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Study the Effect of Triazophos as Plant Growth Regulator in Tissue Culture of Different Plants

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Abstract: The present study was conducted to investigate the effect of triazophos in tissue cultures of three plants *Catharanthus roseus*, *Zizyphus vulgaris* and *Eucalyptus globulus*. Triazophos was compared with 2,4-D on Murashige and Skoog's (MS) medium supplemented with different concentrations of them. Triazophos was tested for it is potential in callus induction and compared its activity with 2,4-D as plant growth regulator .There are no significant differences ($p<0.05$) in callus induction on leaf explants between Triazophos and 2,4-D of all plants. The best concentrations to initiation and maintenance callus were (0.1and 1) mg/L for Triazophos and 2,4-D respectively for all plants.

Keywords: *Catharanthus roseus*, *Eucalyptus globules*, growth regulator, hositation,2,4-Dichlorophenoxy acetic acid, triazophos, *Zizyphus vulgaris*

INTRODUCTION

2,4-Dichlorophenoxy acetic acid (2,4-D) is a common herbicide used in the control of broad leaf weeds. It is the most widely used herbicide in the world (Barnekow *et al*., 2000), absorbed through the leaves and is translocated to the meristems of the plant.2,4-D is a synthetic auxin (plant regulator) and it is often used in laboratories for plant research as a supplement in plant cell culture media such as MS medium. Despite industry efforts claiming the safety of this chemical, there is a large body of evidence indicating major health effects, from cancer to immunosuppressant, reproductive damage cytotoxics , hepatoxic effects to neurotoxicity. Environmental contamination, particularly in wetlands has also been demonstrated. (Blakely *et al*., 1989; Sulik *et al*, 1998; Barnekow *et al*., 2000; Rosso *et al*., 2000; Venkov *et al*., 2000; Charles *et al*., 2001; Madrigal-Bujadar *et al*., 2001; Osaki *et al*., 2001; Tuschl and Schwab,2003).

This study was replaced 2,4-D with triazophos trade name is (Hostathion 40 EC) which an organophosphorus insecticide (systemic pesticide) effective against many insect pests on a wide range of crops. (Hamernik, 2002). Triazophos can penetrate deeply in the plant tissues due to its translaminar properties and can effectively control leaf miner. There has been no report of resistance since inception and has been recommended for use in resistance management program (JMPR, 2007). Triazophos is more safety on human health and less expensive than 2,4-D., Therefore, this study was aimed to compare between Triazphos and 2,4-D as plant growth regulators to produce the callus from *Catharanthus roseus*, *Zizyphus vulgaris* and *Eucalyptus globulus* plants.

MATERIALS AND METHODS

Plant material: *Catharanthus roseus*, *Zizyphus vulgaris* and *Eucalyptus globulus* plants grown in Iraq-Baghdad-Baghdad University gardens were used as a source for plants used in this experimental work. Plants were identified by lush of Science Collage-Baghdad University (Prof. Dr. Ali H. Al-Moosawi).This study was conducted in 2010 at department of biotechnology, college of science, Baghdad University.

Sterilization of media and instruments: Culture medium was sterilized by autoclaving at 121°C under 1.04 Kg/cm² pressure, for 15 min. Glassware and other instruments either autoclaved or placed in electric oven at 200ºC for 2 h. (Ramawat, 2008). The medium in the vessels were left at room temperature to cool and become ready to culture explants.

Sterilization of explants: Mature leaf and petal explants were excised, rinsed with tap water for 30 min., then transferred to a laminar air flow-cabinet where submerged with sodium hypochlorite (NaOCl) at different concentrations (0, 1, 1.5 and 2 %) for 15 min. Sodium hypochlorite (NaOCl) was purchased from a local market at a concentration (6%). To prepare the required concentrations, volumes of (0, 16.6, 25 and 33.3) mL of sodium hypochlorite were taken and the volume was completed to 100 mL with sterilized distilled water (DH_2O) according to the equation $C_1 V_1 = C_2 V_2$. Explants were rinsed with sterilized (DH_2O)

for three times. For each concentration 12 explants were used and both ends of each explant were cut to remove tissues affected by sterilization solution. The final diameter of explants discs was 1 cm using a cork borer (Pierik, 1987). The survival rate was calculated depending on the number of explants show no contamination.

Preparation of culture medium: Murashige and Skoog Medium (1962) (MS) was prepared and used. Sucrose 30 g/l, Myoinositol 100 mg/L and the plant growth regulators (Traizphos and 2,4-D) at different concentrations were added. The pH was adjusted to 5.8 using NaOH or HCl (1 N), then 8 g/L of the agar type (agar-agar) was added to the medium, placed on a hot plate magnetic stirrer till boiling, then aliquots of 20 mL were dispensed into (8×6) cm culture vessels.

Plant growth regulators: Traizophos was purchased from a local market at a concentration (420 g/L) to prepare the required concentrations (0.1, 0.2, 0.4 and 0.6) mg/L and different concentrations of 2,4-diclorophenoxy acetic acid (2,4-D) (1, 2, 4 and 6) mg/L were added to the culture medium after autoclaving.

Incubation of cultures: Leaf explants were sterilized and inoculated into the culture vessels under aseptic conditions, placed in the incubator under 16 h. Light/ 8 hrs. Dark photoperiod at 25ºC for 30 days and then replaced the older media with new media for each growth regulators and incubate the culture vessels in the same conditions for 30 days. The response of these explants to 2,4-D and triazophos was evaluated after 60 days in culture to determine the proper one for callus induction.

Statistical Analysis: Statistical analyses were done using SPSS (version 17) program. Mean and standard deviation were descriptive measures of quantitative data using the analysis of variance test (t-test) for independent samples. P-value $p<0.05$ were considered significant test (Al-Mohammed *et al*,. 1986).

RESULTS AND DISCUSSION

Sterilization of explants: NaOCl was used for explants sterilization leaves of *Catharanthus roseus*, *Zizyphus vulgaris* and *Eucalyptus globules*. The most effective concentration of NaOCl 2% for 15 min, which gave the highest percent 100% of survival for all plants. Using NaOCl is important to eliminate the contaminants. It is used widely for explants sterilization. The selection of sterilizing material depends on the source of explants, roughness of its surface and other factors. The sterilization material should be easy to remove from explants when washed with sterilized DH_2O (Yeoman and Macleod, 1977; Sateesh, 2003).

Pierik, (1987) referred to the importance of NaOCl in explants sterilization and found that increasing the surface sterilization period and concentration often leads to a serious reduction in survival rate. Therefore, optimization experiment is necessary to achieve maximum survival rate with minimum contamination.

Induction of callus cultures: Callus was initiated on leaf explants taken from *C. roseus* and cultured on MS medium with different concentrations of Triazphos and 2,4-D (Fig 1). Results in Table 1 (A, B, C and D) explain the effect of Triazphos and 2,4-D on callus fresh weight (g) initiated on *Catharanthus roses* leaf explants. The concentration (0.1, 0.2, 0.4, 0.6) mg/L of Triazphos showed no significant differences ($p<0.05$) in callus induction when compared with the concentration of (1, 2,4, 6) mg/L of 2,4-D which gave (1.3450, 1.0288, 0.6838 and 1.2888, 0.8375, 0.497) g of callus fresh weight, respectively. The highest callus fresh weight for callus induction on leaf explants was achieved in low concentration (0.1 and 1) mg/L of Triazphos and 2,4-D, respectively which gave (1.345 and 1.2888 g) callus fresh weight (Table 1A) reached to lowest callus fresh weight (0.6838 and 0.4975 g) respectively in high concentration (0.4 and 4 mg/L) of Triazphos and 2,4-D (Table 1C). Results in Table 2 (A, B, C and D) explain the effect of Triazphos and 2,4-D on callus fresh weight (g) initiated on *Zizyphus vulgaris* leaf explants. The concentration (0.1, 0.2, 0.4, 0.6) mg/L of Triazphos showed no significant differences ($p<0.05$) in callus induction when compared with the concentration of (1, 2, 4, 6) mg/L of 2,4-D which gave (0.83113, 0.7250, 0.3438 and 0.7613, 0.6400, 0.3625) g of callus fresh weight, respectively. The highest callus fresh weight for callus induction on leaf explants wasachieved in low concentration (0.1 and 1) mg/L of Triazphos and 2,4-D respectively which gave (0.8113 and 0.7613 g) callus fresh weight (Table 2A) reached to lowest callus fresh weight (0.3438 and 0.3625 g), respectively in high concentration (0.4 and 4 mg/L) of Triazphos and 2,4-D (Table 2C). Results in Table 3 (A, B, C and D) explain the effect of Triazphos and 2,4-D on callus fresh weight (g) initiated on *Eucalyptus globulus* leaf explants. The concentration (0.1, 0.2, 0.4, 0.6) mg/L of Triazphos showed no significant differences ($p<0.05$) in callus induction when compared with the concentration of (1, 2, 4, 6) mg/L of 2,4-D which gave (0.7025, 0.5500, 0.3263 and 0.6288, 0.4713, 0.2488) g of callus fresh weight, respectively.

Table 1: The effect of different concentrations of triazophos and 2,4-D (A, B, C and D) on callus fresh weight (g) initiated on leaf explants of *Catharanthus roseus*, p<0.05

Growth regulators	Conce of growth regulators mg/L	N	Callus fresh weight (gram) (mean±SD)
A			
triazophos	0.1	8	1.3450 ± 0.66606
2,4-D	1	8	1.2888 ± 0.51217
B			
triazophos	0.2	8	1.0288 ± 0.42502
2,4-D	2	8	0.8375 ± 0.08207
C			
triazophos	0.4	8	0.6838 ± 0.26441
2,4-D	4	8	0.4975 ± 0.26168
D			
triazophos	0.6	8	0.00
2,4-D	6	8	0.00

Table 2: The effect of different concentrations of Triazophos and 2,4-D(A, B,C and D) on callus fresh weight (g) initiated on leaf explants of *Zizyphus vulgaris*, p<0.00

Growth regulators	Conce.of Growth regulators mg/l	N	Callus fresh weight (gram) (mean±std.deviation)
A			
triazophos	0.1	8	0.8113 ± 0.16941
2,4,D	1	8	0.7613 ± 0.15570
B			
triazophos	0.2	8	0.7250 ± 0.18103
2,4,D	2	8	0.6400 ± 0.23833
C			
triazophos	0.4	8	0.3438 ± 0.31409
2,4,D	4	8	0.3625 ± 0.28739
D			
triazophos	0.6	8	0.00
2,4-D	6	8	0.00

Table 3: The effect of different concentrations o f triazophos and 2,4-D(A, B, C and D) on callus fresh weight (g) initiated on leaf explants of *Eucalyptus globulus*, p<0.05

Growth regulators	Conce.of Growth regulators mg/L	N	Callus fresh weight (g) (mean± SD)
A			
triazophos	0.1	8	0.7025 ± 0.17982
2,4,D	1	8	0.6288 ± 0.16453
B			
triazophos	0.2	8	0.5500 ± 0.26549
2,4,D	2	8	0.4713 ± 0.16488
C			
triazophos	0.4	8	0.3263 ± 0.23664
2,4,D	4	8	0.2488 ± 0.19187
D			
triazophos	0.6	8	0.00
2,4-D	6	8	0.00

The highest callus fresh weight for callus induction on leaf explants was achieved in low concentration (0.1 and 1) mg/L of Triazphos and 2,4-D, respectively which gave (0.7025 and 0.6288 g) callus fresh weight (Table 3A) reached to lowest callus fresh weight

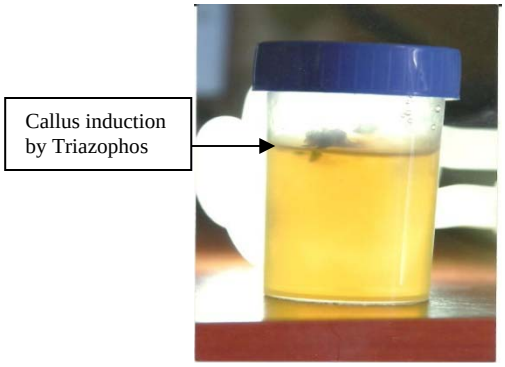

Fig. 1: Callus induction by (0.1 mg/L) of triazophos in MS medium of *Catharanthus roseus* after 60 day

(0.3263 and 0.2488 g), respectively in high concentration (0.4 and 4) mg/L of Triazphos and 2,4-D (Table 3C). The concentrations (0.6 and 6) mg/L of triazophos and 2,4-D respectively (Table 1D, 2D, 3D) showed no callus induction when used in MS media for all plants under experiment. Induction and maintenance of callus cultures in all plants under experiment seem to favor low concentration of triazophos. Callus induction and differentiation are influenced by many factors: medium components, type and concentration of plant growth regulators, plant physiological status, source of explants and environmental conditions (Torbert *et al*., 1998). Callus induction by (0.1 mg/L) of triazophos in MS medium of *Catharanthus roseus* after 60 day.

CONCLUSION

This study was concluded that can use triazophos as plant growth regulator in MS media in low concentration to induce callus production from *Catharanthus roseus*, *Zizyphus vulgaris* and *Eucalyptus globulus* plants in compression with 2,4-D which was used in high concentration.

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