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In Vitro Study of Effect of Sodium Azide on the Callus of Jasmine Plant

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Abstract:- The present paper deals with the callus was raised from the leaf explant of *jasminum sambac* var. *duplex* voigt. The callus was raised on MS media supplemented with 3% sucrose with different concentration 2,4-D and BAP. A maximum callus was obtained at concentration of 2mg/lit maintained for 9 weeks for the growth. This callus was subjected to the chemical mutagen (sodium azide). The effect of sodium azide at 3mm concentration the callus growth was enhance to 1.9 cm.

Keywords:- *Jasminum Sambac. Var Duplex Voigt, Sodium Azide.*

I. INTRODUCTION

Jasmine is highly valued ornamental plant for home gardens and commercial cultivation. flowers and buds are used for making garlands, bouquets and for religious offerings, while veni is used as hair adornment. The flowers are also used for the production of perfumed hair oils and attars. Jasmine essential oil has a sweet and floral aroma. It is regarded as unique, as it blends well with other floral extracts and which is highly valued throughout the world for its high grade perfumes, which is used in soap and cosmetic industries and in flavouring mouth wash liquids. The flowers should preferably be picked at night for extraction of essential oil. Jasmine fragrance is said to give a feeling of optimism, confidence and euphoria, and is helpful against depression, nervous exhaustion and stress related conditions. Jasmine is also used for catarrh, coughs, laryngitis, dysmenorrheal, labor pains, uterine disorders and many skin problems.

India is the largest exporter of jasmine oil in the world accounting for over 40% of total world export. India earned US $329 million by exporting of essential oil in the year 2008 – 09. Rose oil (US$ 3432 per 500g) commands highest price in the international essential oil markets and followed by jasmine oil (US $ 527 per 500gm). The leading export markets for Indian Jasmine oil are France, accounting for over 36% of total jasmine oil exports from India, Followed by UAE, USA , Germany and UK (Indian Horticulture Database, 2009). Other jasmine producing countries are UAR, Morocco, Italy, Algeria etc. (Panda,2006). Among the varieties of jasmine, *Jasminum grandiflorum* (Pichi) is cultivated in more than 60% of the area followed by *Jasmium auiculatum* (Mullai). Tamil Nadu is the leading producer of Jasmine in the country with an annual production of 77,247 tones from a cultivated area of 9360 hectare (Singh,2006). Karnataka is the second highest producer of Jasmine flowers. In the year 2004 Karnataka has produced 20,244 tones of Jasmine flower from 3,451 hectare earning 8,265 lakh rupees. The Tigala community near Devanahalli and Chickaballapur are extremely good at growing flowers (Banumathy and Devi,2004) in Karnataka State. This is ornamental cum medicinal plant, has good economy in our country, therefore to enhance its multiplication, tissue culture mass propagation was studied.

II. MATERIALS AND METHODS

So all experiments were carried out in the department of Botany Smt.K.W.C sangli. The leaves of Jasmine plant were collected from Sangli. The leaf explants of jasmine was excise in the laboratory and callus was developed on M.S media. For callus initiation equal concentration of auxins and cytokines (1:1) were added and 3 different sets of concentrations namely 1mg/ml, 2mg/ml and 3mg/ml were prepared. The M.S media were supplemented with (3% w/v sucrose and 0.8% w/v agar) was used for subsequent experiment. The ph was adjusted to 5.8. All cultures were incubated at 25 $-^{+}$ 2^{0}C under light hours photo period of 30µmol m^{-2} sl of cool white fluorescent tubes (Phillips, India).for chemical treatment sodium azide was used at a 5 different concentration namely 1mM, 2mM,3mM, 4mM and 5mM.

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III. OBSERVATIONS

- *In Vitro Callus Generation*

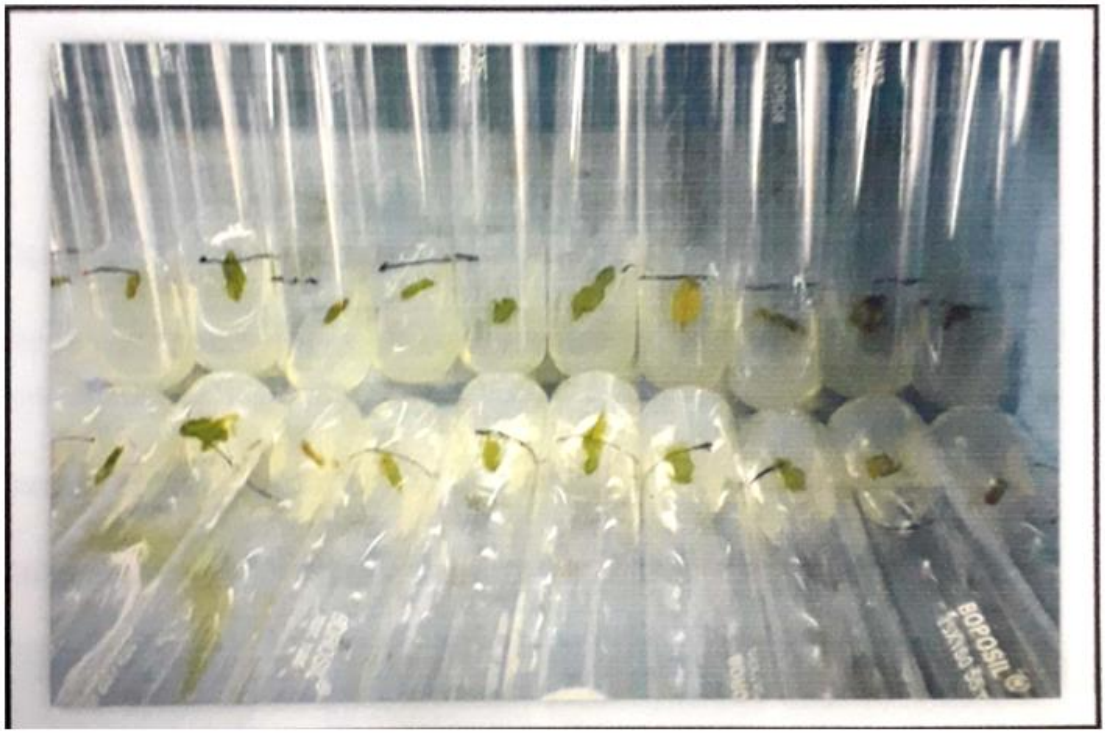

Fig 1:- Explant (leaf) inoculated into sterile MS media tubes containing variable concentration of auxins and cytokines.

After carrying out the surface sterilization procedure the explants (leaf) was inoculated into sterile MS media tubes containing different concentration of growth harmones i.e. 2 gm/lit, 3gm/lit and 1gm/lit respectively. After 15 days the explants started to turn out yellowish brown and approximately after 40-45 days the callus proliferation has started in the inoculated tubes.

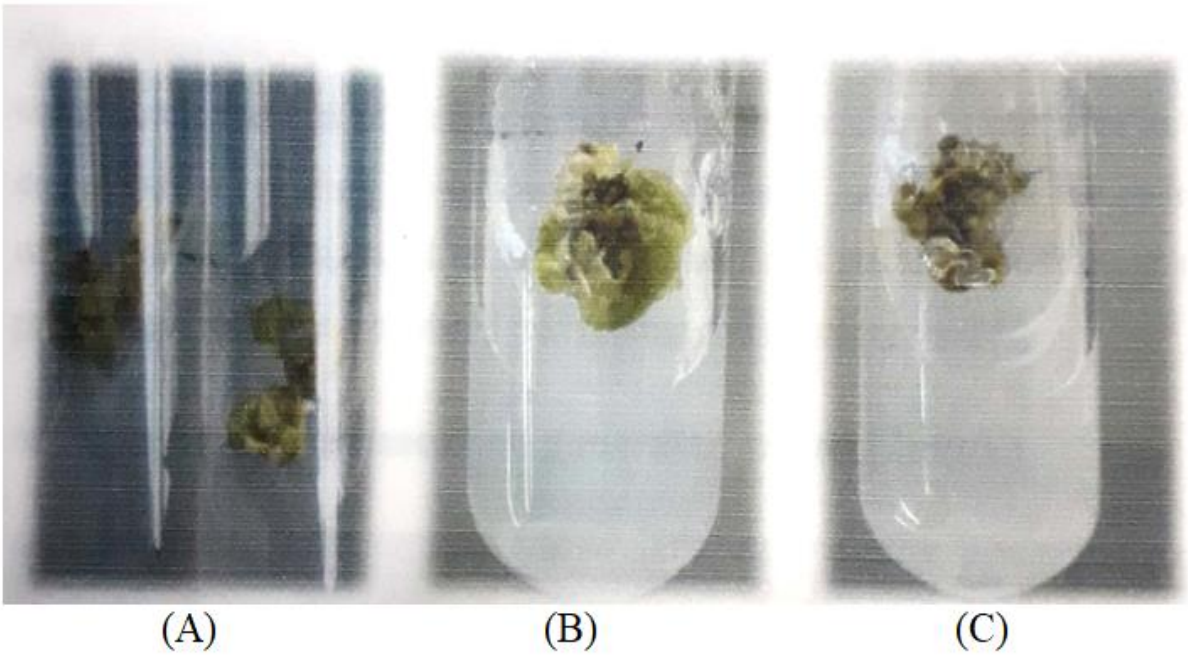

Fig 2:- Callus regeneration after six weeks. [(A) 1 mg/lit, (B) 2 mg/lit, (C) 3 mg/lit.]

The above photos shows the callus generation after 40-45 days at different harmone concentration i.e. 1 mg/lit, 2 mg/lit and 3 mg/lit.

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➢ *Sodium Azide Treatment on Generated Callus [2 mg/lit]:*

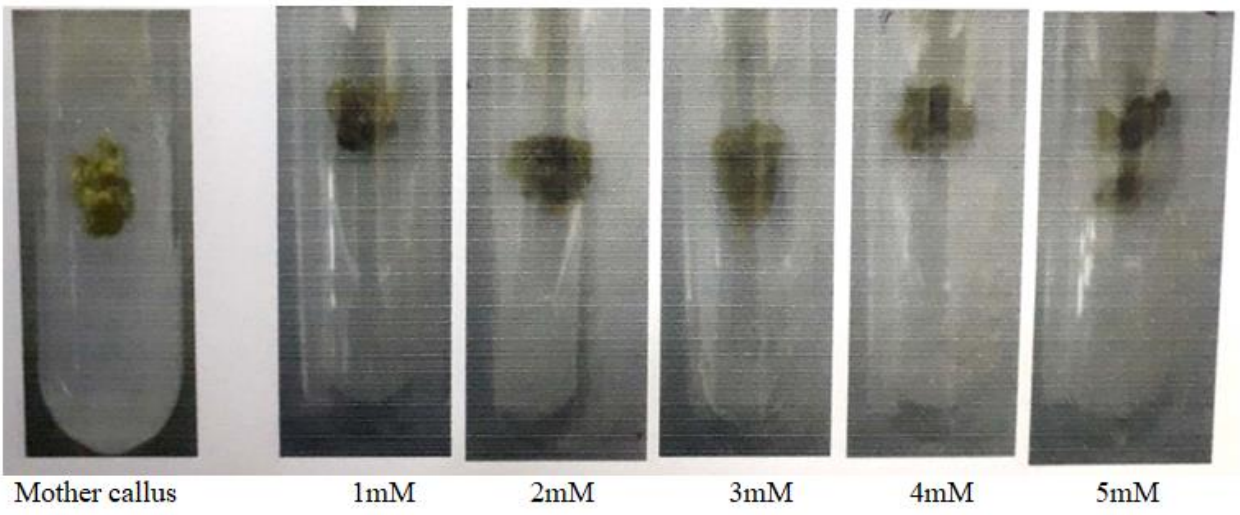

Fig 3:- The callus was treated with sodium azide.

The mother callus which was inoculated in 2mg/lit of hormone concentration was used for further treatment and study the effect of sodium azide on the proliferated callus. Different concentration of sodium azide was added into MS media after sterilization of media later after solidification the mother callus was distrupted into 5 pieces and was inoculated and kept for generation.

Soon after inoculation the green coloured callus turned out to brown within 2 weeks and later the proliferation started. The callus having 3mM concentration of sodium azide gave positive response and started proliferating within 2 weeks the other callus having different concentration in the media responded later. This proliferated callus was further inoculated for organogenesis.

IV. RESULT AND DISCUSSION

The different types of explant showed various responses to media and hormones after 6 weeks the highest amount 89 % of greenish friable callus was obtained from leaf explant cultured on MS medium supplimented with 2mg/lit 2,4-D and BAP then followed by 3mg/lit and 1mg/lit respectively. then sodium azide treatment with different 5 concentration 1mM, 2mM, 3mM, 4mM,5mM was given then it showed that all different concentrated harmonal callus were survived and it is greenish and fragile and root initiation has been started. The aim of present invetigation was to study the growth condition for in vitro micropropagation of jasmine from leafs. To achive this goal different concentration of growth regulator was used sodium azide has been used in wide range of application to improve crops ability in their resistance against their harmfull pathogen or to produce desired variation for salt tolerence and other abiotic stresses, in our research using sodium azide at various concentration results either negative or possitive responses at high or low concentrations respectively.

➢ *Callus was Formed after Weeks Supplemented with 2mg/lit, 3mg/lit, 1mg/lit.*

Concentrations	Hormones	Days
2mg/L	2,4-D and BAP	42
3mg/L		52
1mg/L		70

Table 1:- Days of callus proliferated in In Vitro culture condition at different concentration and hormone 2,4-d and BAP.

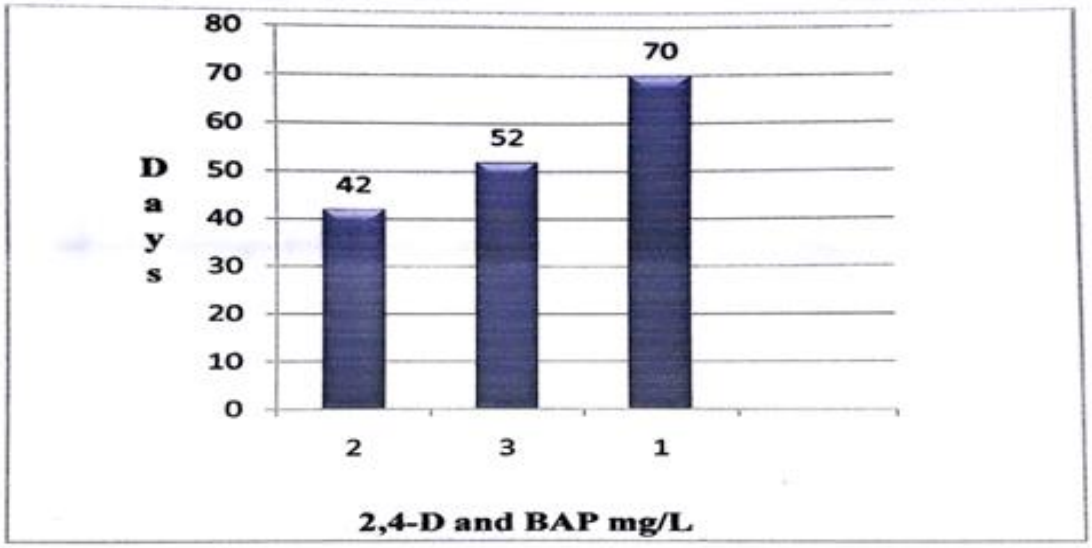

Fig 4:- Callus formation of *j.sambac* on MS media at various concentration of 2,4-d and BAP in days.

	Size in cm								Mean total
Test tube no.	1	2	3	4	5	6	7	8	
2 mg/L	2.3	2.1	2.3	2.1	2.1	2.0	2.2	2.3	2.17
3 mg/L	1.7	1.7	1.6	1.3	1.4	1.5	-	-	1.15
1 mg/L	0.8	0.9	0.6	0.8	0.7	0.8	0.7	-	0.66

Table 2:- Size of callus proliferated in In Vitro culture condition at different concentration of auxin and cytokines.

- *Sodium Azide*

Concentration Hormones	Size in cm					Size of Mother Callus(cm)
	1mM	2mM	3mM	4mM	5mM	
1mg/L	0.4	0.5	0.6	0.5	0.4	0.9
2mg/L	1.7	1.6	1.9	1.6	1.5	
3mg/L	0.8	0.7	0.6	0.7	0.6	

Table 3:- Size of callus proliferated in in vitro culture condition and different concentration of auxin and cytokines treated with and variou concentration 1mM,2mM, 3mM, 4mM, 5mM.

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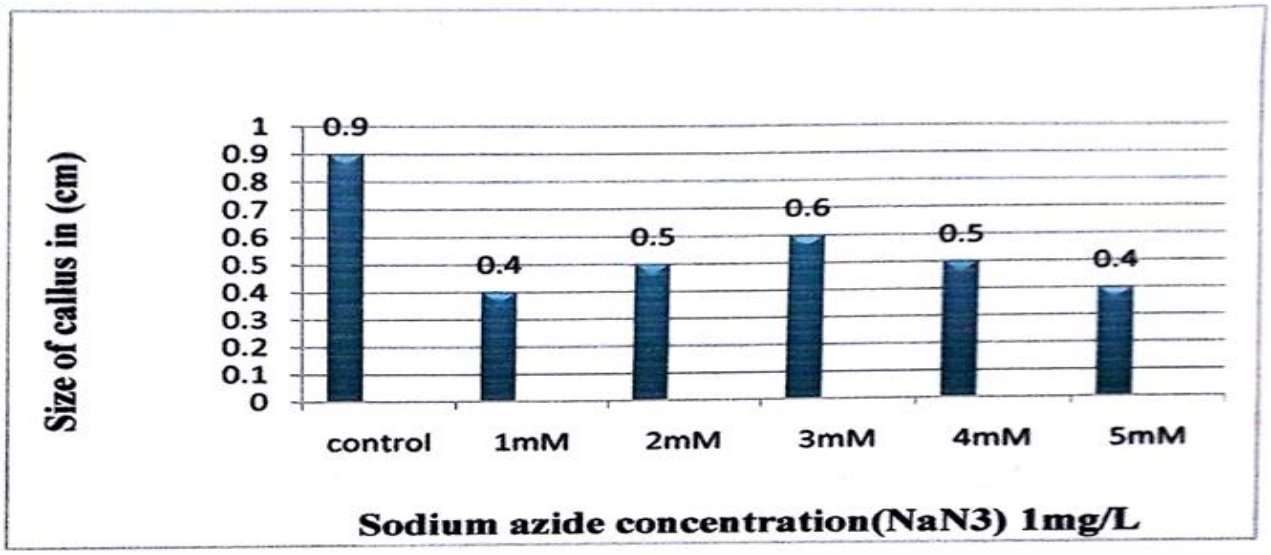

Fig 5:- Comparative chart of sixe of callus treated with sodium azide concentration 1mg/lit controled and (untreated).

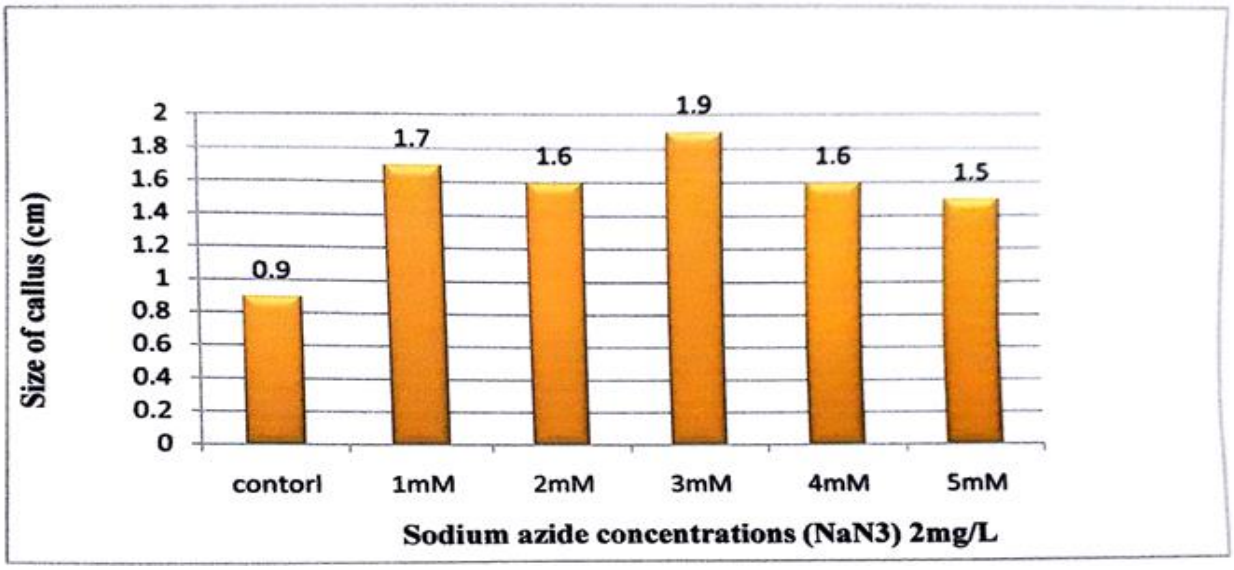

Fig 6:- Comparative chart of sixe of callus treated with sodium azide concentration 2mg/lit controled and (untreated).

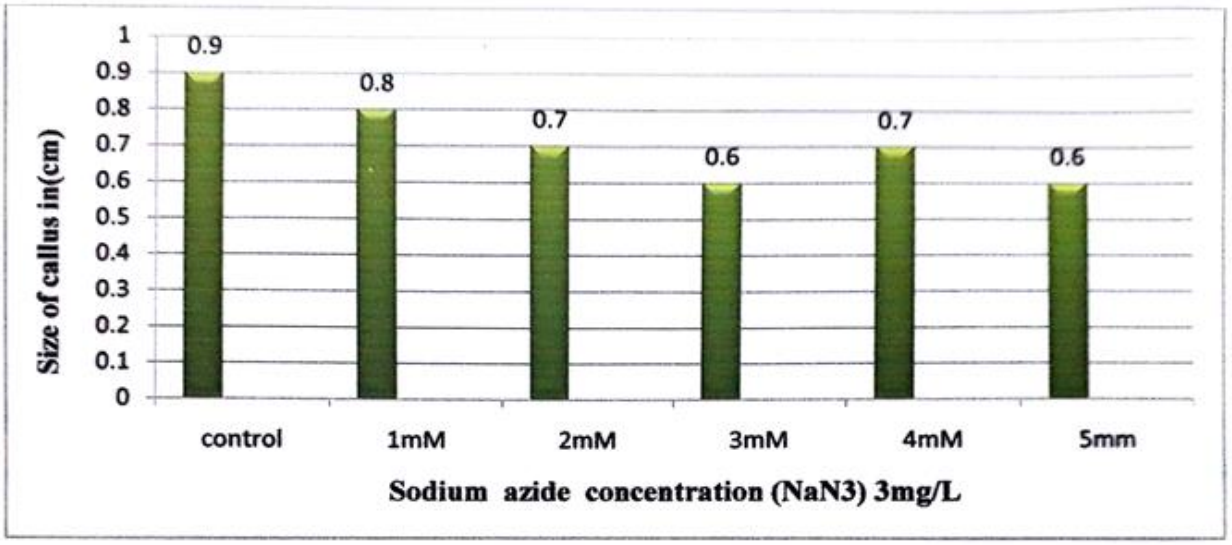

Fig 7:- Comparative chart of sixe of callus treated with sodium azide concentration 3mg/lit controled and (untreated).

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V. CONCLUSION

The present study showed the highest amount of callus production on MS media supplimented with 2.0mg/lit, 2,4-D concentration. The treatement at different time interval but radiation concentration is same and sodium azide treatment at different concentration within two weeks of culture maximum callus yeild was obtained from 2.0mg/lit of 2,4-D after 6 weeks the callus was subcultured in the same medium at regular interval of times.

It was noticed that upto 2.0mg/lit of 2,4-D shows the best growth of callus in *jasminum sambac* and root initiation has been started. The application of sodium azide on crop is easy and inexpensive for improvement. Different concentrations of growth regulator were used.Sodium azide has been used in wide range of application to improve crop ability. In their resistance aganst harmfull pathogen or to produce salt tolerence and other abiotic stresses. Sodium Azide (NaN_3) should be used to create mutation in those crops which are highly susceptible for harmful pathogens and made economically in expensive and benificial for farmers.

The main aim was to study the growth conditon for in vitro propagation of Jasmin from leafs. To achive this different concentration of growth regulator was used and using this we studied the different growth condition in 1mM, 2mM, 3mM, 4mM, 5mM at 1mg/lit, 2mg/lit and 3mg/lit respectively. We conclude that maximum average growth of callus of *jasminum sambac* at 1mg/lit,2mg/lit and 3mg/lit the 3mM was the highest.

FUTURE ASPECTS

- Screening for production of Secondary metabolites.
- Hardening of callus.
- Phytochemical anlysis of Wild plant and proliferated callus

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