertilizers and control (without organic fertilizers).

CONCLUSION

Therefore, the present work demonstrated that *in vitro* propagation of *Psoralea corylifolia* was successfully done by using different explants. Different combinations of growth regulators in MS media were used to promote shoot and root formation. Maximum (80.0%) shoot induction of *Psoralea corylifolia* through mature meristem was observed on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA. while as induced shoots shows highest (85.0%) and profused shoot multiplication rate on medium supplemented with 1.5 mg/l BAP with 0.5 mg/l NAA.Regenerated and multiplied shoots shows maximum (75.5%) root induction on MS medium supplemented with 1.0 mg/l IBA within 20 days. The present research also focused on the methods and benefits of organic farming of the medicinal plant prevalent nowadays, although more better and profitable techniques can be introduced by carrying out further research in this regard. Maximum (85%) plant growth were observed in compost containing soil, sand and vermiculite (1:2:1) combination. About 9.0±0.1 number of shoots were achieved having length of about 15.3.±0.2 centimetres within 45 days **Figure.2 (j).** However least growth (40%) growth was observed in control condition i.e. soil and sand (1:2) was observed about 3.5±0.6 number of branches emerges obtaining the length of about 5.0 ± 0.1 centimetres after 65 days. **Figure.2 (m). (Table. 4.)**. Hence it concludes that application of organic application of organic and biofertilizers acts as substitute of inorganic fertilizers. in order to grow the medicinal and aromatic plants, should not be considered as a simple objective and short term benefits, but as a mean to improve the environmental conditions and human health. Therefore the present investigation was undertaken with the aim to prescribe the panacea by developing an effective method for rapid and large scale multiplication with protocol for effective organic farming to boost the vigour and other quantitative traits of the plant. Therefore the protocol developed could be used for conservation of elite

germplasm and true to type mass propagation of *Psoralea corylifolia* of immense pharmaceutical relevance. This is highly advantageous for the production of uniform source of *Psoralea corylifolia* plants for a range of further biotechnological applications and will also help in the production of improved plants.

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