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The effects of plant activators on total protein and peroxidase levels in *ın vivo* and *ın vitro* growth *Capsicum annuum* L.

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ABSTRACT

In this research, two plant activators were sprayed on ten weeks old plantlets of Capsicum annuum L. varieties grossum and longum which growth in vivo and in vitro controlled conditions . Crop-Set plant activator was sprayed to the leaf in recommended dose, two and four fold doses (0,6-1,2-2,4 ml/L) and Auxigro plant activator was sprayed to the leaf in recommended dose, two and four fold doses (0,3-0,6- 1,2 g/L) with one week interval for two times. Changing of protein and peroxidase [EC 1.11.1.7] levels in both varieties which is grown under in vivo and in vitro conditions were compared after 24-48-72 hours after second application of plant activators. All of the experiments were realized tree times. After plant activators application POX activity changing in grossum seedlings were determined in different levels. After 24 hours 1,2 ml/L dose of Crop-Set application to the in vivo growth seedlings, POX activity increased 319% while after 72 hours 1,2 g/L dose of Auxigro application to the in vitro growth seedlings, POX activity increased as 140%. In vivo growth longum seedlings POX activity increased 48 hours after 2,4ml/L dose of Crop-Set application as 180%. In vitro growth seedlings POX activity increased 72 hours after 1,2g /L dose of Auxigro application as 123%. According to our research results, both of the plant activators significantly increased POX activity in both plant varieties according to the exposure time.

Keywords : Crop-Set, Auxigro, Protein, POX, *Capsicum annuum L.*

INTRODUCTION

People have applied to a variety of plant protection methods in order to cope with plant diseases and insect pests for years. 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]. Most of all natural plants of economic importance with these preparations, various bacteria, nematodes, viruses and fungi on preventing diseases brought occurred resulting product losses are underway not to make a negative impact on human health also increases the desirability[9].

Moreover when considering the harmful effects of the pesticides used, it is an unavoidable fact that many advantages such as minimizing the use of pesticides[10].

C. annuum L. species have been used as research material. This species have economic importance in the world and well-known antioxidant properties [11] This plants also has a high rate regeneration ability , has not huge genome and more easy germination than other plants in vitro conditions [12]. It has advantogeus aspects in vivo and in vitro experiments and it is more attractive for researches.

MATERIALS AND METHODS

Plant Material

In this research, *grossum* and *longum* varieties of *Capsicum annuum* were used as a plant source. The certified pepper seeds and plant activators have been provided from Ova Agricultural Companies.

In Vivo Assay

Seeds were germinated in plastic pots (60x20x15 cm) containing a mixture of 3:1 soil-peat under sterile conditions. Plantlets were grown in growth chambers 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 30 plantlets. Growing periods of these plantlets were 10 weeks. Plantlets which getting growth to 8-10 leaf stage were harvested for extraction.

In Vitro Assay

C. annuum L. seeds were surface sterilized with 70% ethanol for 1 minute and 1% sodium hypochlorite for 20 minutes. After that seeds rinsed with sterile distilled water three times. After surface sterilization, seeds were germinated aseptically on MS medium. MS basal medium supplemented with 3% (w/v) sucrose and 0.8 % (w/v) agar were used for *in vitro* experiments. The pH of medium was adjusted 5.75 before adding agar, then autoclaved at 121˚C for 15 min. All of the cultures were kept in growth chambers at 25±2˚C under 16/8 h photoperiod with 72 µmol m^{-2} s^{-1} During the experiments five seeds were placed each petri dishes. 30 petri dishes were used for each varieties of C. *annuum*. Four weeks old plantlets were transferred to magenta culture dishes under sterile conditions. Plantlets were kept in growth chambers 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 30 plantlets. In vitro plantlets which getting growth to 8-10 leaf stage were harvested for extraction.

Plant Activator Applications

Crop- Set and Auxigro plant activators sprayed on the leaf of ten weeks old in vivo and in vitro growth plantlets. In vitro growth plantlets were acclimatized for one week in growth chamber conditions. Crop-Set application were realized as recommended dose 0.6 ml/L, twice dose 1.2 ml/L and 2.4 ml/L. Auxigro application were realized as recommended dose 0.3 g/L, twice dose 0.6 g/L and 1.2 g/L with one week interval two times. Samplings were made 24, 48 and 72 hours after the second plant activator application. Each experiments were organized with three replicates.

Analysis Procedure

Preparation of Leaf Extracts

Healthy terminal leaves were harvested in eight, ten true leaf stage. 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 4^0C. After centrifugation, the supernatants were collected and their protein concentrations were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard [13].

Protein and Enzyme Analyses

Plant specific proteins were analyzed according to Bradford (1976) with bovine serum albumin (BSA) as a standard [13]. 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 [14]. The kinetic enzyme reaction was allowed to proceed for 3 minutes and peroxidase measurements were taken in every 15 seconds with modified methods of Lurie et al. (1997). One unit of peroxidase activity is defined as mg/mL/min.

RESULTS AND DISCUSSION

In this research, different doses of the two plant activators were applied to ten weeks old plantlets which grown in vivo and in vitro conditions. Physiological responses were determined as total protein and POX enzyme level.

Total Protein Results

In vivo protein changing in Capsicum annuum L. var. grossum

When application groups compared with the control groups, total protein amount were increased. The maximum percentage of total protein exchange was 32% in Crop-Set application of the recommended dose after 24 hours. Auxigro application of the recommended dose after 72 hours total protein increased as 25% when compare with control group.

In vitro protein changing in Capsicum annuum L. var. grossum

When application groups compared with the control groups, total protein amount were increased. The maximum percentage of total protein exchange was 28% in Crop-Set application of the x2 dose after 72 hours. Auxigro application of the recommended dose after 48 hours total protein increased as 18 % when compared with the control group.

In vivo protein changing in Capsicum annuum L. var. longum

When application groups compared with the control groups, total protein amount were increased. The maximum percentage of total protein exchange was 58% in Crop-Set application of the x2 dose after 72 hours. Auxigro application of the recommended dose after 24 hours total protein increased as 49 % when compared with the control group.

In vitro protein changing in Capsicum annuum L. var. longum

When application groups compared with the control groups, total protein amount were increased. The maximum percentage of total protein exchange was 19% in Crop-Set application of the 42 dose after 72 hours. Auxigro application of the x4 dose after 48hours total protein increased as 21 % when compared with the control group.

Peroxidase Results

Peroxidase enzyme activities were increased in two varieties of *C.annuum* after plant activator applications when compared with control plants. 24 hours after 1,2 ml/L dose of Crop-Set application to the *in vivo* growth seedlings, POX activity increased 319% while 72 hours after 1,2 g/L dose of Auxigro application to the *in vitro* growth seedlings, POX activity increased 140%. In *vivo* growth *Capsicum annuum L. var longum* seedlings POX activity increased 180%, 48 hours after 2,4ml/L dose of Crop-Set application. *In vitro* growth seedlings POX activity increased 123%, 72 hours after 1,2g /L dose of Auxigro application. Changes in pox activities were given in figures 1-4.

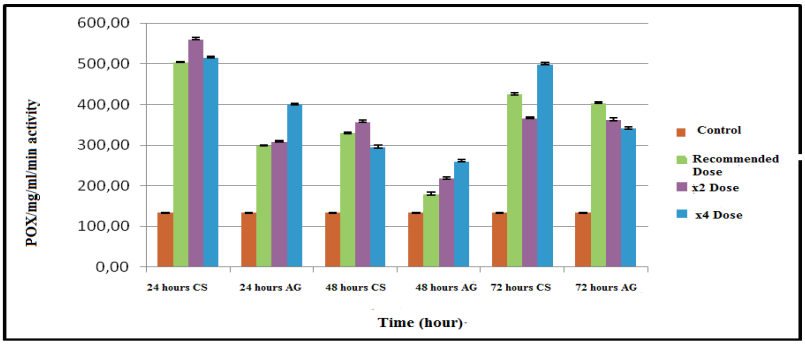

Figure 1- In vivo POX changing in *C. annuum* L. var. *grossum*.

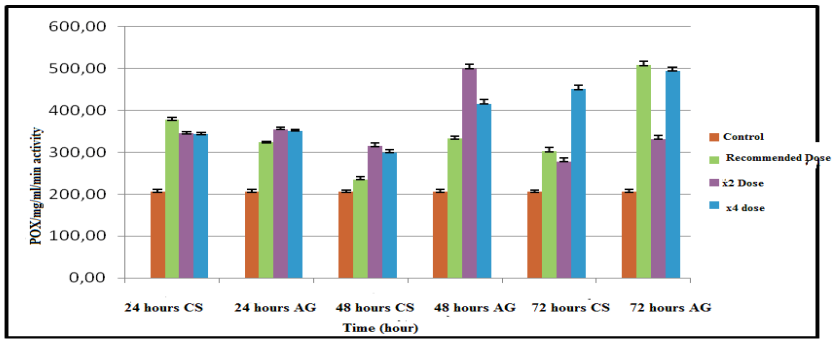

Figure 2- In vitro POX changing in *C. annuum* L. var. *grossum*.

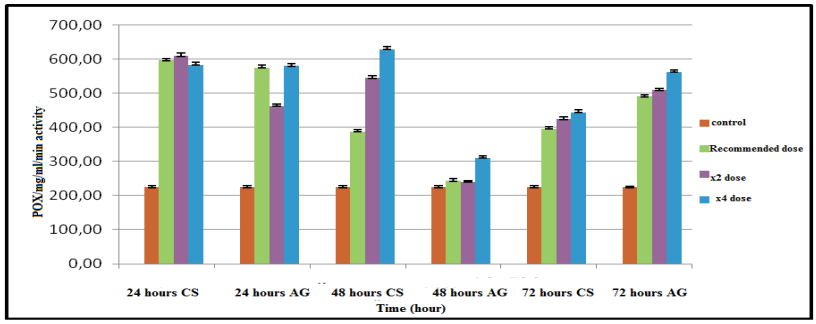

Figure 3- In vivo POX changing in *Capsicum annuum* L. var. *longum*

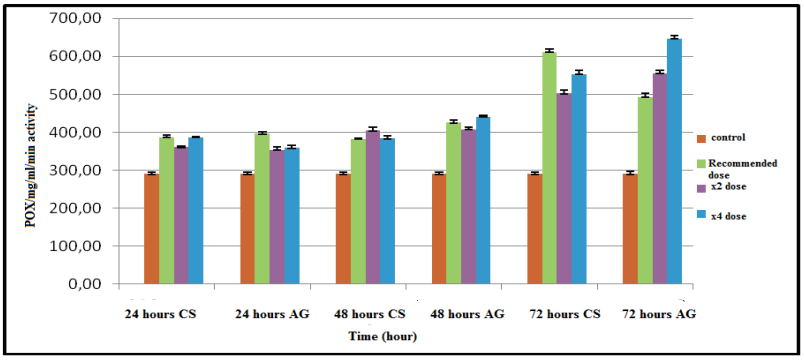

Figure 4- In vitro POX changing in *Capsicum annuum* L. var. *longum*

Considering the scientific research carried out to date, there are many research about the use of plant activators in the fight against pests. Aminuzzaman Bremen and Hossain (2007) Bio their study 50 WG trade is plant activator , Tilt 250 EC and Amistar alone and the trade mark fungicides have implemented against leaf blight disease of wheat in a mixture of one another , statistically have achieved the highest efficiency Bio - Amistar mix application. Bion, productivity has increased by 53.33 % [15].

In Pavlista (2011) , in his study that was introduced recently , our in which the plant activator produced by GABA technology we use in our research Auxigro of 140-700g / ha applied practice groups to create potato tubers, leaves , 420g / HA treatment groups was seen that the yield increases . It reported an increase of 27% compared to control yield . POX plant with an increase in the application of our research results have been observed in Auxigro [16].

When recently in different plant groups in our research evaluated the results obtained from scientific research on plant activators that we use , they warned in a good way the defense system of the actuator and consequently the diseases they reduce the risk of transmission plant of factors , the increase in productivity in the economic product is found to bring about . Research has also grown in different media *Capsicum annuum* variety of different durations and activator defense systems at different levels at doses it has been found to stimulate positive.

CONCLUSION

In conclusion, the application of both plant activators in different doses and exposure times to the *C. annum* L. varieties which *in vitro* cultivated, POX enzyme activity was found to be effective then *in vivo* cultivated plants. In addition, this method due to the disease , instead of using imported seedling resistance genes are harmful natural genomes preserved plant activator application carried by healthy seedlings, before and after in vitro in the ecosystem cultivated under field conditions and the way to grow products they can consume with consumers peace of mind will be opened .

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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] O. Aşçıoğul N. Tosun, *Ege Üniv. Ziraat Fak. Derg.*,**2010,** 47 (3), 231-240.
[10] C. A. Damalas, I.G. Eleftherohorinos, *Int. J. Environ. Res. Public Health*, **2011**, *8*(5), 1402-1419.
[11] D. H. Hernandez, S.G. Sayago, I.goni, *J. Agric. Food Chem.*, **2010**, *58* (6), 3399–3406.
[12] C. Akı, *Journal of Biological Science*, **2005,** 5 (3):,257-259.
[13] M. Bradford, *Analytical Biochemistry*, **1976,** 72, 248-254.
[14] J. Kanner, J. E Kinsella , *J.Agric.Food Chem*., **1983**, 31,370-376.
[15] F.M. Aminuzzaman I. Hossain, *Pakistan Journal of Biological Sciences*,**2007,** 10 (11), 1797-1803.
[16] D.A.Pavlista, *Am. J. Pot Res.*,**2011,** 88, 479-484.