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Effects of *Echinacea angustifolia* Hell. extracts on total protein and peroxidase changing in *Solanum lycopersicum* L.

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ABSTRACT

Echinacea angustifolia Hell. is an endemic, medicinal and economically important plant which is belonging to Asteraceae family. In this research, the effects of ethanolic and methanolic extracts of E. angustifolia (narrow-leaved echinacea) on total protein and peroxidase [EC 1.11.1.7] levels changing on Solanum lycopersicum L. were determined. Echinacea powder extract were prepeared as a stock solution by DMSO solvent as 0.01 g/mL and 0.03 g/mL concentrations. Extracts were applied to the S. lycopersicum L. plantlets by leaf spraying. 24 and 48 hours after applications, healthy leaf of 16 weeks old plantlets were harvested for protein and peroxidase analyses. Changing in total protein and peroxidase levels were measured spectrophotometrically. Changing in both protein and peroxidase levels were occurred after echinacea applications acoording to concentrations and exposure time.
Amount of total protein changing after echinacea application in S. lycopersicum plantlets when compare with control group and 24 hours application. Protein levels were decreased 48 hours after 0.03g/mL echinacea application in DMSO, DMSO+methanol, DMSO+ethanol groups respectively as %43.16, %29.32 ve %27.26.
Peroxidase activity changing in S. lycopersicum plantlets when compare with control group and 24 hours application. Peroxidase levels were increased 48 hours after 0.03g/mL echinacea application in DMSO, DMSO+methanol, DMSO+ethanol groups respectively as %43.16, %62.60, %38.20, %38.95. As a result of our research, echinacea extracts which is prepeared with two different concentrations and exposure times were stimulate the plant defense system as a plant activator as well.

Keywords: *Echinacea angustifolia*,total protein, peroxidase, *Solanum lycopersicum*

INTRODUCTION

Plant protection against all kind of biotic and abiotic stress factors is very important. Because of that specially last decades researchers try to find the best protection method for solving this problem. These methods include cultural measures, physical battle, mechanical war, quarantine measures, biotechnical methods, biological warfare, chemical warfare [1]. The new methodology that is being used in the plant health is using the plant activator is activated by plant health the plant activators that increasing the resistance to diseases and pests occupies an important place in biological control Plant activators do not directly impact on disease factors like classic pesticides, fungicides, insecticides [2-3].The natural defense mechanisms in plants by stimulating plant activators in case of an armed and wait for the plant maintenance and possible pathogen attack provides the act. This mechanism SAR is a defense mechanism [4-5-6-7]. Plant activators has been found that many plants strength enhancing direction so that the resistance applied to the plant enhancer stimulates gene carried use of plant activator has opened the path of a new technology in crop protection [8]. Various enzymes working in defense mechanism in plants are synthesized and protect the plant if necessary. Free electron as a reaction to stress and accordingly a significant increase in free radical levels have occurred. The primary enzyme that is actively included in the functioning of the defense mechanism in all of them is peroxidase. Peroxidase [EC 1.11.1.7] has a role in the plant defense reactions against potential pathogens. Peroxidase is synthesized in chloroplasts in many plants [9]. It provides catalyzing lignin

polymerization for cinnamyl group in the plants and it provides mechanical support to the plant tissues. Also, it plays a significant role in protecting the plants against pathogenic attacks [10].

Asteraceae family have an medicinal and economically importance in the plat kingdom. *E. purpurea, E. angustifolia* ve *E. pallida* species which are belonging to this family have widely using area as immunoregulator, immunostimulator, antimicrobial, antienflamatuar effects. Extracts of *Echinaceapurpurea* are among the most widely used herbal medicines throughout Europe and North America for the prevention or treatment of common cold, coughs, bronchitis and other upper respiratory infections. Popular preparations include expressed juice from the aerial parts of the plant (which contain polysaccharides) and alcoholic tinctures from roots (containing caffeic acid derivatives and alkylamides) [11].

Because of this kind of specifications, in our research the effects of ethanolic and methanolic extracts of *E. angustifolia* (narrow-leaved echinacea) on total protein and peroxidase levels in *Solanum lycopersicum* L. were determined.

MATERIALS AND METHODS

Plant Material

Solanum lycopersicum L. SC-2121 seeds were used as a plant material. The certified pepper seeds have been provided from Ceylan Agricultural Companies. Crude extracts of *E. angustifolia* which is taken from İstanbul Spice Company were used for the application.

Plant Growing

S. lycopersicum seeds were germinated in violes (capacity 28) containing a mixture of 3:1 soil-peat under controlled conditions. Plantlets were grown in growth chamber at 25±2 ˚C under 16/8 h photoperiod with 72 µmol m^{-2} s^{-1} and organized with three replicates, each of which included 168 plantlets. Growing periods of these plantlets were 16 weeks. Plantlets which getting growth to 8-10 leaf stage were harvested for extraction.

Preperation of Echinacea Extracts and Application Solution

Commercial dried echinacea crude extracts (totally 80 g) were divided in two parts which each one is 40 g. After that each part of this crude extract mixed with 200 mL ethanol and 200 mL methanol seperately for solving procedure in shaking incubator in 50^{o}C, 150 rpm for 5 hrs. After solving procedure, ethanolic and methanolic extracts has been put in to evaporator 60^{o}C for 30 minutes. After evaporation process 8 g of echinacea powder were obtained for both ethanolic and methanolic extracts. This powder solved in 40 mL of DMSO for prepearing the stock solution. Application concentrations were diluted with distilled water as 0.01 g/m L and 0.03 g/mL.

Application of Echinacea Extracts to Solanum lycopersicum

Diluted echinacea extracts sprayed on the leaf of sixteen weeks old in vivo growth *S. lycopersicum* plantlets as 0.01 g/mL and 0.03 g/mL concentrations. Control group were sprayed only with distilled water.

Figure 1. Concentrations of e*chinacea*

Stock Solution (0,2g/mL)	Concentration (0,01g/mL)	Concentration (0,03g/mL)
DMSO alone	5mL DMSO + 95mL dw	15mL DMSO + 85mL dw
Methanolic Extract	5mL extract + 95mL dw	15mL extract+ 85mL dw
Ethanolic Ectract	5mL extract + 95mL dw	15mL extract + 85mL dw

Analysis Procedure

Preparation of Leaf Extracts

Leaf samples of *S. lycopersicum* were harvested 24, 48 hours after application. For the preparation of leaf extracts, 0.5 g of leaf was homogenized with 5 mL of cold sodium phosphate buffer (0.05 M, pH 6.5), centrifugated at 13000 rpm for 15 minutes at 4^{0}C. After centrifugation, the supernatants were collected and their protein concentrations were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard [12]. Each experiments were organized with three replicates.

Protein and Enzyme Analyses

Amount of total protein was measured spectrophotometrically at 595_{nm}. Peroxidase (POX) [EC 1.11.1.7] activity in the leaf extracts was assayed spectrophotometrically. 1 ml of assay mixture containing 0.05 M sodium acetate buffer (pH 6.5), 0.2 ml of 0.1 M pyrogallol, 0.1 ml of 90 mM H_2O_2 and an aliquot of the crude leaf extract containing 10-40 µg proteins were mixed together immediately before detecting. The peroxidase enzyme activity was measured at 300 nm according to Kanner and Kinsella [13]. One unit of peroxidase activity is defined as µmol/mgprot/min.

RESULTS AND DISCUSSION

In this research, two different concentrations of ethanolic and methanolic extracts of echinacea were applied to sixteen weeks old *S. lycopersicum* plantlets which grown in vivo conditions. Physiological responses were determined by the meaning of total protein and peroxidase enzyme changing levels.

Protein Results

It has been observed that total protein amounts were decrease in *S. lycopersicum* when compare with control group and 24 hrs. application. protein levels were decreased 48 hrs. after 0.03g/mL echinacea application in DMSO, DMSO+methanol, DMSO+ethanol groups respectively as %43.16, %29.32 ve %27.26. Changing in peroxidase activities were given in figures 2-3.

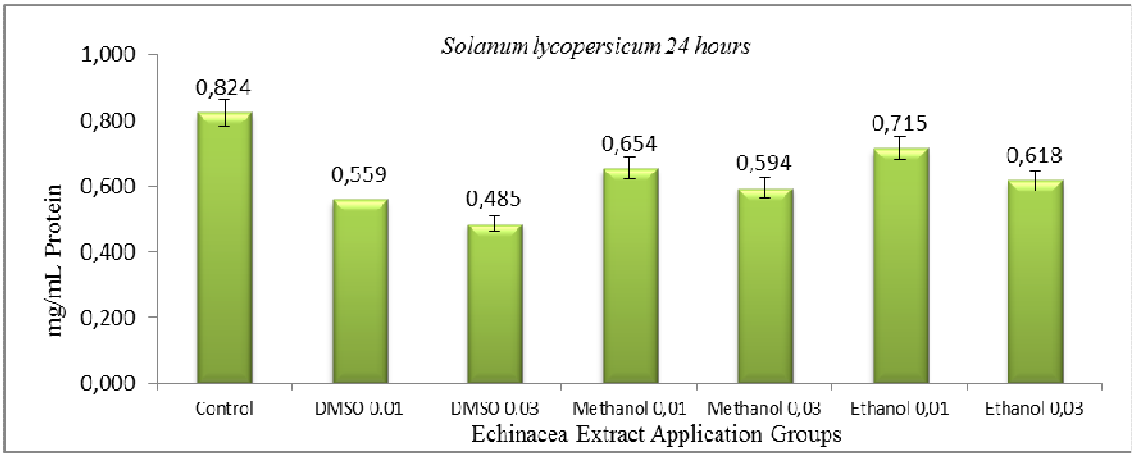

Figure 2- Protein changing in *S. lycopersium* L. 24 hrs. after echinacea application

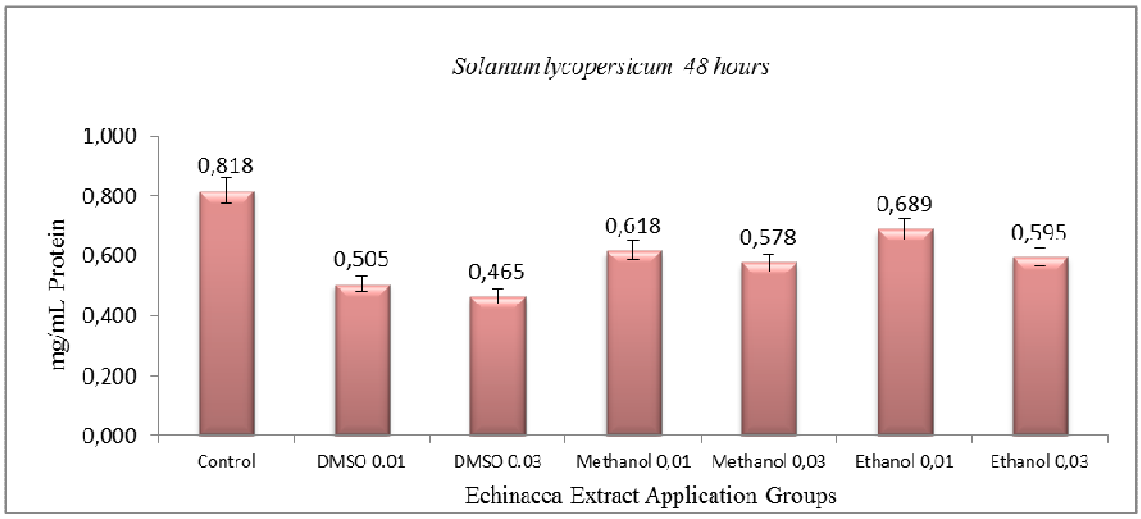

Figure 3- Protein changing in *S. lycopersium* L. 48 hrs. after echinacea application

Peroxidase Results

Peroxidase activity changing in *S. lycopersicum* plantlets when compare with control group and 24 hrs. application, POX acivity were decreased 48 hrs. after 0.03g/mL echinacea application in DMSO, DMSO+methanol, DMSO+ethanol groups respectively as %43.16, %62.60, %38.20, %38.95. Changing in peroxidase activities were given in figures 4-5.

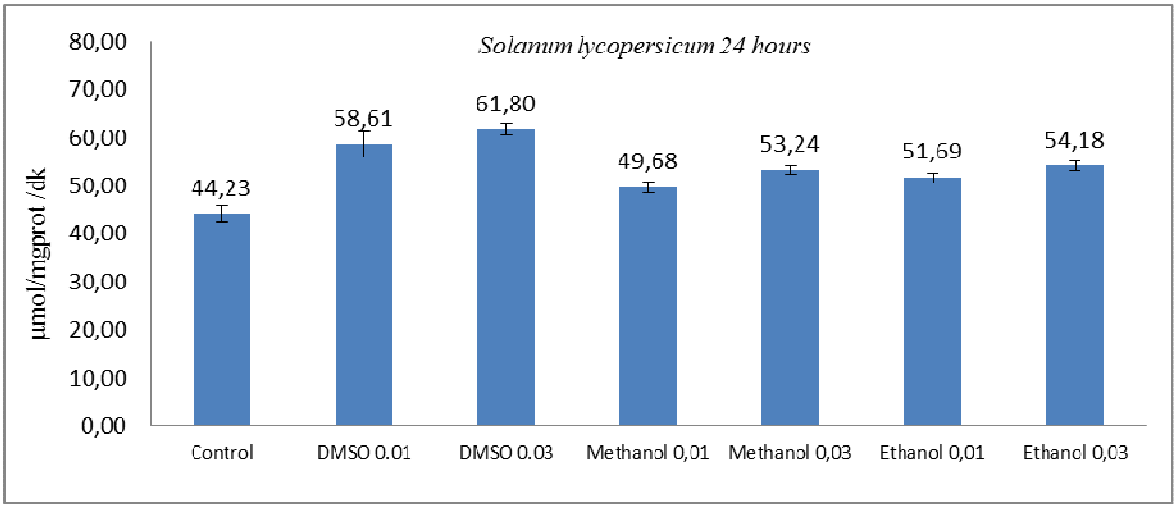

Figure 4- Peroxidase changing in *S. lycopersium* L. 24 hrs. after echinacea application

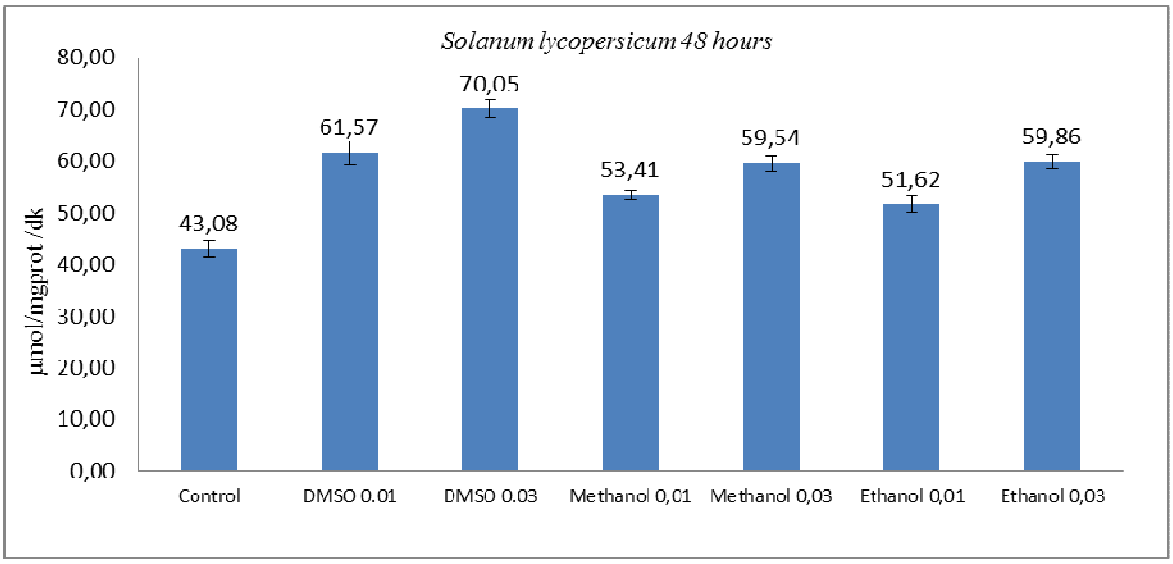

Figure 5- Peroxidase changing in *S. lycopersium* L. 48 hrs. after echinacea application

In our research, it has been determined that ethanolic and methanolic extracts of echinacea causes the degradation of protein and stimulating of peroxidase activity depending on concentration and exposure time. One of the research, researchers have applied the spraying method to the plant leaves to determine metabolic parameters in the plants *Nicotiana* and *Vigna* which have economic significance of the different doses of waste pesticides. In the result of it, it has been observed that the increasing doses of the waste pesticides cause the decrease in the amount of protein and also, the increase in the peroxidase and catalase enzyme activities of both plants [14].

In other research were shown that echinacea extracts were given damage to the different plant's leaf mesophyll cells [15]. Antioxidant activity of *E. purpurea* ve *E. angustifolia* on the oil systems were determined by Hall et al. Researchers were prepared hexan and ethanol extracts of echinacea, and then they applied this solution on corn oil. As a result of this application thet fount that this extract inhibited the corn oil oxidation [16]. As a result of our research, we found possitive stimulating effects of echinacea extracts on *S. lycopersicum* defense system belonging to three factors which including solvent, concentration and exposure time.

CONCLUSION

In conclusion, the application of ethanolic and methanolic extract of echinacea in different concentrations and exposure times to the *S. lycopersicum* plantlets, amount of protein decreased and peroxidase activity increased. Our results are showing that appropriate concentrations of echinacea extracts can be use as a plant activator for stimulate the plant defense system. Instead of synthetically prepared or microbial originated plant activators/biostimulators,

this kind of natural extracts can be use for growth healthy seedlings. As a result of this, people will gain unpolluted ecosystem and agricultural products for future.

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REFERENCES

[1] İ. Öztürk, N. Tosun, *Ege Univ. Faculty of Agriculture Journal* , **2004**, 41 (3),77-87.
[2] G.E. Vallad, R.M. Goodman, *Crop Science*, **2004**, 44, 1920-1934.
[3] A. Dereboylu, PhD Thesis, Ege University (İzmir, Turkey, **2005**).
[4] M. Oostendorp, W. Kunz, B. Dietrich, T. Staub , *European Journal of Plant Pathology*, **2001**,107, 19-28.
[5] R. Bari, J. Jones, *Plant Mol Biol.*, **2009**, 69, 473-478.
[6] J. Shah, *Current Opinion in Plant Biology*, **2009**, 12, 459-464.
[7] T. Gaffney, L. Friedrich, B.Vernooij, D.,Negrotto, G.Nye, S. Uknes, E.Ward, H. Kessmann , J. Ryals, *Science*, **1993**, 261, 754-756.
[8] A. Erkılıç, B. Güven, D.S. Akgül, . *J. Turk. Phytopath.*, **2006**, 34 (1-3), 15-28.
[9] Maleopsa, U. and U. Urbanek..J., *of Phytopathology*, **1994**, 141: 314-322.
[10] Lagrimni, L. M., J. Vaughn, W. A. Erb, and S. A. Miller., *Hort.Sci.*,**1993**, 28: 218-221.
[11] M. Sharma, J. T. Arnason, A. Burt., J. B. Hudson. *Phytotherapy Research*, Vol. 20, issue 2, 147-152, **2006**
[12] M. Bradford, *Analytical Biochemistry*, **1976,** 72, 248-254.
[13] J. Kanner, J. E Kinsella , *J.Agric.Food Chem*., **1983**, 31,370-376.
[14] Gupta, K., Mishra K., Sanwal E., and P.K. Tandon., *International Journal of Environment*, **2015**, 2091-2854
[15] Panwar S.G., Guru S.K., , *J Plant Biochem Biot*., 10. DOI 10.1007/s13562-013-0235-5, **2013**
[16]Hall C., Schwarz J., Shultz K., **2001**, The antioxidant activity of the purple coneflower (Echinacea). Abstracts of the 92nd AOCS Annual Meeting & Expo, May 13–16, Minneapolis,MN.