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Research Article

EFFECTS OF GINGER EXTRACTS ON TOTAL PROTEIN AMOUNT AND PEROXIDASE ACTIVITY IN SOLANUM LYCOPERSICUM L.

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ABSTRACT

Zingiber officinale Roscoe, belonging to the Zingiberaceae family, is a medicinal plant that also spreads in our country. In this research, extracts of *Z. officinale* rhizomes were prepared with different solvents (ethanol, methanol, distilled water, DMSO). *Zingiber* rhizome extracts were sprayed on the leaves of rio grande and marmande varieties of 10-12 weeks old *Solanum lycopersicum* plantlets. Total protein amount and peroxidase activity were examined by spectrophotometrically 24 and 48 hours after the application. According to the results, it was determined that the maximum increase in the total protein amount compared with the control group was 31.31% and 25.53% in marmande and rio grande varieties in ginger rhizome extract prepared with methanol, respectively. The increase in peroxidase activity was determined as 144.14% and 124.85% in marmande and rio grande varieties in ginger rhizome extract prepared with ethanol, respectively. As a result of our research, it was determined that rhizome extracts of *Z. officinale* can be used as natural plant activator.

Key words: *Zingiber officinale*, *Solanum lycopersicum*, total protein, peroxidase

1. INTRODUCTION

Humans have used the protective and therapeutic properties of plants extensively for a long time (Dündar, 2001). After the 1900s, people discovered the side effects of synthetic drugs and the harm caused by synthetic substances in food and beverages to human health with the awareness, the demand for natural products has increased (Acibuca and Budak, 2018).

As in the whole world, the plants in the natural flora in our country are among the people for therapeutic purposes, food, tea, spice, dye, insecticide, treatment of animal diseases, resin, use of gum, volatile oils, long use in soft drinks and cosmetic industry has been a part of our traditional cultural wealth that has been going on for years (Faydaoğlu and Sürücüoğlu, 2011).

Ginger is a species belonging to the Zingiberaceae family. This family of up to 24 genera and around 300 species. The genus Zingiber has also about 20 species. Ginger plant perennial has tuber or rhizome roots. Plant up to 60-90 cm high, single with dark green leaves forms an annual stalk. There are two rows of 8-12 leaves on the stem. The leaves are long sheathed, alternate, lanceolate, strip-lanceolate, pointed, flat and stemless sheathed, 10-21 cm long, 2-2.5 cm wide. The flowers are small and pale yellow (Felter and Loyd, 2002).

The homeland of the tomato plant is Peru, one of the countries of South America. In the 19th century, tomato plant arrived to Turkey after via Syria. (Kaya et al., 2018). Tomatoes are a rich source of minerals, vitamins, organic acids, essential amino acids and nutrients (Kabelka et al., 2004). It is the richest and most important source of lycopene with antioxidant effect. Lycopene does not act as a vitamin, it is a precursor when taken into the body. However, its antioxidant property is very valuable. It has been scientifically recorded that high lycopene value in the blood prevents prostate cancer (Gann et al., 1999). As it can be consumed fresh in our country as in the whole world; It is also widely consumed as tomato paste, sauce and ketchup (Sönmez and Ellialtıoğlu, 2014).

Plants are exposed to negative effects of pathogenic organisms such as viruses, bacteria, protozoa, nematodes, fungi and various environmental conditions. They have developed many defense mechanisms to protect themselves from pathogen attacks. During this defense, while using chemical compounds such as pathogenic hydrolytic enzyme, toxin, plants have a large number of defense substances, including physical barriers, defense peptides, antioxidants, secondary metabolites and antimicrobial proteins. Antioxidants are involved in preventing cellular damage. Compounds in this defense minimize the damage caused by pathogens (Koç and Üstün, 2008).

Jana et al. (1999) observed that ginger had an anti-inflammatory effect compared to control as a result of their study with *Zingiber officinale*, *Vitex negundo* and *Tinospora cordifolia* to treat inflammation in the feet of albino rats.

Liu and colleagues, who developed new strategies to improve the response to chemotherapy in recurrent endometrial cancer, showed that terpenes found in ginger extract obtained by steam distillation have the potential to inhibit proliferation of endometrial cancer cells (Liu et al., 2012).

Raaof et al. (2013) evaluated the activity of the three plants (*Zingiber officinale*, *Thymus vulgaris* and *Acacia arabica*) as the coagulating agent using the removal of the crude alkaloids and three fragmented concentrations of each plant extract, and observed them in laboratory mice.

Türküsay and Tosun (2005) used copper hydroxide 361.1 g/L (champ formula) and hydrogen peroxide 580 g/L (HuwaSan TR50) against *Clavibacter michiganensis* as a plant activator. In their study with HuwaSan TR50+Champ Formula, they determined that the effect

obtained in co-administration was higher than the use of activator alone, and that the total protein and peroxidase enzyme activities were found at the highest level in the plants where this application was performed.

As a result of the literature review, there are no studies have been found on how the ginger rhizome extracts which we used in our research affected on the total protein amount and peroxidase activity on another plant. In our research, it has been demonstrated that the rhizome extracts of ginger obtained by using different solvents have different stimulating effects on the defense system in marmande and rio grande varieties of *S. lycopersicum*, depending on the variety, type of extract, exposure time. Effects of ginger rhizome extracts have been calculated by total protein amount and peroxidase activity changing.

2. MATERIAL AND METHODS

2.1. Plant Growth

Marmande and Rio Grande varieties of *S. lycopersicum* used in the research have been regularly watered with distilled water in pots with 1:3 perlite:soil at the temperature of $24\pm 2^{\circ}\text{C}$, 28.000 lux light, under the conditions of 16 hours light and 8 hours dark. Plantlets were grown in controlled plant chamber.

2.2. Preparing of Ginger Rhizome Extracts

Rhizomes of *Z. officinale*, originating in China, were obtained from a local market. After the rhizomes are dried in the shade at room temperature, they are powdered with the help of a warden blender. Powdered rhizome extracts were prepared with water, ethanol, methanol, and DMSO solvents. Extraction was done by taking the prepared solutions into the flasks and stirring at 110 rpm for 24 hours at 50°C in the shaker. The solvents were then kept in a water bath for 24 hours at 55°C for evaporation. The powder from the extracts removed from the water bath was weighed on a scale. Stock solution was prepared with 100 mL of DMSO by taking 10 g of the ginger rhizome powder. Two different concentrations were prepared by diluting with distilled water from the stock solution as 0.01 mg/mL and 0.02 mg/mL.

2.3. Application of Ginger Rhizome Extracts to The Plantlets

The prepared ginger rhizome extracts were applied to the leaves of 12 weeks old *S. lycopersicum* seedlings (Marmande and Rio Grande varieties) in equal amounts with the help of a pulverizator. Healthy and young leaves of the seedlings were harvested 24 and 48 hours after application. From each application groups 0.5 g of the fresh leaf have been weighted.

2.4. Leaf Homogenization

These leaves were crushed together with 5mL cold 0.05M sodium acetate buffer (pH 6.5) in cooled porcelain mortar for one minute. The homogenates were transferred to the 3 eppendorfs after being filtered from the filter paper. Then these homogenants were centrifuged 13000 rpm at $+4^{\circ}\text{C}$ for 15 minutes. After the centrifugation, the supernatant were used for determination of total protein amount and peroxidase (POX) activity spectrophotometric analysis. All trials were carried out in triplicate.

2.5. Determination of Total Protein Amount

For the protein amount measuring, homogenates were transferred to the glass test tubes as 100 μL from the eppendorfs and 5 mL of Protein Reagent Blue G-250 was added to each test tube. The total protein amount in the homogenates was determined by according to Bradford (1976)'s method using bovine serum albumin (BSA) as a standard. All trials were carried out in triplicate.

2.6. Determination of Peroxidase (POX) Activity

POX activity changes were performed by spectrophotometric analysis according to Kanner and Kinsella (1983). During the determination of the POX kinetic reaction, the spectrophotometer measured at 300nm for 2 minutes. The biggest differences between the absorbance values taken in every 10 seconds for 2 minutes periods are determined for each group. These determined differences have been converted to mg protein level and given as mg/ml/min POX enzyme activity. All POX activity measurements were performed in three replicates.

3. RESULTS AND DISCUSSION

3.1. Total Protein Results

The effects of *Z. officinale* rhizome extracts on the total protein amounts in marmande and rio grande varieties were given in Figure 1 and Figure 2.

Fig 1. Effects of *Z. officinale* extracts on Total Protein Amount in *S. lycopersicum* var. Marmande *Blue: 24 hours, *Red: 48 hours

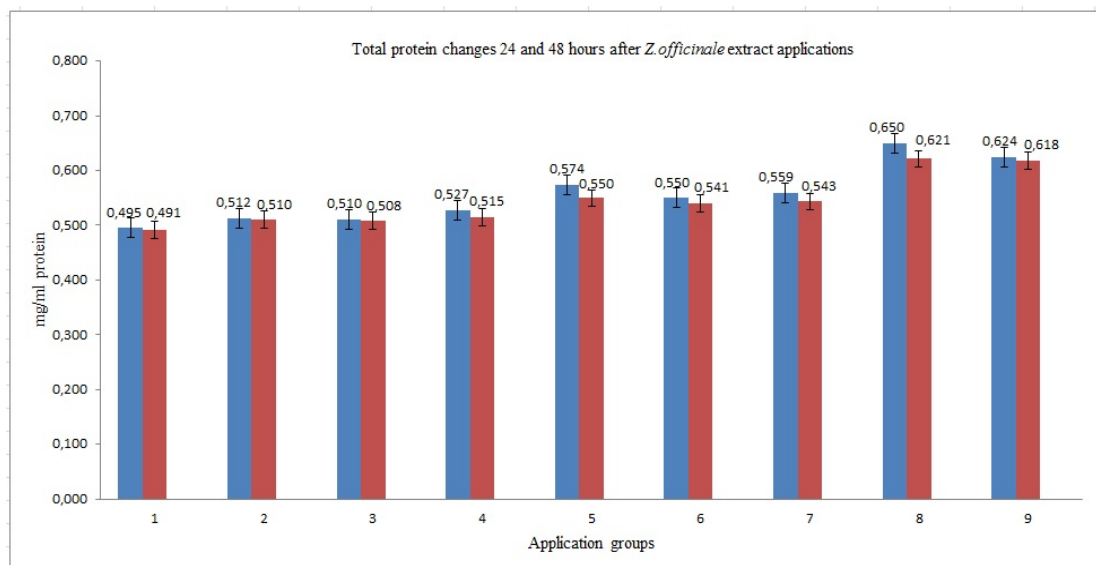
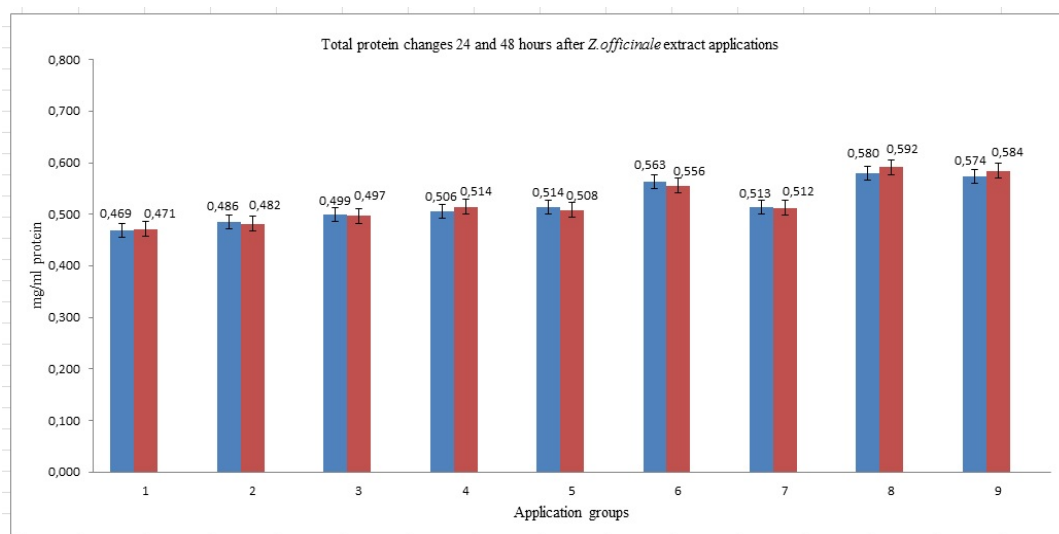


Fig 2. Effects of *Z. officinale* extracts on Total Protein Amount in *S. lycopersicum* var. Rio Grande *Blue: 24 hours, *Red: 48 hours



When compared with the control group, the highest increase in the amount of protein 24 and 48 hours after application have been measured as 31.31% and 25.53% respectively in the 8th group (methanol extracts of rhizome) in the marmande and rio grande varieties of *S. lycopersicum*.

When compared with the control group, it was determined that all ginger extracts which prepared with different solvents such as distilled water, DMSO, ethanol, methanol caused an increasing in the total protein amounts.

3.2. Peroxidase Results

The effects of *Z. officinale* rhizome extracts on the peroxidase activity in marmande and rio grande varieties were given in Figure 3 and Figure 4.

Fig 3. Effects of *Z. officinale* extracts on POX activities in *S. lycopersicum* var. Marmande
*Red: 24 hours, Green: 48 hours

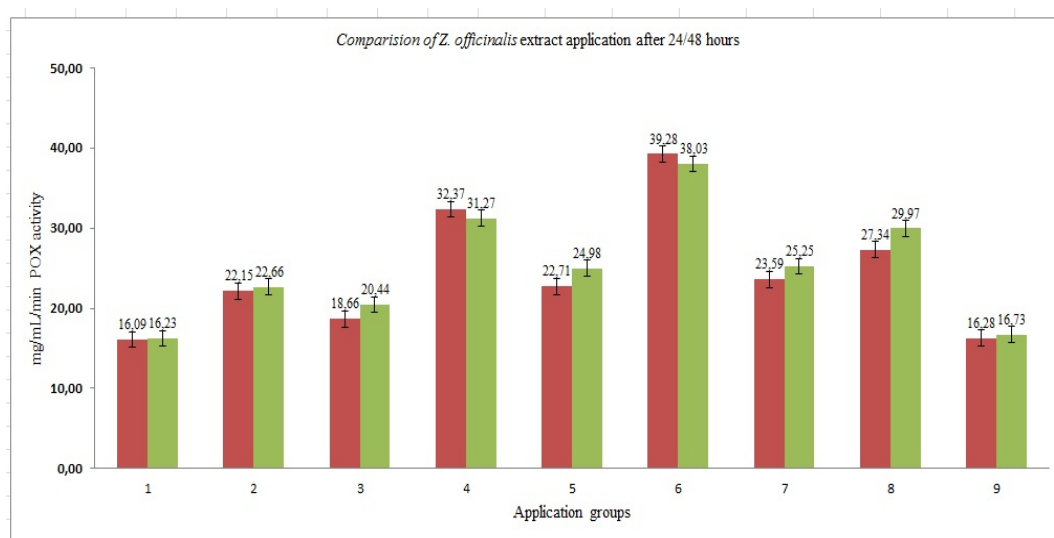
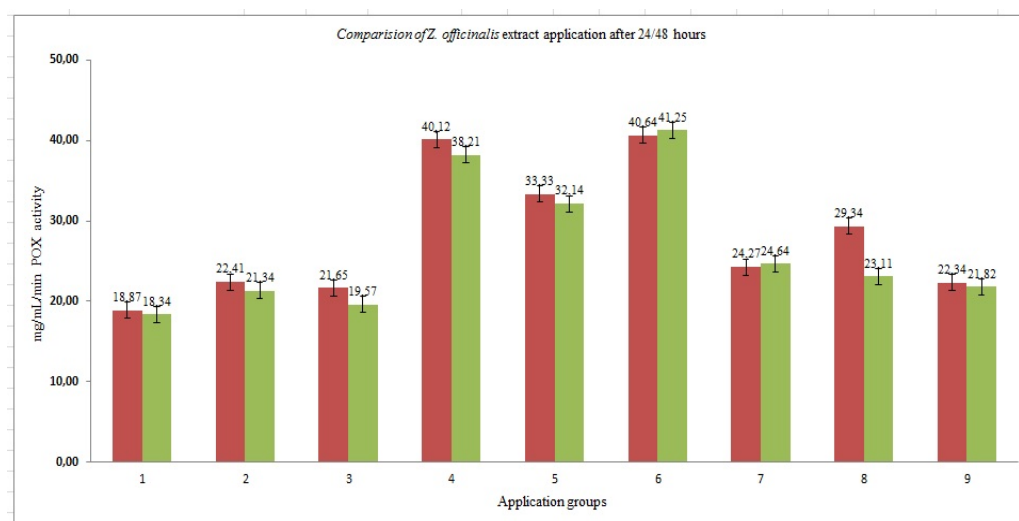


Fig 4. Effects of *Z. officinale* extracts on POX activities in *S. lycopersicum* var. Rio Grande
*Red: 24 hours, Green: 48 hours



After the application of *Z. officinale* rhizome extracts, peroxidase activity increased in all application groups of marmande and rio grande varieties when compared with control group. The highest increase in the peroxidase activity 24 and 48 hours after application have been measured as 144,14% and 124,85% respectively in the 6th group (ethanol extract) of marmande and rio grande varieties of *S. lycopersicum*.

3.3. Discussion

As a result of our research, it was observed that ginger rhizome extracts which prepared using different solvents, affected plant defense system in different degrees both tomato varieties.

In the research of Yıldız and Akı (2019), it was shown that *Prunus spinosa* and *Rubus sanctus* fruit extracts have been stimulated the plant defense system in grossum and conoides varieties of pepper at different levels within the scope of total protein amount and peroxidase activity according to the control group.

There are another research that was determined the effect of *P. spinosa* and *R. sanctus* leaf extracts on the total protein amount and POX activity in pepper varieties (Yıldız and Akı, 2018).

In another research on *S. lycopersicum*, changing in both protein and peroxidase levels were occurred after *Echinacea angustifolia* extract applications according to concentrations and exposure time. Amount of total protein changing after Echinacea application in total protein levels were decreased 48 hours after 0.03g/mL Echinacea extract applications in DMSO, DMSO+methanol, DMSO+ethanol groups respectively as 43.16%, 29.32% and 27.26%. Peroxidase activity were increased 48 hours after 0.03g/mL Echinacea applications in DMSO, DMSO+methanol, DMSO+ethanol groups respectively as 43.16%, 62.60%, 38.20%, 38.95%.

As a result of this research, echinacea extracts which is prepared with two different concentrations and exposure times were stimulate the plant defense system as a plant activator as well (Dinç and Akı, 2015).

All of this researches are showing that some other researchers are trying to find natural plant activators. Our research results also parallel with other research results.

In our research, it has been demonstrated that extracts prepared from the rhizomes of *Z. officinale* plant using distilled water, DMSO, ethanol and methanol solvents can be used as a natural plant activator in certain proportions.

4. CONCLUSION

In conclusion, the application of ethanolic and methanolic extract of ginger rhizome in different concentrations and exposure times to the *S. lycopersicum* plantlets have been stimulated the tomato defence system. Our research results are showing that appropriate concentrations of ginger rhizome extracts can be use as a natural 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.

NOTES

The codes of the application groups prepared from the 100 mg/mL stock solution of ginger rhizomes used in the research are as follows;

- (1) Control Only distilled water application
- (2) 0.01 mg/mL Only DMSO application
- (3) 0.02 mg/mL Only DMSO application
- (4) 0.01 mg/mL Ginger rhizome prepared with distilled water
- (5) 0.02 mg/mL Ginger rhizome prepared with distilled water
- (6) 0.01 mg/mL Ginger rhizome prepared with ethanol
- (7) 0.02 mg/mL Ginger rhizome prepared with ethanol
- (8) 0.01 mg/mL Ginger rhizome prepared with methanol
- (9) 0.02 mg/mL Ginger rhizome prepared with methanol

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