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EFFECT OF USNIC ACID ON MITOTIC INDEX IN ROOT TIPS OF ALLIUM CEPA L.

S. ÖZTÜRK*, S. GÜVENÇ*, N. ARIKAN** & Ö. YILMAZ*
Uludag University, Science and Art Faculty, Department of Biology*
& Department of Chemistry**, Görükle - Bursa, Turkey.
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Resumen. Se ha ensayado el efecto del ácido úsnico, obtenido a partir de *Usnea filipendula* Stirton, en el índice mitótico de meristemos de *Allium cepa* L. El índice mitótico disminuye a dosis de 100, 200 y 300 ppm de ácido úsnico. El efecto inhibidor del ácido úsnico aumenta con la dosis y el tiempo de tratamiento. Este efecto inhibidor es estadísticamente significativo, $p<0,05$ y $p<0,001$, para 12 y 24 h. de tratamiento, respectivamente.

Summary. Usnic acid obtained from *Usnea filipendula* Stirton has been tested for its effect on mitotic index in root tips of *Allium cepa L.* Mitotic index is decreased 100, 200 and 300 ppm doses of usnic acid. The inhibition effect of usnic acid is increased with doses and time of treatment. The inhibition effect is statistifically significant, $p < 0.05$ and $p < 0.001$, for 12 and 24 hour treatment, respectively.

INTRODUCTION

Lichens produce a highly diverse variety of organic compounds that can be lumped into two groups called primary metabolites and secondary metabolites (ZEYBEK & JOHN, 1992). Primary metabolites are the carbohydrates, proteins, lipids and other organic compunds vital to the lichen's structure and metabolism. Some are produced by the lichen's fungal partner and others by the liche's algal / or cyanobacterial partners. Secondry metabolites are produced by the fungus alone and secreted onto the surface of its hyphae in either on amorphous form or as crystals. If they are found in only lichens, then they are called lichen substances (MALCOM & GALLOWAY, 1997).

The chemistry of about one third of all lichen species has now been studied and about 350 secondary compunds are known from lichens. The chemical structure of approximately 200 of them has been established. They are extracellular products of relatively low molecular weight, crystallize on the hyphal zell walls, are usually insoluble in water, and can be extracted only withn

organic solvents. They amount to between 0.1 and 10% of the dry weight of the tallus, sometimes up to 30 % (GALUN, 1988).

The polysaccharides isolated from *Umbilicaria sp., Evernia prunastri, Alectoria sp.* and *Lasallia sp.* have been observed to be have antitumour activity against sarcoma 180 in mice (TAKEDA & al., 1972; ZEYBEK & JOHNS, 1992). Also anti - HIV activity of GE - 3 - S, a partially acetylated β (1 → 6) glucan of the lichen *Umbilicaria esculenta*, was being determined (HIRABAYASHI & al., 1989).

Usnic acid is one of the most common secondary metabolites found in lichens (ASAHINA & SHIBATA, 1971). *Usnea sp.* is known to be a rich source of usnic acid which have antimicrobial activity (DÜLGER & al., 1997; HARMALA & al., 1992; ÖZTÜRK & al., 1998). *l* - usnic acid and *d* - usnic acid isolated from *Cetraria sp.* and *Usnea sp.*, respectively is tested in mice against the Lewis lung carcinoma and is found to be have antitumour activity (KUPCHAN & al., 1975).

According to information by Autors, major effects in the plants of usnic acid are inhibition of root growth and dwarfism. When the plants is treated with usnic acid, in photosynthesis and transpiration rates are presented a decrease (VAVASSEUR & al., 1991).

MATERIAL AND METHOD

Powdered *Usnea filipendula* (60 g) is extracted with ether at 4 hours. It is crystallized from benzene and galcial acetic acid according to ASAHINA & SHIBATA (1971). When usnic acid obtained from *Usnea filipendula* compared with reference acid (U-7876, SIGMA), melting point and IR spectrum of usnic acid is similar to reference.

Treatment solutions were prepared by dissolving usnic acid in 1 % DMSO (usnic acid is dissolved in 1 ml DMSO and subsequently diluting in distilled water). The used concentrations were 100, 200 and 300 ppm solutions. As a control solutions is used 1% DMSO and distilled water.

Root tips of 2-3 cm length were exposed to the test solutions for 12 hour and 24 hour. Following all treatments roots were detached, washed and fixed in 3 : 1 alchol - acetic acid for 24 hours and stored in 70 % alchol in the refrigerator until use.

Cytological preparations were carried out using Acetocarmen squash technique. 25 slides of each treatment were examined for the effect on mitotik index (MI). The treatments have been repeated five times. Mitotic index was estimated according to below formule:

$$\% \text{ MI} = \frac{\text{Mitotic cells}}{\text{Mitotic cells + Normal cells}} \text{ X } 100$$

For each one slide was counted 1500 cells. Statistical analysis was done by computer program STATISTICA for Windows 4.3. The determination the effect of various concentrations of usnic acid on mitotic index for 12 hour and 24 hour treatment is used multiple regression test and the determination difference between 100, 200 and 300 ppm concentrationof usnic acid in each one treatment is used t test. Probability level for significance was set at $p < 0.05$.

RESULTS AND DISCCUSION

In this study, the effect of the treatment solutions prepared by DMSO of pure usnic acid obtained as a crystal form from *Usnea filipendula* have been investigated depend on doses and time of treatment increase on mitotic index in root tips of *Allium cepa*.

For dissolve usnic acid which is insoluble in water is used DMSO as a solvent. It is clearly appeared that DMSO has inhibition effect on miotic index when compared with distilled water (Table 1).

Treatment time	Treatment	MI ± SE	Number of Slides	t
24 hour	Distilled water	7.71 ± 0.87	25	
12 hour	% 1 DMSO	4.47 ± 1.10	25	4.34*
24 hour	% 1 DMSO	4.56 ± 1.88	25	3.33*

Table 1. Effect of 1% DMSO and distilled water on mitotic index in root tips of *A. cepa*. * $p < 0.05$.

Treatment time	Treatment conc.	MI ± SE	Number of Slides	t
12 hour	DMSO	4.47 ± 1.10	25	
	100ppm	3.74 ± 1.16	25	0.95
	200ppm	3.68 ± 1.59	25	0.95
	300ppm	2.94 ± 0.35	25	3.13*
24 hour	DMSO	4.56 ± 1.88	25	
	100ppm	3.24 ± 0.89	25	1.76
	200ppm	1.97 ± 1.03	25	2.52*
	300ppm	1.74 ± 0.47	25	2.98*

Table 2. Effect of the different concentrations of usnic acid on mitotic index in root tips of *A. cepa*. * $p < 0.05$.

The effect of 100, 200 and 300 ppm concentrations of usnic acid on mitotic index is shown Table 2 and Fig. 1.

Only 300 ppm concentration of usnic acid has significant effect on mitotic index for 12 hour, while 200 and 300 ppm concentrations of usnic acid has significant effect for 24 hours (Table 2).

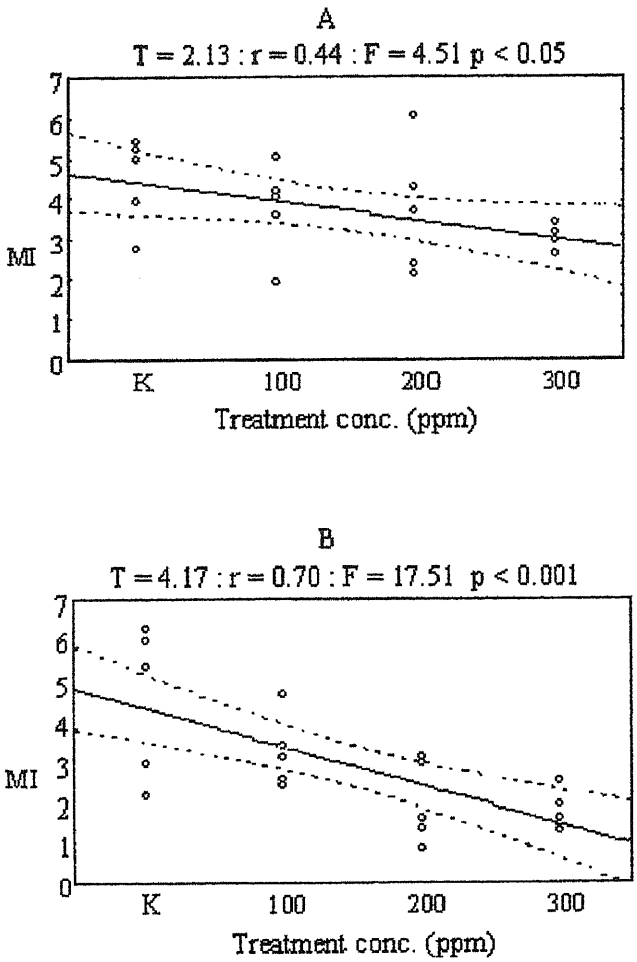

Fig. 1. The effect depend up to increase concentration of usnic acid and time of treatment on mitotic index in root tips of *A. cepa*.
A: 12 hour teatment ($p < 0.05$). B: 24 hour treatment ($p < 0.001$).

The inhibition effect of usnic acid on mitotic index is increased whith time of treatment increas (Fig. 1).

When compared with the difference between 12 hour and 24 hour treatments, 100 and 200 ppm concentrations of usnic acid is not significant effect on mitotic index, while 300 ppm concentration is statisficaly significant (Table 3, Fig. 2).

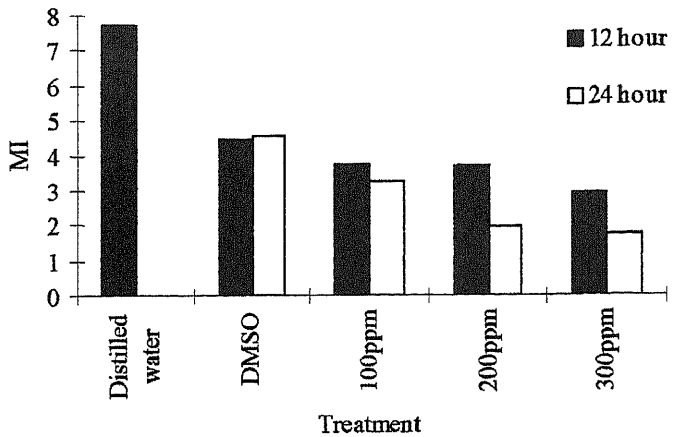

Fig. 2. Effect of concentrations of usnic acid and treatment time on mitotic index in root tips of *A. cepa*.

Treatment	Treatment time	MI ± SE	Number of Slides	t
Control (DMSO)	12 saat	4.47 ± 1.10	25	–0.09
	24 saat	4.56 ± 1.88		
100 ppm	12 saat	3.74 ± 1.16	25	0.75
	24 saat	3.24 ± 0.89		
200 ppm	12 saat	3.68 ± 1.59	25	2.00
	24 saat	1.97 ± 1.03		
300 ppm	12 saat	2.94 ± 0.35	25	4.53*
	24 saat	1.74 ± 0.47		

Table 3. Effect of time of treatment on mitotic index in root tips of *A. Cepa*. * $p < 0.05$.

Usnic acid showed an ability to inhibit mitotic activity in root tips of *A. cepa*. The reduction in mitotic activity was clearly doses dependant. Mitotic index value was progressively decreased with the increas of usnic acid concentration and time of treatment.

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