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Annals of Biological Research, 2016, 7 (2):13-18
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

The effects of pesticides on total protein amount and peroxidase activity in *Capsicum annuum* L. varieties

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ABSTRACT

In this research, two pesticides were sprayed on twelve weeks old plantlets of Capsicum annuum L. varieties grossum and longum which growth in vivo conditions. Insecticide was sprayed to the leaf in recommended dose, two and four fold doses (0,2-0,4-0,8 ml/L) and fungicide was sprayed to the leaf in recommended dose, two and four fold doses (2-4- 8 ml/L) with twelve 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 conditions were compared after 48-96 hours after second application of pesticides. All of the experiments were realized tree times.Changing of peroxidase activity levels in Capsicum annuum L. varities in seedlings after pesticides application was compared with control groups. . Capsicum annuum L. var grossum seedlings POX activity increased 170,2%, 48 hours after 0,4ml/L dose of insecticide application. POX activity increased 161,6%, 48 hours after 4m/L dose of fungicide application. Capsicum annuum L. var longum seedlings POX activity increased 96,6%, 48 hours after 0,4ml/L dose of insecticide application. POX activity increased 89,2%, 48 hours after 8m/L dose of fungicide application. According to our research results, both of the plant activators significantly increased POX activity in both plant varieties according to the exposure time.

Keywords : Insecticide, Fungicide , Protein, POX, *Capsicum annuum L.*

INTRODUCTION

It increases the potential danger in the environmental pollution that the human being constantly creates waste products and rapid industrial development especially in recent years. Biodiversity and human health are under serious threat from pesticides [1]. However, the chemical warfare where the pesticides are used is the most used method in agricultural struggle [2]. The pesticides can have been turned into the mutagenic and carcinogenic agents that show effects as toxic agent vectors on people by vegetation [3]. Many researchers have informed that the pesticides have the mutagenic and carcinogenic effects [4-5].

This threat is more on the vegetation; because it -as primary producers in and ecosystem- takes place in the bottom rungs of the food chain, can also affect other creatures and has economic importance. The plant's transpiration, photosynthesis, enzyme action, nucleic acid construct, chlorophyll biosynthesis have affected many physiological events such as membrane damage and the disruption of hormonal balance [6].

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 [7]. 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 [8].

This study has used as a research material that is a type of *Capsicum annuum L.* This type has economic significance in world and is one of the types where product losses are much because of the pathogens. Therefore, in our research the changes that the overdoses in addition to the time cause on the basis of plant physiology have been examined within the scope total protein and POX enzyme changes.

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 pesticides have been provided from Ceylan Agricultural Companies.

In Vivo Assay
Seeds were germinated in plastic viols 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 organized with three replicates, each of which included 122 plantlets. Growing periods of these plantlets were 12 weeks. Plantlets which getting growth to 8-10 leaf stage were harvested for extraction.

Application of Pesticides
Insecticide and fungicide pesticides sprayed on the leaf of ten weeks old in vivo growth plantlets. Insecticide application were realized as recommended dose 0.2ml/L, twice dose 0.4 ml/L and 0.8 ml/L. Fungicide application were realized as recommended dose 2 m/L, twice dose 4 m/L and 8 m/L with one week interval two times. Samplings were made 48 and 96 hours after the second pesticide 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 [9].

Protein and Enzyme Analyses
Plant specific proteins were analyzed according to Bradford (1976) with bovine serum albumin (BSA) as a standard [9]. 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 [10]. 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 µmol/mgprot/min.

RESULTS AND DISCUSSION

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

Total Protein Results
It has been observed that total protein results decrease in two varieties of *Capsicum annuum L.* after the pesticide application when are compared with the control group.

It has been detected that a decrease- respectively 28,2% and 33,3%- occurs in the plants that are reaped after 48 hours from the application of the concentration of insecticides (0,8 ml/L) and the concentration of fungicide (8 ml/L) in *Capsicum annuum L.var grossum* seeds.

It has been determined a decrease- respectively 31% and 24,3%- occurs in the plants that are reaped after 48 hours from the application of the concentration of insecticides (0,8 ml/L) and the concentration of fungicide (8 ml/L) in *Capsicum annuum L.var longum* seeds.

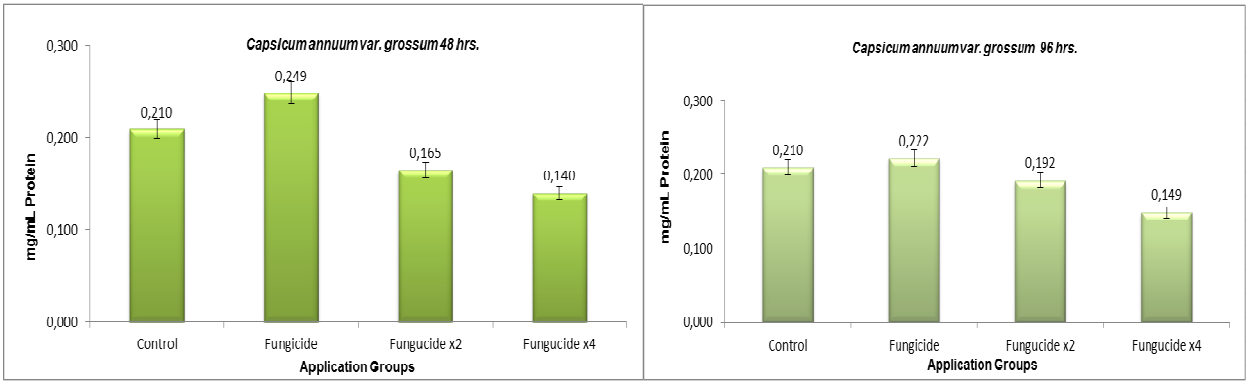

Figure 1 - The effects of fungicide application on total protein amount after 48 and 96 hours

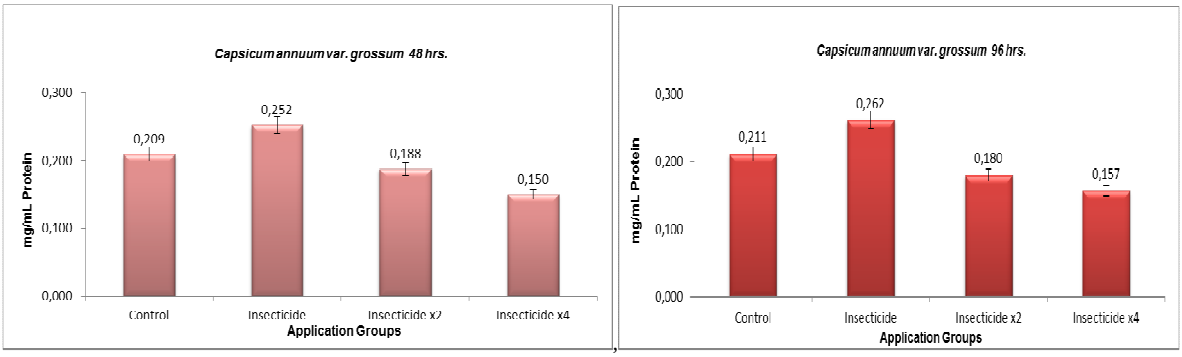

Figure 2 - The effects of incecticide application on total protein amount after 48 and 96 hours

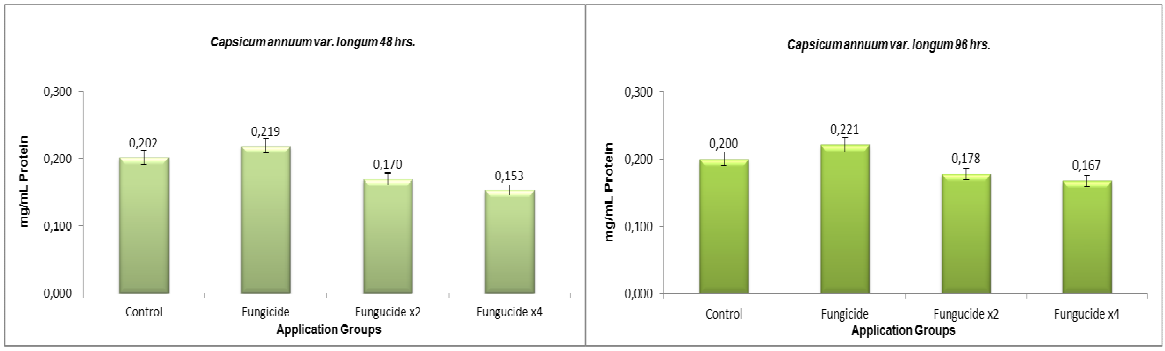

Figure 3 - The effects of fungicide application on total protein amount after 48 and 96 hours

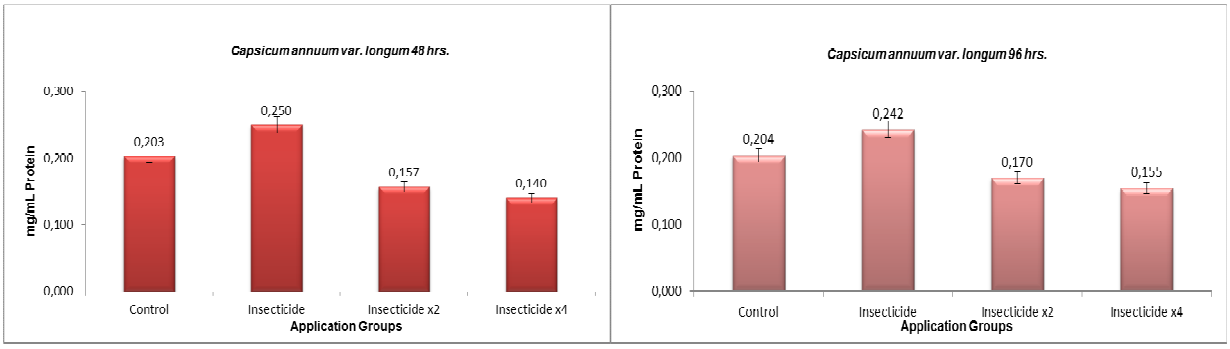

Figure 4 - The effects of insecticide application on total protein amount after 48 and 96 hours

Changing in total protein amount after fungicide and insecticide applications were given in figures 1-2 for *Capsicum annuum L. var grossum* and figures 3-4 for *Capsicum annuum L. var longum*.

Peroxidase Results

Peroxidase enzyme activities were increased in two varieties of *C. annuum* after pesticide applications when compared with control plants.

Peroxidase activity increased 48 hours after 0,4ml/L insecticide application and 4 ml/L fungicide application in var. *grossum* seedlings as 170.2% and 161,6% respectively.

Peroxidase activity increased 48 hours after 0,4ml/L dose of insecticide application and 8ml/L fungicide application in var. *longum* seedlings as 96,6% and 89,2% respectively.

Changing in peroxidase activities after fungicide and insecticide applications were given in figures 5-6 for *Capsicum annuum L. var grossum* and figures 7-8 for *Capsicum annuum L. var longum*.

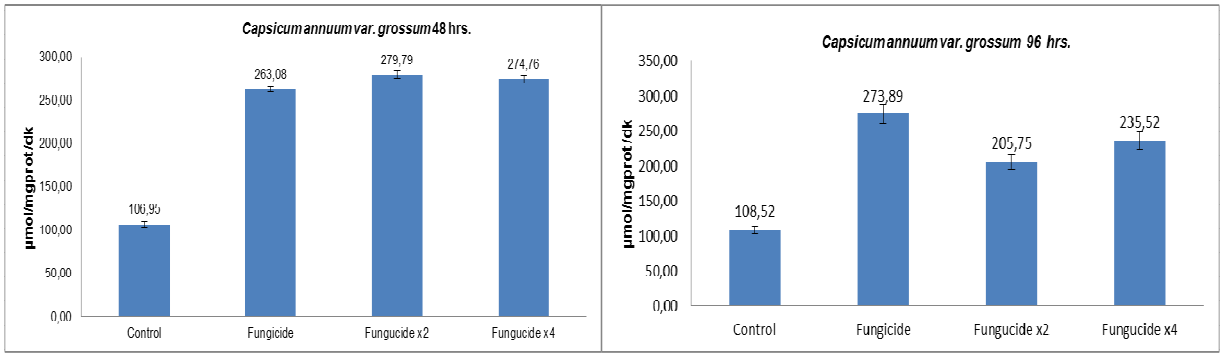

Figure 5 - The effects of fungicide application on peroxidase activity after 48 and 96 hours

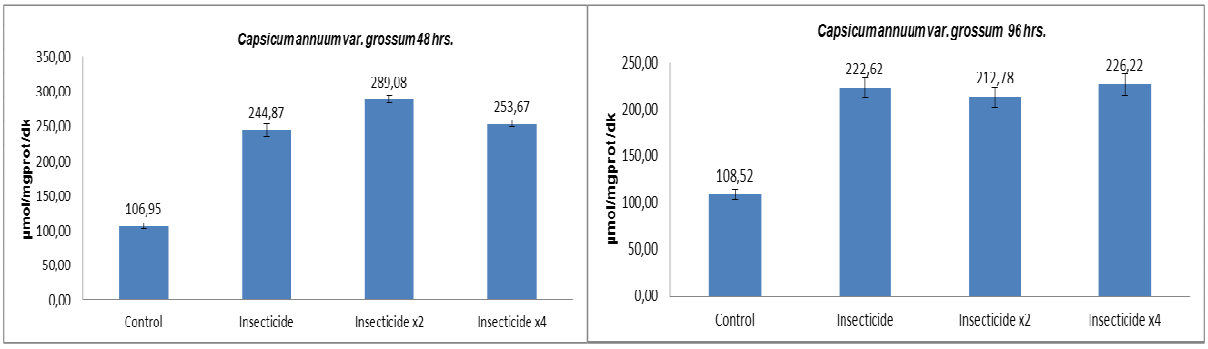

Figure 6 - The effects of insecticide application on peroxidase activity after 48 and 96 hours

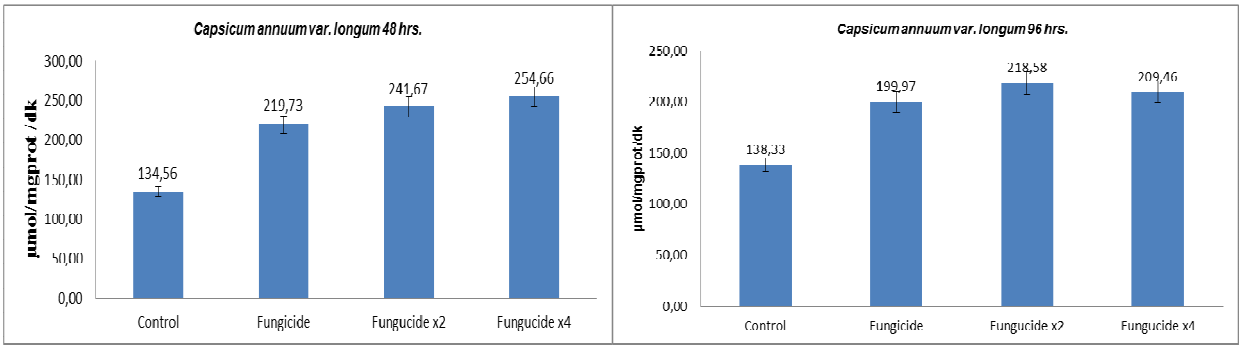

Figure 7 - The effects of fungicide application on peroxidase activity after 48 and 96 hours

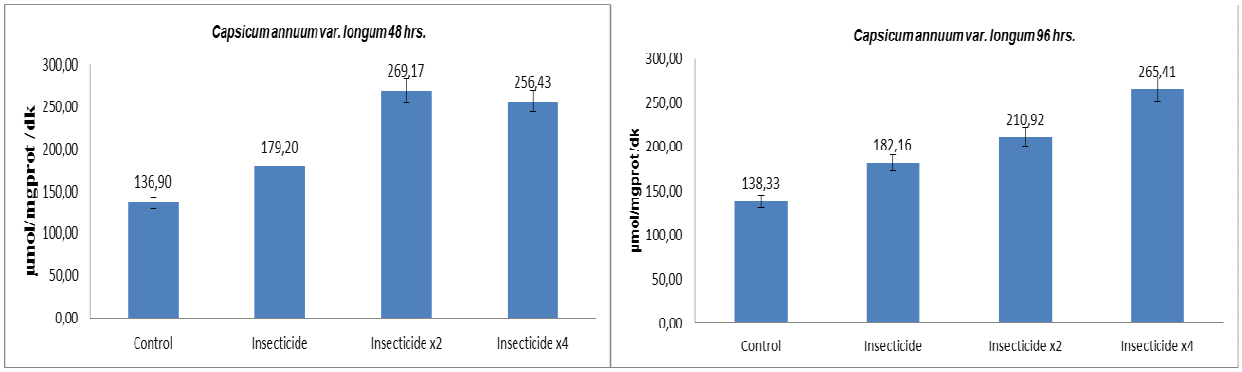

Figure 8 - The effects of insecticide application on peroxidase activity after 48and 96 hours

There are many researches about the changes that the use of pesticide in the struggle against pests causes on the plant physiology when the scientific researches until today have been considered.

Kaya et al. (2015) have detected the physiological changes by applying the different concentrations of the insecticides called as abamectin, thiamethoxam, pyriproxyfen and acetamiprid in *Solanum tuberosum*. They have observed that the catalase, peroxidase and superoxide dismutase amount of enzyme depending on the increasing concentrations in all of insecticides as a result of his research [11].

Gupta et al. (2015), in their research, 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 enzyme activities of both plants (peroxidase and catalase)[12].

In addition, it has been observed that peroxidase, polyphenol oxidase and total phenol concentration have increased as a result of the fungicide application in the research done to induce the resistance in wheat to rust infection[13].

When compared with the results of the researches, it has been determined that it causes the degradation in total protein and increases in defense enzymes when the pesticides that are used in our research are excessively used in not-recommended way.

CONCLUSION

In the result of the research it has been determined that both pesticides cause the increases in different levels on POX enzyme activity in two peepr varieties. It has been detected that insecticide application is more effective than fungicide application in the two varieties. With this study it has been demonstrated the effects of the unconsciously used of these kinds of agrochemicals on ecosystem and plant physiology.

Acknowledgements
This research is a part of Miss. Bahar GÖK's TUBİTAK Project which is supported with the frame of Undergraduate Students Grant in Biological Science. This project has been completed succesfully under the supervisory of Prof. Dr. Cüneyt AKI.

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