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STUDIES ON EFFECT OF CERTAIN ANTIBIOTICS ON CALLUS GROWTH IN TERMINALIA CHEBULA

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ABSTRACT

The study was undertaken to identify suitable antibiotic and their required concentrations for enhancement of callus production and for further transformation studies. The effect of antibiotics such as carbenicillin, cefotaxime and streptomycin were studied on the callus growth of *Terminalia chebula*. Different antibiotics were added at different concentrations to the callus culture media and maintained up to 45 days. The experiments exhibited varied growth stimulation over controls. Cefotaxime and carbenicillin exhibited higher growth index when compared to control at different concentrations whereas streptomycin showed insignificant effect on growth index of *Terminalia chebula* callus culture. The results could be attributed to the plant hormone like chemical nature of cefotaxime and carbenicillin. However streptomycin did not exhibited higher callus production. Hence, it is concluded that the addition of antibiotics at optimum concentrations to the culture media could increase the growth of callus and the higher yield of callus.

Key Words: *Terminalia chebula*, Callus growth, Cefotaxime, Carbenicillin, Streptomycin

INTRODUCTION

Terminalia chebula is an important medicinal plant having various bioactive compounds such as alkaloids, tannins, flaovanoids, terpinoids (Rathinamurthy and Thilagavathi, 2014). And it is secondary food plant for wild silkworms. Since, *Terminalia chebula* had hard seed coat, heavy pest and disease infestation of seeds and low survival rate of stem cuttings, grafting and layering, it is the need of the hour to go for alternative methods of plant propagation. Unorganized cell mass and the development of callus is necessary for the development of transgenic plants. It is known that callus could be initiated by hormones such as cytokinins and auxin. Purohit et.al., (1995) exhibited high frequency of regenerative of callus, when supplemented with α-indole acetic acid (IAA) and kinetine (Kn). Antibiotics are used to suppress or to eliminate bacteria in Invitro plant tissue cultures. Furthermore antibiotics are being used to induce to calli, however a little is known about the mechanism of induction. Apart from controlling and removal of microbial contamination in plant tissue cultures (Pollock et.al., 1983) certain antibiotics exhibited growth stimulation in different plants. Carbenicillin and cefotaxime both belong to the β-lactam group, have minimal toxicity on most plants (Mathias and Boyed, 1986) and these antibiotics are widely used in agrobacterium mediated transformation. Streptomycin is an amino aminoglycoside antibiotic which inhibits peptide elongation and protein synthesis resulting in bactericidal activity (Biswas and Gorini, 1972).Hence the present study was carried out to develop callus production by addition of antibiotics to callus cultures of *T. chebula* from leaf explants to know the effect of different antibiotics on callus growth and development.

MATERIALS AND METHODS

Plant material *Terminalia chebula* of the family Combretaceae was used as plant material collected from one year old *T. chebula*. The leaves were washed in water containing 5 ml laboline detergent and thoroughly rinsed in distilled water for 5-6 times. These were treated with 0.1% (w/v) Bavistan (fungicide) solution for 10 min, and rinsed for 5-6 times with sterile distilled water. The tender leaves of approximately 5 cm in length were cultured in 25×150 mm tubes containing 15ml of solid 0.9% (w/v) agar, MS media (Murashige and Skoog 1962). The basal medium was supplemented with 30

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g/l sucrose and 2 mg/l 2-4-D to achieve callus. The pH of the medium was adjusted to 5.8 before autoclaving at 121ºC for 15 min. Collected explants such as invitro leaves were used for callus initiation.

Small piece of callus of known initial fresh weight 50±10 mg/l were kept on the medium containing Control 100,200,300,400,500,700mg/l of carbenicillin, cefotaxime and streptomycin for each experiment included eight replication for treatment. After 45 days of incubation the final fresh weights were measured to calculate the growth index (GI). Callus growth (GI) was represented according to the equation as described by (Dung et al., 1981).

$$\text{Growth index (GI)} = \frac{\text{Final callus fresh weight - Initial callus fresh weight}}{\text{Initial callus fresh weight}} \times 100$$

Antibiotic treatments of callus cultures carbenicillin, cefotaxime and streptomycin were dissolved in double distilled water (DDW), filtered-sterilized and added to the liquid medium after autoclaving all the above antibiotics were added to the callus culture medium at different concentrations i.e., 100 mg/l, 200 mg/l, 300mg/l, 400mg/l, 500mg/l 700mg/l. Control were maintained without antibiotics, the effects of antibiotics and the growth of callus on solid medium after 45 days. Duncan's Multiple Range Test(1955) was used to determine the statistically significance among control and antibiotic treated callus. All quantification measurements were expressed as mean ± SEM for each experiment 8 replications were cited.

RESULTS

From the date presented in the Table 1 and Figure 1, it is observed that relative to growth index of callus in *Terminalia chebula*. After 45 days significantly increased on cefotaxime treatment. The increase in the callus growth index was highest at the concentration of 500 mg/l. However, at the concentration of 700 mg/L the growth index was less when compare to controls. The order of increase was 100<200<300<400<500. Carbenicillin treated callus cultures also (Table 2 and Figure 2) showed significant increase in callus growth index and highest at 500 mg/l and negative impact exhibited at 700 mg/l of carbenicillin. The order of increase in callus growth index was 100>200>300>400>500. Streptomycin added callus culture also showed (Table 3 and Figure 3) an increase growth index but degree of increase was less when compared to cefotaxime and carbenicillin. The increase in growth index was concentration dependent up to 500 mg/l and growth index decreased at 700 mg/l of streptomycin. The order of growth increase was 100<200<300<400<500.

DISCUSSION

In the present investigation scope of high production of callus from the T. chebula was examined. It is known that callus induction and growth is dependent on different growth hormones and explants and relationship existed between growth hormones and biomass production. Kim et. al., 2001, supplemented culture media with 0.5mg/L of 2, 4-dichlro phenoxy acetic acid (2-4-D) and observed highest callus yield in Agastache rugosa O Kuntaze. However, realizing the importance of antibiotics in plant tissue culture we attempted to apply antibiotics ability to enhance T. chebula callus culture yield at higher level. In our study, β-lactam antibiotics such as cefotaxime and carbenicillin exhibited positive effects on callus growth. Further, more aminoglycoside antibiotics such as streptomycin also exhibited positive effect on callus growth but not significant effect when compared to controls. Danilova and Dolgikhi, (2004) exhibited a stimulatory effect in maize callus cultures. The activity of cefotaxime was attributed to the fact that plant esterases might have degraded into new metabolites and these metabolites might have showed growth regulation activity (Mathias and Boyed,1986). Quin et al., (2011) also reported that carbenicillin exhibited both stimulation and inhibitory effect. According to Holford and Newbury, (1992), carbenicillin molecular structure possess a structure similar to auxin and in media the carbenicillin is broken down to physiologically active phenyl acetic acid which can induce auxin like effect. It was noticed that very little callus growth was observed the concentration of 300 mg/l^{-1} of carbenicillin in carrot (Chang and Schmidt, 1991). Streptomycin is one of the antibiotic belonging to the category of aminoglucoside antibiotic which inhibits the growth of plant cells by binding to the 30S ribosome subunit (Biswas and Gorini, 1972). In contrary with earlier report our results exhibited positive effect at all concentrations of streptomycin, however, the effect was insignificant. This could be due to the stress of streptomycin on the callus cells as reported in the earlier studies.

On the whole it is concluded that addition of antibiotics such as cefotaxime, carbenicillin and streptomycin were not toxic to callus formation. Further these antibiotics enabled to enhance the callus culture when compared to controls in the range of 100-500 mg/L. Hence these antibiotics can be used for enhancement of callus production. However, further studies are needed to elucidate the biochemical mechanism of antibiotics in relation to enhancement of callus growth and development.

CONCLUSION

Broad spectrum antibiotics are being used in plant tissue cultures to reduce *in vitro* contamination. Our studies revealed

that antibiotics such as cefotaxime and carbencillin significantly increased the callus production at critical concentrations. It is concluded that carbencillin and cefotaxime acts as growth promoting substances and this could be attributed to the chemical structured similarities of antibiotics with some growth hormones.

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Table 1: Effect of Cefotaxime on the growth index (GI) of callus of T. Chebulla after 45 days of incubation in solid media.

Cefotaxime (mg/L)	Callus initial Fresh weight (mg)	Callus final Fresh weight (mg)	Growth index (GI)
0	50±10	925	1750 [b] ±0.07
100	50±10	1045	1990 [b] ±0.06 (+ 13.71)
200	50±10	1150	2200 [c] ±0.05 (+ 29.02)
300	50±10	1179	2258 [c] (+ 26.50)
400	50±10	1220	2340 [c] ±0.3 (+33.71)
500	50±10	1740	3380 [d] ±1.1 (+93.14)
700	50±10	470	910 [a] ±1.0 (-48.0)

[*] Each value is a mean of eight estimations.
[**] Per cent decrease over control is given in parenthesis.
*** Mean within a column followed by the same letter are not significantly different (p>0.05) from each other according to Duncan's Multiple range test.
Mean ± SEM

Table 2: Effect of carbenicillin on the growth index of callus(GI) of T. Chebulla after 45 days of incubation in solid media.

Carbenicillin (mg/L)	Callus initial Fresh weight(mg)	Callus final Fresh weight (mg)	Growth index (GI)
0	50±10	0874	1648[b] ±0.5
100	50±10	0978	1856[b] ±1.2 (12.62)
200	50±10	1100	2100[c] ±4.4 (27.42)
300	50±10	1252	2404[d] ±1.7 (45.87)
400	50±10	1565	3030[e] ±2.9 (85.07)
500	50±10	1607	3114[e] ±0.6 (88.95)
700	50±10	0534	680[a] ±5.0 (-58.73)

* Each value is a mean of eight estimations.
** Per cent decrease over control is given in parenthesis.
*** Mean within a column followed by the same letter are not significantly different (p>0.05) from each other according to Duncan's Multiple range test.
Mean ± SEM

Table 3: Effect of streptomycin on the growth index of callus of T. Chebulla after 45 days of incubation in solid media.

Streptomycin (mg/L)	Callus initial Fresh weight (mg)	Callus final Fresh weight (mg)	Growth index (GI)
0	50±10	947	1754[a] ±3.9
100	50±10	1070	2040[b] ±4.7 (+16.30)
200	50±10	960	1820[b] ±2.4 (+3.76)
300	50±10	1097	2094[b] ±4.8 (+19.38)
400	50±10	1120	2140[c] ±0.3 (+22.00)
500	50±10	1137	2174[c] ±2.4 (+23.94)
700	50±10	849	1598[a] ±1.8 (-8.89)

* Each value is a mean of eight estimations.
** Per cent decrease over control is given in parenthesis.
*** Mean within a column followed by the same letter are not significantly different (p>0.05) from each other according to Duncan 's Multiple range test.
Mean ± SEM

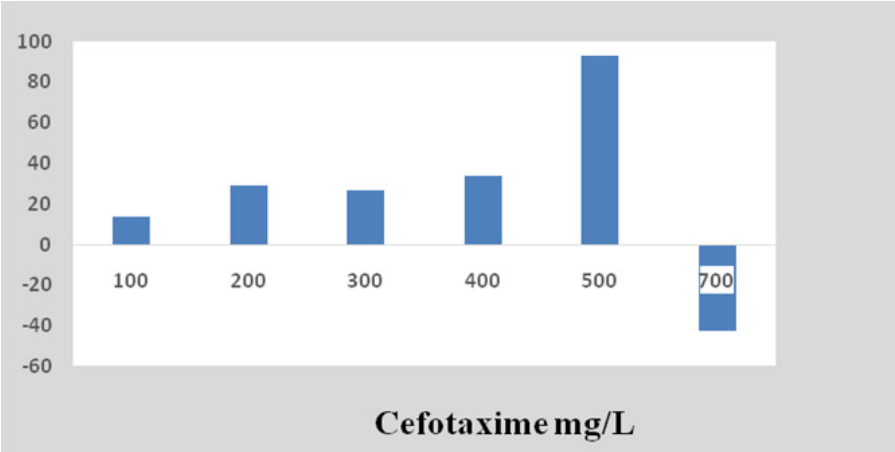

Figure 1: Percent change increase/decrease in callus growth index(GI) of T. cheulla on adding to different concentrations of cefotaxime at 45 days.

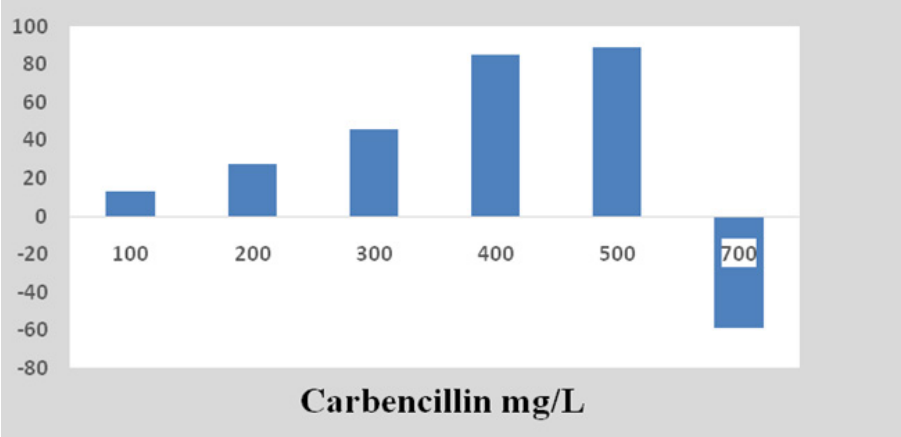

Figure 2: Percent change increase/decrease in callus growth index(GI) of T. cheulla on adding to different concentration of carbencillin at 45 days.

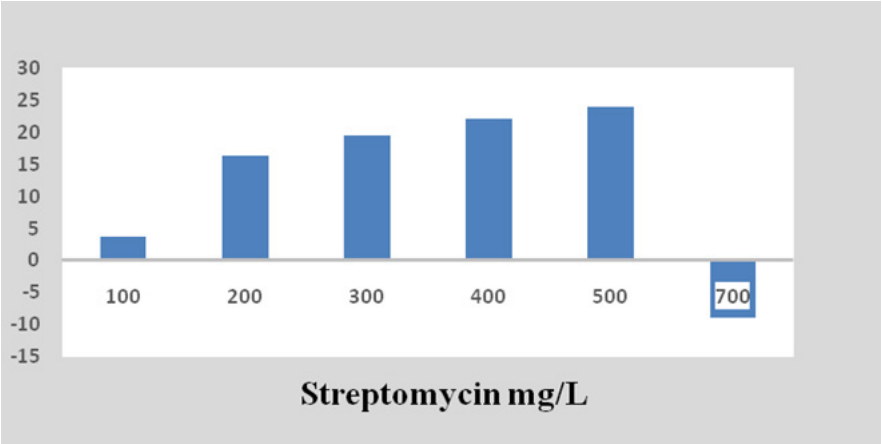

Figure 3: Percent change increase/decrease in callus growth index(GI) of T. cheulla on adding to different concentration of streptomycin at 45 days.