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Callus induction treatments influence antimicrobial effect of tissue culture-derived *Thuja orientalis* L.

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Abstract

Thuja orientalis belonging to the family Cupresseaceae is an important forest tree species for timber and wood production. Its wood is light in weight and highly resistant to decay. The leaf of explants of this plant was cultured for callus induction and plant regeneration. The explants of this plant were cultured onto Murashig and Skoog (MS) medium supplemented with different concentrations of (α- naphtalene acetic acid (NAA), p-chlorophenoxyisobutyric acid (PCIB) alone and in combination with 6- benzyladenine (BA) for callus induction. The highest callus was induced in medium containing 3 mg/l PCIB. The lowest callus was found to be on MS medium containing (3 mg/l NAA +0.1 mg/l BA). Callus of explants were grown on MS media contained 3 mg/l PCIB +0.1 mg/l BA extract is the most active fraction against *E. coli* also it is active against *Streptococcus pneumonia* and *Bcilis subtilis*. Callus of explants were grown on MS media (3 mg/l NAA +0.1 mg/l BA) has higher activity against *Streptococcus pneumonia*, Callus of explants were grown on MS media supplemented with (3 mg/l PCIB +0.1 mg/l BA) has higher activity against *Bacilis subtilis*. These results provide a basis for future studies on genetic improvement and could be applied to production of secondary metabolites through cell culture in *Thuja orientalis*.

Keywords: *Thuja orientalis*, Callus formation, *Pseudomonas aeruginosa*, *E. coli*, *Bacilis subtilis*, *Streptococcus pneumonia*.

INTRODUCTION

Thuja orientalis (Commonly- Morpankhi, Family- Cupressaceae) is a genus of coniferous trees. *T. orientalis* is an evergreen, monoecious trees or shrubs growing to 10-60 feet tall. The shoot are flat, leaves are scale like. The leaves are arranged in flattened fan shaped growing with resine glands [1]. Their leaves contain essential oils used to treat fungus infections, cancer, moles and parasitic worms. The essential oil derived from the leaves is toxic. α-thujone is useful as an insecticide and an antihelminthic agent for the treatment of parasitic worms [2]. However, α -thujone is a toxic substance that disrupts neurological signals in the brain. Ingestion of the essential oils of Thuja leaves can cause death [2]. Seed with a pair of narrow lateral wings, seedlings produce 2 cotyledons. The wood is light, soft and aromatic. Thuja poles also often used to make fence posts and rails. The wood of Thuja is commonly used for guitar sound boards [3]. It is used as a medicinal plant in various forms of traditional medicines like folk medicine, homeopathy and treatment of bronchial catarrh, enuresis, cystitis, psoriasis, uterine carci-nomas, amenorrhea and rheumatism [4-6]. Oil of Thuja contains thujone which has been studied for its GABA (gamma-aminobutyric acid) receptor antagonistic, with po-tentially lethal properties [2]. A yellow dye is obtained from the young branches [7]. Thuja is also occasionally used for treating diseases of skin, blood, gastrointestinal tract, kid-ney, brain, warty excrescences, and spongy tumors [6]. Dubey and Batra [8,9] reported that the hepato-protective activities and antioxidant activity of *Thuja occidentalis*. Anti-prolif-erative and apoptosis- inducing properties of *Thuja occidentalis* has been evaluated by Biswas *et al.*[6].

Thuja orientalis apical buds from adult trees and seedlings were collected in 3 seasons. After pre sterilization, the best method for sterilizing of buds was identified. The method was: Cleaning and brushing the buds between and ethanol 70% solution as the pretreatment, dipping them in ethanol 70% for 1minutes and cleaning buds with 0.1% $HgCl_2$ solution in different periods (4 for spring, 6 for summer, and 9 minutes for fall). The suitable medium for establishment was DKW medium with 0.5 mg/lit 2iP.Emam Mitra [5].

The essential oil from seed coats of *Thuja orientalis L*. (Family Cupressaceae) obtained in an yield of 1% has been studied for its antimicrobial activity against 6 bacteria and 5 fungi using filter paper disc agar diffusion technique. Rakesh Kumar Jain *et.al* [10]. The composition of the hydro distilled essential oils from the fruits and leaves of *Thuja orientalis L*. grown in Iran was analyzed by GC/MS. Nineteen and twenty-eight compounds have been identified in the volatile oils of the fruit and leaf Bahman Nickavara *et.al* [11]. Antibacterial compounds from Aeglemarmelos and *Thuja orientalis* extracted with chloroform proved effective against two-gram positive pathogenic bacteria, *Staphylococcus aureus* and *Bacillus thuringiens* is infecting mulberry silkworm, *Bombyx mori* L., S. Manimegalai1 *et.al* [12].

MATERIALS AND METHODS

Plant material, sterilization and preparation of explants

This work was done in the Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt.

Source of Leave

The Leave of (*Thuja Orientalies L*) (Hera) obtained from *Thuja orientalis trees at Sadat city*, the seeds were identified to the representative herbarium specimens in Cairo University.

Sterilization steps:

The leave were carefully washed with detergent and rinsed with tap water. They were washed with disinfectant agent sodium hypochloride 3% for 20 minutes.

Culture media:

These explants were then cultured aseptically on basal solid MS-medium with several treatments. The pH was adjusted to 5.7 with 1 N KOH or 1 N HCl before adding gel rite and prior to autoclaving at 121 ˚C (0.1. MPa) for 20 min. The cultures were kept in a growth chamber at 21 ± 1 ˚C, and a photoperiod of 16.h (30 µE m-2s-1, Philips TL 33 light)

Leave were cultured on media containing half strength MS media (half MS), full strength MS (full MS), half MS with 3 ml p- chloro iso butyric acid (PCIB), full MS with 3mg/l p- chloro iso butyric acid (PCIB).

Callus formation

Leave were cultured for callus formation on different concentrations of growth regulators including auxins as NAA (1 mg/l NAA with 0.1 mg/1 (BA). Auxin transport inhibitor p- chloro iso butyric acid (PCIB) was used with different concentrations (1, 3 mg/l PCIB + 0.1 mg/l benzyl adinine)

The cultivation was done in 300 ml glass jars containing 50 ml of basal MS-medium.

Antibacterial and antifungal activities

Extracts under investigation were individually tested against a panel of Gram-positive and Gram-negative bacterial pathogens, yeast and fungi.

Antimicrobial tests were carried out using the agar well diffusion method, with 100 ml suspension containing 1 x 10^8 colony forming units (CFU)/ml for tested bacteria, 1 x 10^6 CFU/ml yeast and 1 x10^4 spore/ml fungi spread on nutrient agar (NA), Sab. dextrose agar (SDA), and malt extract agar (MA), respectively[13].

After the media had cooled and solidified, wells (6 mm in diameter) were made in the solidified agar and loaded with 100 µl of tested compound solution prepared by dissolving 1 mg of the chemical compound in 1 ml dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37°C for bacteria and yeast, 48 h at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ampicillin, were used as standards for Gram positive bacteria, Gram negative bacteria and fungi respectively. After incubation, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard.

Antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Statistical methods

The experiment was carried out in triplicate and the data was expressed as mean ± SD.

RESULTS AND DISSCUTION

In our research, the beginning of callus production by the explants on all media was observed after 4 weeks.

The amount of callus depended on the composition of the medium. After 6 weeks, the mean ratings of the amount of callus ranged from 0.5–5.0°. The most intense callus development was observed in the leaves explants grown on the medium containing MS + p- chloro iso butyric acid (3 mg/l PCIB). The lowest callus amount explants were observed in the leaves explants grown on the medium containing MS+ (3 mg/l NAA + 0.1 mg/l benzyl adinine).

Callus derived from the previous experiments used in this experiment to study the effect of media component on callus formation. Callus formation from leaves explants could be observed on MS medium supplemented with 3 mg/l PCIB) and half MS supplemented with 3 mg/l PCIB) after four weeks whereas callus initiated on other medium formation after 6 weeks. On the present study data obtained clearly showed that MS medium supplemented with 3 mg/l PCIB) and half MS supplemented with 3 mg/l PCIB) gave the highest callus formation as it gave the highest number of either explant formed callus, callus percentage, callus fresh weight and callus index. While MS medium supplemented with (1 mg/l PCIB+ 0.1 mg/l BA) and 3 mg/l PCIB+ 0.1 mg/l BA had moderate results. Lower frequency of callus was observed on MS medium supplemented with (3 mg/l NAA+ 0.1 mg/l BA).

Table 1: Effect of component of plant tissue culture nutrient medium on callus formation of *Thuja orientalis* after 6 weeks

Medium component	**No of initial Explants**	**No of explants formed callus**	**Callus %**	**Callus fresh weight mg/explant**	**Callus Index***
MS+3 mg/l PCIB	20	15	75	37.41	2805.7
Half MS +3 mg/l PCIB	10	9	90	20.73	1865.7
MS + 1 mg/l PCIB+ 0.1 mg/l BA	10	6	60	33.38	2002.8
MS + 3 mg/l PCIB+ 0.1 mg/l BA	10	4	40	10.17	406.8
MS+3 mg/l NAA+ 0.1 mg/l BA	10	4	40	8.93	357.2

Table 2: Determination of antimicrobial effect of samples A, B, C, D and E extracts

Sample Tested microorganisms	**MS + 1 mg/l PCIB + 0.1 mg/l BA (A)**	**MS + 3 mg/l PCIB + 0.1 mg/l BA (B)**	**Half MS +3 mg/l PCIB (C)**	**MS+3 mg/l PCIB (D)**	**MS+3 mg/l NAA+ 0.1 mg/l BA (E)**	St.
Fungi						Amphotericin B
Aspergillus fumigatus (RCMB 02568)	14.3±15	17.4±0.58	13.6±0.58	15.2±0.58	16.3±1.5	23.7±0.1
Candida albicans (RCMB 05036)	NA	NA	NA	NA	NA	25.4±0.1
Gram Positive Bacteria						Ampicillin
Streptococcus pneumoniae (RCM 010010)	16.2±2.0	19.2±0.58	16.9±0.44	17.4±0.64	19.6±1.0	23.8±0.2
Bacilis subtilis (RCMB 010067)	17.2±1.2	20.3±0.53	19.2±0.63	20.2±0.58	20.0±0.58	32.4±0.3
Gram Negative Bacteria						Gentamicin
Pseudomonas (RCMB 010043)	NA	NA	NA	NA	NA	17.3±0.1
Escherichia coli (RCM 010052)	14.4±2.0	17.8±0.63	16.4±058	17.3±0.63	15.9±0.63	19.9±0.3

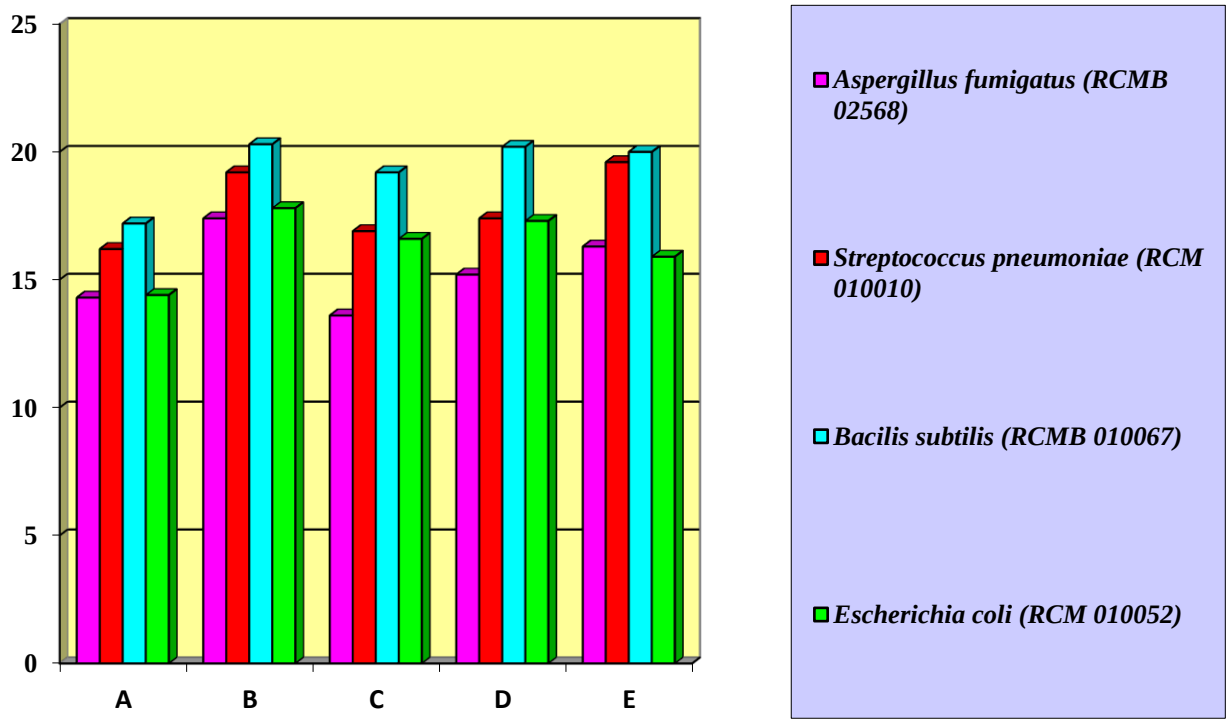

Figure 1: Graphical representation of antimicrobial

The test was done using the diffusion agar technique, well diameter: 6.0 mm-

Data are expressed in the form of mean ± SD -

Tested samples concentration is (10 mg/ml)

(Sample B) extract is the most active fraction against *E. coli* also it is active against *Streptococcus pneumonia* and *Bcilis subtilis.* The tested sample has no activity against *Pseudomonas aeruginosa* and *Candida albicans*. (Sample E) has higher activity against *Streptococcus pneumoniae*, (Sample B) has higher activity against *Bacilis subtilis*.

CONCLUSIONS

1. The *Thuja Orientalis L* callus formation can be obtained from different concentrations of growth regulators.
2. The growth regulators also had a significant impact on the amount of callus produced.
3. The efficiency of callus formation depended on the hormone concentrations and the proportion between PCIB and BA. The best results were achieved on the medium containing 3 mg/l PCIB and 0.1 mg/l BA.
4- Sample(B) Callus of explants were grown on MS media +(3 mg/l PCIB +0.1 mg/l BA) extract is the most active fraction against *E. coli* also it is active against *Streptococcus pneumonia* and *Bacilis subtilis.*

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