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Evaluation of the Effects of Some *Raphanus sativus* Tuber Extracts on Certain Antioxidant Enzyme Activity and Lipid Peroxidation Level Against Gray Mold (*Botrytis Cinerea*) in *Vicia Faba Leaves*

Nergis KAYA*[1], Tayfun KAYA[1], Soner YİĞİT[1]

Abstract

The antioxidant defense system of the medicinal plant *Raphanus sativus* L. tuber root in terms of certain antioxidant enzymes (ascorbat peroxidase, glutathion reductase, catalase, superoxide dismutase) and lipid peroxidation against gray mold disease caused by *Botrytis cinerea* fungus on *Vicia faba* L. (bean) leaves was analyzed spectrophotometrically. *V. faba* plants were grown from seed under controlled conditions. A spore suspension of 10^5 spores/ml of *B. cinerea* was prepared. The leaves of *V. faba* were treated with 1-) only distilled water, 2-)only DMSO, 3-) only *B. cinerea* (positive control), 4-) only extract applications (distilled water, ethanol and methanol extract),5-) *B. cinerea* treatment after extract applications (extract:fungus). The leaves of *V. faba* were harvested 24 and 48 hours after the applications. Analyzes of the supernatants obtained from the extracts were performed spectrophotometrically. It has been revealed that the activity of all investigated enzymes generally increased in the extract:fungus application groups compared to the fungus application group. It was determined that the application of fungus alone caused an increase in the activity of enzymes compared to application groups other than the extract:fungus application groups. It was observed that there was an increase in lipid peroxidation (MDA) only in the fungus application group compared to all extract:fungus application groups except 10mg/ml distilled water extract:fungus treatment. As a result of the research, it was determined that the extract:fungus applications generally increased the antioxidant enzyme activity levels and decreased the MDA content when compared to the negative control and positive control groups. In extract:fungus applications, enzyme activities were obtained from 10mg/ml distilled water extract:fungus, 10mg/ml EtOH extract:fungus, 10mg/ml MeOH extract:fungus applications. From this point of view, it can be stated that *R. sativus* distilled water, ethanol and methanol extracts generally give an increased antioxidant defense response in *V. faba* leaves compared to *B. cinerea* inoculation alone.

Keywords: Vicia faba L., *raphanus sativus* L., *botrytis cinerea*, antioxidant enzymes, lipid peroxidation

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1. INTRODUCTION

In agricultural areas, it is tried to increase the resistance of plants against various biotic stress factors with many synthetic chemicals such as synthetic plant activators and elicitors. In addition, although it is stated that these synthetic chemicals are not harmful, nowadays, secondary metabolites, which are safer and produced naturally in plants, have been determined instead of them. These secondary compounds are easy to biodegrade. These compounds are therefore healthier for both consumers and the environment. Plant physiologists are investigating application methods by dissolving secondary compounds in plants in suitable solvents. It is suggested that these secondary compounds in plants should be used instead of synthetic plant activators and elicitors for biological control. This is especially important in organic farming [1].

One of the most important stress factors causing crop loss in the agricultural sector is biotic stress. One of the biotic stress factors is pathogens. Fungi take almost the most important place among pathogens. Fungi are biotic stress factors that are difficult to combat because they reproduce quickly, spread rapidly and are resistant to many adverse conditions [2].

A plant exposed to stress can overcome oxidative stress by activating the antioxidant defense system [3]. Among the hosts of *Botrytis cinerea* (gray mold) are plants such as broad beans [4], cucumbers [5] and tomatoes [6]. *B. cinerea* fungus, which affected *V. faba* (broad bean), causes gray mold disease in the plant [7]. The effect of natural substances obtained from plants against different phytopathogens was investigated and it was determined that they were effective against these phytopathogens [8]. Today, researches are carried out to reduce the use of synthetic chemicals against plant diseases. Alternative possibilities have been provided by using products that can be more effective on target organisms and based on a biological basis [9].

The Brassicaceae family includes very important vegetables of economic importance. Radish (*Raphanus sativus* L.) is important for human health and nutrition. It belongs to the Brassicaceae (Cruciferea) family and is a type of vegetable. The antibiotic activity of its extracts has been proven in microbial diseases as indicated in traditional medicine. It has been proven that ethanolic and aqueous extracts show antimicrobial activity. The aqueous extract of its roots has been reported to have antimutagenic activity [10]. It was revealed that radish root extract has antioxidant activity in vitro [11]. It has been revealed that the most abundant phenolic compounds in radish roots are pyrogallol, vanillic acid, epicatechin and coumaric acid [12]. It was revealed that root extracts have much higher antioxidant capacity than leaf extracts [13].

Today, synthetic plant activator and elicitor are used in the fight against plant diseases. In this study, it was aimed to investigate the use of certain extracts of *R. sativus* tuber, which may be biodegradable in nature and not harm living organisms, as a plant defense system stimulant, instead of these chemicals. Thus, the potential to be an alternative to these chemicals was sought to be investigated. In this research, certain extracts (distilled water, ethanol and methanol extracts) potential of *R. sativus* tuber, which have antioxidant activity in activating plant defense system elements, in order to eliminate the effect of gray mold caused by *B. cinerea* fungus, which causes significant product losses in *V. faba* species in agricultural areas was investigated. Thus, it is planned to investigate possible changes in physiological resistance in *V. faba* against *B. cinerea*. In the study, certain extracts of *R. sativus* tuber were treated to *V. faba* in order to overcome gray mold disease with minimum product loss. The

purpose of applying certain extracts of *R. sativus* on *V. faba* is to increase the stimulation of the antioxidant defense system of *V. faba.* For this purpose,*V. faba* treated with distilled water only (negative control)), dimethyl sulfoxide (DMSO) only, *B. cinerea* only (positive control) (), ,,the certain extracts of *R. sativus* tuber only, the certain extracts of *R. sativus* and then *B. cinerea* inoculation (extract:fungi treatments) are compared. In this study, it was aimed to investigate the level of ascorbate peroxidase (APOX), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) activity level and lipid peroxidation (MDA) content in *V. faba* leaves with extracts:fungi treatments compared to other treatment groups on *V. faba* leaves.

2. MATERIALS AND METHODS

2.1.Materials

Vicia faba (faba bean) and *Raphanus sativus* (radish) were used. *V. faba* seeds were purchased from Zengarden as certified seed and its seedlings were grown. *R. sativus* tuber was purchased from the grocery.

2.2.Methods

2.2.1. The cultivation of *vicia faba* plants

Vicia faba seedlings were grown at 24 ± 2º C in 12 hours of light and 12 hours of darkness. Seed sowing was done in pots containing peat and perlite.

2.2.2. Extraction of *raphanus sativus* tuber

Crushed radishes were extracted with soxhlet in 300 ml solvent (distilled water, EtOH, MeOH) as 70 grams in each group. The material obtained from Soxhlett was taken into beakers and subjected to a water bath at 60°C for 24 hours. The extracts from the water bath were weighed on a precision balance. The remaining radish extract was stored in the refrigerator until analysis.

From the prepared *R. sativus* distilled water, ethanol and methanol extracts, 100 mg/ml stock solutions were prepared with DMSO (dimethyl sulfoxide). Dimethyl sulfoxide is an organosulfur compound. The compound, which is colorless and liquid, is an important polar solvent. For this reason, in order to dissolve the medically important secondary metabolites in the obtained extracts, DMSO was used in the preparation of stock solutions from the extracts in accordance with the literature [14].

2.2.3.Treatments to *vicia faba* leaves

1-) Only distilled water, 2-) only DMSO, 3-) only fungi were applied to the leaves of *V. faba*. 4-) The other group *V. faba* leaves were applied on the 3rd day after the first (1st day) application of certain extracts of *R. sativus* tuber (distilled water, ethanol and methanol extracts). The leaves of *V. faba* were harvested 24 and 48 hours after the application of the extracts. 5-) The leaves of the other group *V. faba* were infected by spraying with *B. cinerea* (gray mold) a spore suspension at a concentration of 10^5 spores/ml developed in potato dextrose agar (PDA) after extract applications. The spore suspension was prepared in sterile distilled water to which 0.03% tween-20 was added to ensure uniform spore distribution [5]. All applications were carried out by spraying. All applications to *Vicia faba* leaves were carried out for 24 and 48 hours. At the end of these periods, *V. faba* leaves were harvested.

2.2.4.Extraction of *vicia faba* leaves

It was taken from the leaves of the young *V. faba*. It was weighed 0.5 grams on a precision balance. These leaves were extracted with different extraction buffers to measure the total protein content and peroxidase (POX) activity to be analyzed. Each 0.5 gram leaf sample was crushed with

a pestle in a porcelain mortar with extraction buffer.

Extraction processes for SOD (superoxide dismutase), CAT (catalase), GR (Glutathione reductase) analysis were performed according to the method [15]. According to this method, the extraction buffer was prepared as 50 mM sodium phosphate buffer (pH=7.8) containing 1mM $EDTANa_2$.

Extraction for lipid peroxidation (MDA) analysis was performed according to the method [16]. According to this method, the extraction buffer was prepared by completing 0.10 g Trichloroacetic acid (TCA) with distilled water to a final volume of 100 ml. Leaf samples were crushed in a porcelain mortar with extraction buffer.

2.2.5. Spectrophotometric analysis of antioxidant enzymes and lipid peroxidation

Spectrophotometric analyzes and calculations were performed according to the methods for ascorbate peroxidase (APOX) [17], glutathione reductase (GR) [18], and catalase (CAT) [19]. The analysis and calculations of superoxide dismutase (SOD) in spectrophotometer were carried out by the appropriate method [20, 21]. Spectrophotometric analysis of lipid peroxidation (MDA) was performed in accordance with the method [16].

The analysis of ascorbate peroxidase (APOX) enzyme in spectrophotometer was carried out according to appropriate method. For the blank, sodium phosphate buffer, $EDTA.Na_2$, ascorbate, H_2O_2 were added to the cuvette. In the cuvette containing the plant sample, in addition to the chemicals added to the cuvette for the blind, supernatant was added. The kinetic measurement between zero and ninety seconds in the spectrophotometer was made at a wavelength of 290nm. Measurements were taken every 10 seconds.

For the purpose of analysis of glutathione reductase (GR) enzyme according to appropriate method, kinetic measurement was made in spectrophotometer. In the spectrophotometer, a measurement was taken every 10 seconds between the 0th and 90th seconds at a wavelength of 340 nm. For the blank, sodium phosphate buffer, GSSG buffer, and $NaDPNa_4$ buffer were added, respectively. Supernatant was added to the quartz cuvette prepared with the plant sample, in addition to the buffers in the cuvette with the blank.

In order to analyze the catalase (CAT) enzyme, kinetic measurements were made in the spectrophotometer. The wavelength in the spectrophotometer is set to 240nm. Measurements were taken every 10 seconds between 0 and 90 seconds in the spectrophotometer. Reading buffer, supernatant and distilled water were placed in the quartz cuvette for the blank. The cuvette containing the plant sample was prepared by adding the reading buffer, supernatant and 3% H_2O_2.

The analysis of superoxide dismutase (SOD) in spectrophotometer was carried out with the appropriate method. For this purpose, the reaction mixture was prepared. This reaction mixture contains sodium phosphate buffer, nitrotetrazolium blue, L-methionine, $EDTANa_2$ and riboflavin. Riboflavin was added to the reaction mixture last in the dark. First of all, the blank was formed in test tubes by adding 0 µl, 50 µl, 100 µl, 150 µl, 200 µl supernatant, extraction buffer and this prepared reaction mixture. Blank and test tubes with samples were stored away from light. Then the reaction was carried out by keeping the test tubes under high light for 10 minutes. At the end of ten minutes, the light source was turned off. Then, the plant samples were read in the spectrophotometer at 560 nm.

The spectrophotometric analysis of lipid peroxidation (MDA) was performed in appropriate method. Before reading in the

spectrophotometer, pipetting into the tubes was carried out in the dark. Supernatant and a mixture of trichloroacetic acid and thiobarbutyric acid are added to each test tube. The blank was prepared with extraction buffer and a mixture of trichloroacetic acid and thiobarbutyric acid. In the second stage, the prepared test tubes were covered with aluminum foil and placed in a hot water bath at 95°C. The tubes, which were removed from the hot water bath, were then taken into the cold water bath. After cooling, the mixture in the tubes was transferred to plastic Eppendorf tubes. It was centrifuged in a cooled centrifuge (10000 rpm, +4°C, 15 min). Then, 1 ml of supernatant was transferred to a quartz cuvette. 1 ml blank was also transferred to the quartz cuvette. Reading was performed by scanning in the spectrophotometer from 600nm to 532nm.

2.2.6.Statistical analysis

In order to examine the effect of group and time together on APOX, GR, CAT, SOD and MDA, the Analysis of Variance Technique in factorial order was used. Tukey's multiple comparison test was used to determine which group or subgroup the differences originated from. All statistical analyzes considered in the study were made using the R-Project programming language.

3. RESULTS AND DISCUSSION

As a result of the research, it can be stated that antioxidant enzyme activities increased with fungi applications after extract applications (extract:fungus) compared to only distilled water, only fungus and only dimethyl sulfoxide (DMSO) application groups. It was determined that lipid peroxidation (MDA) content decreased MDA content with extract:fungus applications (except 10mg/ml water extract:fungus application for 24 hours) compared to alone fungus application. APOX, GR, CAT, SOD activities and lipid peroxidation content are shown in Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, respectively.

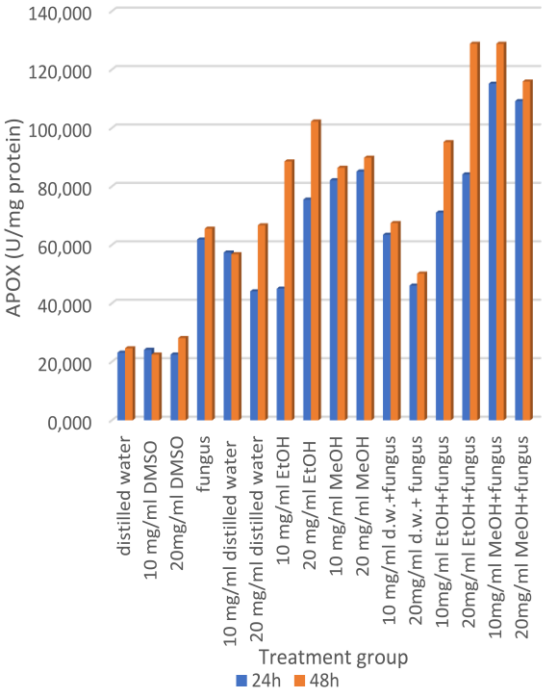

Figure 1 Ascorbate peroxidase activity

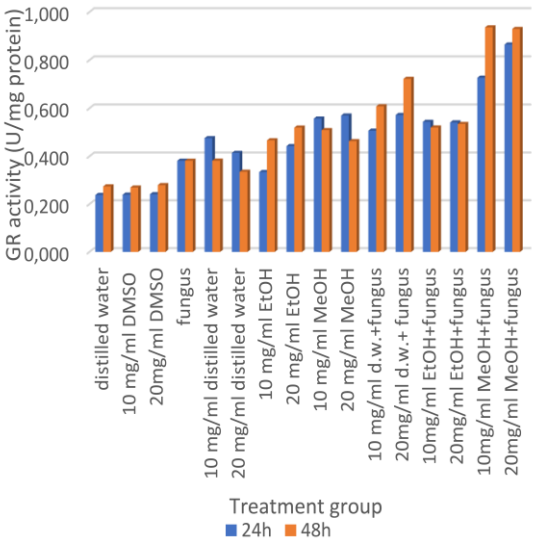

Figure 2 Glutathion reductase activity

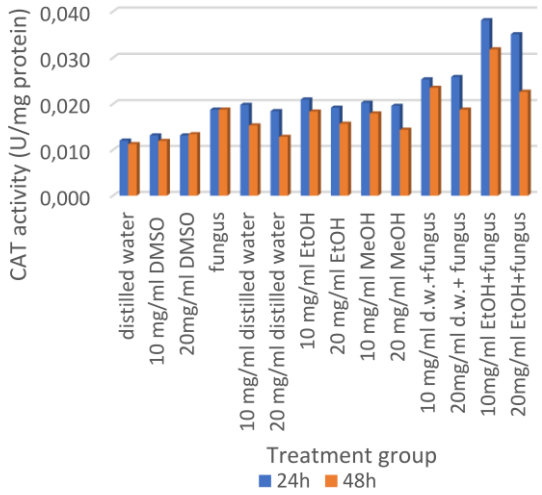

Figure 3 Catalase activity

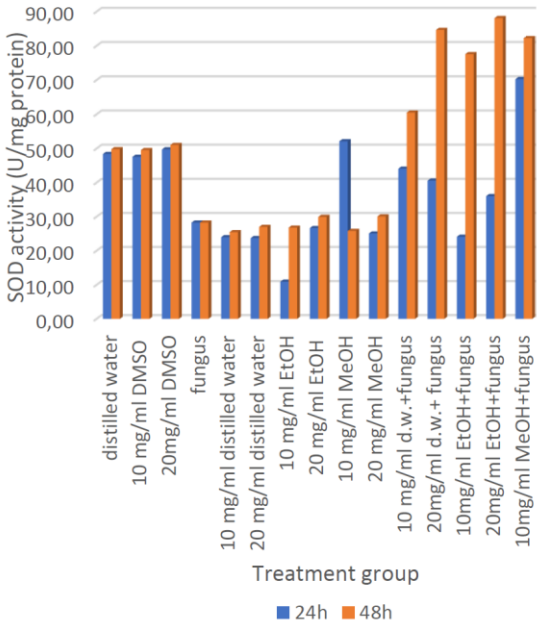

Figure 4 Superoxide dismutase activity

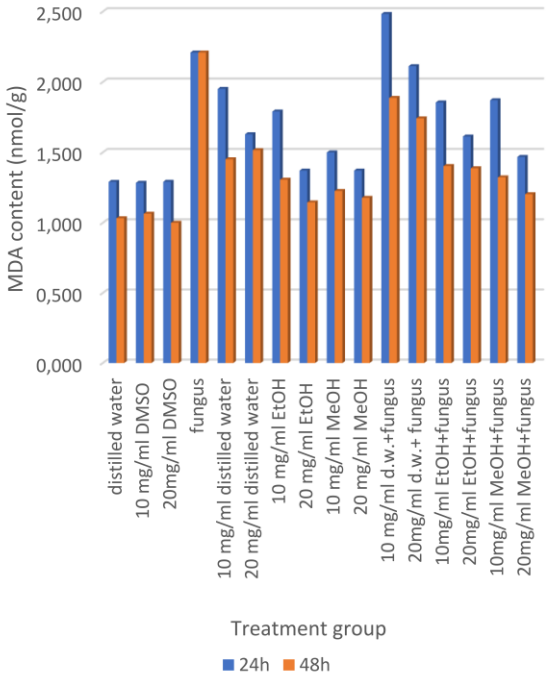

Figure 5 Lipid peroxidation (MDA) content

Ascorbate peoxidation, glutathion reductase, catalase, superoxide dismutase acitivity and lipid peroxidation content was shown in Table 1, Table 2, Table 3, Table 4 and Table 5, respectively.

Enzyme activity related to plant defense can indicate host resistance against plant pathogens. In addition, the increase in enzyme accumulation and activity depends on the plant species, physiological conditions and pathogen type. The synthesis of defense chemicals against plant pathogens is triggered by a series of morphological and biochemical changes initiated by certain fungal species [22]. Reactive oxygen species (ROS; O2-, OH-, H_2O_2) are constantly produced during metabolic processes in plant cells. In addition, they may react rapidly with other subcellular components and molecules and cause oxidative damage [23]. The superoxide radical is converted to H_2O_2 by the dismutation reaction catalyzed by SOD [24]. It can be stated that a pathogen causes an increase in SOD activity [25].

Table 1 Tukey test results by group and time in terms of ascorbate peroxidase activity (U/mg protein)

		24h		48h		Total	
		Mean	Standard error	Mean	Standard error	Mean	Standard error
Treatment group	d.w. (NC)	23.152 Fa	2.316	24.641 Ga	0.791	23.897	1.088
	10 mg/ml DMSO	24.143 Fa	0.606	22.521 Ga	1.397	23.332	0.778
	20mg/ml DMSO	22.521 Fa	1.880	28.136 Ga	0.425	25.328	1.802
	Fungus (PC)	61.852 CDa	2.769	65.562 Ea	3.189	63.707	2.030
	10 mg/ml dw	57.402 DEa	2.393	56.830 EFa	1.683	57.116	1.206
	20 mg/ml dw	44.119 Eb	3.502	66.702 Ea	0.997	55.411	6.686
	10 mg/ml EtOH	45.042 Eb	0.321	88.578 CDa	0.525	66.810	12.570
	20 mg/ml EtOH	75.526 BCb	1.926	102.274 BCa	2.266	88.900	7.817
	10 mg/ml MeOH	82.154 Ba	2.030	86.421 Da	2.708	84.288	1.851
	20 mg/ml MeOH	85.120 Ba	4.996	89.851 CDa	1.650	87.486	2.545
	10 mg/ml d.w+fungus	63.469 CDa	3.679	67.481 Ea	1.700	65.475	2.020
	20mg/ml d.w.+ fungus	46.070 Ea	1.962	50.185 Fa	4.473	48.127	2.321
	10mg/ml EtOH+fungus	71.059 BCDb	1.057	95.186 CDa	4.315	83.123	7.197
	20mg/ml EtOH+fungus	84.158 Bb	1.049	128.867 Aa	3.674	106.512	13.000
	10mg/ml MeOH+fungus	115.193 Ab	5.169	128.185 Aa	5.389	121.689	4.833
	20mg/ml MeOH+fungus	109.229 Aa	4.221	115.950 ABa	3.379	112.590	2.939
	Total	63.138	4.970	76.086	5.999	69.612	3.949

Differences between group means, shown in different capital letters at the same time, are significant (P≤0.05).
The difference between the time averages shown with different lowercase letters in the same group is significant (P≤0.05).
Abbreviations: d.w: Distilled water, DMSO: Dimethyl sulfoxide, EtOH: Ethanol, MeOH:Methanol

Table 2 Tukey test results for glutathione reductase activity (U/mg protein) by group and time

		24h		48h		Total	
		Mean	Standard error	Mean	Standard error	Mean	Standard error
Treatment group	d.w. (NC)	0.238 Kb	0.003	0.274 Ia	0.003	0.256	0.011
	10 mg/ml DMSO	0.239 Kb	0.009	0.269 Ia	0.002	0.254	0.009
	20mg/ml DMSO	0.241 Kb	0.004	0.279 Ia	0.001	0.260	0.011
	Fungus (PC)	0.381 Ib	0.006	0.392 Ga	0.002	0.387	0.004
	10 mg/ml dw	0.476 Fa	0.006	0.382 Gb	0.003	0.429	0.027
	20 mg/ml dw	0.414 Ha	0.003	0.335 Hb	0.004	0.375	0.023
	10 mg/ml EtOH	0.334 Jb	0.004	0.467 Fa	0.002	0.400	0.038
	20 mg/ml EtOH	0.443 Gb	0.004	0.520 DEa	0.001	0.481	0.022
	10 mg/ml MeOH	0.557 CDa	0.007	0.509 Eb	0.001	0.533	0.014
	20 mg/ml MeOH	0.569 Ca	0.006	0.463 Fb	0.002	0.516	0.031
	10 mg/ml d.w+fungus	0.507 Eb	0.005	0.608 Ca	0.003	0.558	0.029
	20mg/ml d.w.+ fungus	0.572 Cb	0.005	0.722 Ba	0.002	0.647	0.043
	10mg/ml EtOH+fungus	0.726 Bb	0.003	0.936 Aa	0.002	0.831	0.060
	20mg/ml EtOH+fungus	0.865 Ab	0.004	0.930 Aa	0.003	0.897	0.019
	10mg/ml MeOH+fungus	0.544 Da	0.004	0.520 DEb	0.002	0.532	0.007
	20mg/ml MeOH+fungus	0.541 Da	0.002	0.535 Da	0.003	0.538	0.002
	Total	0.478	0.030	0.509	0.036	0.493	0.023
	20mg/ml MeOH+fungus	0.541 Da	0.002	0.535 Da	0.003	0.538	0.002
	Total	0.478	0.030	0.509	0.036	0.493	0.023

Differences between group means, shown in different capital letters at the same time, are significant (P≤0.05).
The difference between the time averages shown with different lowercase letters in the same group is significant (P≤0.05).
Abbreviations: d.w: Distilled water, DMSO: Dimethyl sulfoxide, EtOH:Ethanol, MeOH:Methanol

Table 3 Tukey test results for catalase enzyme activity (U/mg protein) by group and time

		24h		48h		Total	
		Mean	Standard error	Mean	Standart Hata	Mean	Standard error
Treatment group	d.w. (NC)	0.012	0.002	0.011	0.001	0.012 F	0.001
	10 mg/ml DMSO	0.013	0.002	0.012	0.001	0.012 F	0.001
	20mg/ml DMSO	0.013	0.003	0.014	0.001	0.013 F	0.001
	Fungus (PC)	0.019	0.002	0.021	0.003	0.020 CDEF	0.002
	10 mg/ml dw	0.020	0.001	0.016	0.003	0.018 DEF	0.002
	20 mg/ml dw	0.019	0.003	0.013	0.002	0.016 EF	0.002
	10 mg/ml EtOH	0.021	0.002	0.019	0.003	0.020 CDEF	0.002
	20 mg/ml EtOH	0.019	0.002	0.016	0.002	0.017 DEF	0.001
	10 mg/ml MeOH	0.020	0.001	0.018	0.003	0.019 CDEF	0.002
	20 mg/ml MeOH	0.020	0.002	0.015	0.003	0.017 DEF	0.002
	10 mg/ml d.w+fungus	0.029	0.002	0.026	0.002	0.028 BC	0.001
	20mg/ml d.w.+ fungus	0.026	0.001	0.024	0.002	0.025 CD	0.001
	10mg/ml EtOH+fungus	0.038	0.003	0.035	0.002	0.037 A	0.002
	20mg/ml EtOH+fungus	0.038	0.003	0.032	0.002	0.035 AB	0.002
	10mg/ml MeOH+fungus	0.023	0.003	0.021	0.002	0.022 CDE	0.002
	20mg/ml MeOH+fungus	0.020	0.002	0.019	0.002	0.020 CDEF	0.001
	Total	0.022 a	0.001	0.019 b	0.001	0.021	0.001

Differences between group means, shown in different capital letters at the same time, are significant (P≤0.05). The difference between the time averages shown with different lowercase letters in the same group is significant (P≤0.05). Abbreviations: d.w: Distilled water, DMSO: Dimethyl sulfoxide, EtOH:Ethanol, MeOH:Methanol

Table 4 Tukey test results for superoxide dismutase activity (U/mg protein) by group and time

		24h		48h		Total	
		Mean	Standard error	Mean	Standard error	Mean	Standard error
Treatment group	d.w. (NC)	23.979 Fa	1.992	25.455 Ea	2.308	24.717	1.316
	10 mg/ml DMSO	23.717 Fa	2.005	26.991 Ea	0.760	25.354	1.288
	20mg/ml DMSO	25.142 Fa	1.998	29.154 DEa	3.267	27.148	1.946
	Fungus (PC)	49.730 CDa	1.511	51.020 Ba	1.549	50.375	0.959
	10 mg/ml dw	48.377 Da	1.136	49.790 BCa	1.950	49.083	1.008
	20 mg/ml dw	47.560 Da	2.458	49.523 BCa	2.584	48.542	1.562
	10 mg/ml EtOH	32.125 EFa	2.002	26.789 Ea	1.318	29.457	1.825
	20 mg/ml EtOH	30.088 EFa	2.616	29.935 DEa	1.776	30.011	1.291
	10 mg/ml MeOH	10.910 Gb	2.270	25.833 Ea	1.617	18.371	4.456
	20 mg/ml MeOH	25.069 Fa	1.939	26.650 Ea	0.869	25.859	0.980
	10mg/ml d.w+fungus	70.280 ABb	2.786	82.250 Aa	2.143	76.265	3.742
	20mg/ml d.w.+ fungus	71.724 Ab	2.683	88.037 Aa	1.441	79.880	4.871
	10mg/ml EtOH+fungus	77.551 Ab	1.594	88.113 Aa	2.597	82.832	3.293
	20mg/ml EtOH+fungus	60.497 BCb	1.390	84.649 Aa	1.142	72.573	7.011
	10mg/ml MeOH+fungus	44.095 Da	1.920	39.113 CDa	3.457	41.604	2.162
	20mg/ml MeOH+fungus	40.580 DEa	2.290	36.032 DEa	2.113	38.306	1.828
	Total	42.589	3.457	47.458	4.273	45.024	2.744

Differences between group means, shown in different capital letters at the same time, are significant (P≤0.05). The difference between the time averages shown with different lowercase letters in the same group is significant (P≤0.05). Abbreviations: d.w: Distilled water, DMSO: Dimethyl sulfoxide, EtOH: Ethanol, MeOH:Methanol

Table 5 Tukey test results by group and time in terms of lipid peroxidation content (nmol/g)

		24h		48h		Total	
		Mean	Standard error	Mean	Standard error	Mean	Standard error
Treatment group	d.w. (NC)	1.291 Ka	0.004	1.033 Lb	0.002	1.162	0.075
	10 mg/ml DMSO	1.284 Ka	0.005	1.065 Kb	0.004	1.174	0.063
	20mg/ml DMSO	1.291 Ka	0.001	1.000 Mb	0.005	1.146	0.084
	Fungus (PC)	2.210 Ba	0.005	2.215 Aa	0.004	2.212	0.003
	10 mg/ml dw	2.484 Aa	0.003	1.887 Bb	0.005	2.186	0.172
	20 mg/ml dw	2.113 Ca	0.003	1.742 Cb	0.007	1.928	0.107
	10 mg/ml EtOH	1.855 Ea	0.003	1.403 Fb	0.005	1.629	0.131
	20 mg/ml EtOH	1.613 Ga	0.003	1.387 Fb	0.006	1.500	0.065
	10 mg/ml MeOH	1.871 Ea	0.004	1.323 Gb	0.002	1.597	0.158
	20 mg/ml MeOH	1.468 Ia	0.002	1.203 Hb	0.003	1.335	0.076
	10mg/ml d.w+fungus	1.952 Da	0.002	1.452 Eb	0.002	1.702	0.144
	20mg/ml d.w.+ fungus	1.629 Ga	0.008	1.516 Db	0.005	1.572	0.033
	10mg/ml EtOH+fungus	1.791 Fa	0.006	1.307 Gb	0.004	1.549	0.140
	20mg/ml EtOH+fungus	1.371 Ja	0.006	1.145 Jb	0.004	1.258	0.065
	10mg/ml MeOH+fungus	1.500 Ha	0.005	1.226 Hb	0.006	1.363	0.079
	20mg/ml MeOH+fungus	1.371 Ja	0.004	1.178 Ib	0.004	1.274	0.056
	Total	1.693	0.064	1.380	0.057	1.537	0.047

Differences between group means, shown in different capital letters at the same time, are significant (P≤0.05). The difference between the time averages shown with different lowercase letters in the same group is significant (P≤0.05). Abbreviations: d.w: Distilled water, DMSO: Dimethyl sulfoxide, EtOH:Ethanol, MeOH:Methanol

In this study, it was determined that the level of lipid peroxidation was 2.210nmol/g and 2.215nmol/g for 24 and 48 hours, respectively and increased in V. faba leaves infected with B. cinerea alone compared to negative control and DMSO application. Apart from this, it was determined that the MDA content of *V. faba* leaves treated with only 10mg/ml and 20mg/ml R. sativus purified water extracts increased compared to the fungus application. The highest MDA content was obtained first from the 10mg/ml distilled water extract:fungi application, followed by the fungus alone application. Except for the 10mg/ml distilled water extract:fungi application, the other extract:fungi applications were found to be less than the MDA content in the leaves of V. faba infected with the fungus alone. In the study, parallel results were obtained with [26] who stated that after infection with Fusarium:plant extract applications reduced the lipid peroxidation content compared to both the uninfected and the unextracted control group. In addition, when the results of the study are examined in terms of an increase in the MDA level after infection when compared to the control group, which was not infected and did not apply the extract, it is also consistent with the results of our research. Compared to the control group, the results of our study are consistent with the results of [26].

The increase in MDA (malondialdehyde) content, which occurs as a result of the oxidative damage of unsaturated fatty acids in the cell membranes when plants are under stress, indicates that the structural integrity of the cell membranes is impaired. This is also known as LPO (lipid peroxidation) [27]. LPO level similar to MDA content was induced in tomato varieties after B. cinerea inoculation by comparison with control [28, 29].

It was stated that infecting vine leaves with *B. cinerea* alone increased the MDA contencompared to the control group. However, essential oil treatments have been

shown to reduce the high MDA content caused by B. cinerea infection. Consistent with these results of [30] in our study, it was determined that MDA content decreased in extract:fungus applications when B. cinerea application to bean leaves alone and *R. sativus* extract:fungus applications were compared. In addition, it shows parallelism with our research results in that alone fungus application causes an important increase in APX, CAT, GR and SOD activities in grapevine leaves by comparison with the control [30]. In our study, it was determined that fungal applications alone increased the activities of APOX, GR, CAT, SOD enzymes compared to the negative control group. It has been stated that oxidative stress stimulates the activity of antioxidant defense enzymes to reduce oxidative destruction caused by fungus treatment [30].

Results of determination of an increase in antioxidant defense enzyme activities (CAT, SOD) after only Fusarium infection, plant extract: Fusarium infection by [16] is in line with the results of our research.

In our study, it was determined that antioxidant defense enzymes (APOX, GR. CAT, SOD) activity increased both after only *B. cinerea* infection and as a result of *R. sativus* extract and then *B.cinerea* applications compared to the control group. The results of our study are compatible with the results of [16] in terms of determining an increase in the antioxidant enzyme activities with fungus:extract applications compared to the application of alone fungus.

In our study, it was determined that the high APOX, GR, CAT, SOD enzyme activities detected in many extract: fungal applications compared to only the fungus application were found in the plant leaves at high values for the removal of ROS. In this way, it can be stated that in the plant defense system, *B. cinerea* creates defense resistance against the stress created in the leaves of *V. faba*.

It was found that after the application of biocontrol treatments (*Azadirachta indica* and *Salix babylonica*) with *Fusarium oxysporium*, the lipid peroxidation level decreased significantly and stimulated the high activity of antioxidant defense enzymes (POX, CAT, SOD) compared to the uninfected seedlings in the control group. It was stated that high CAT and SOD activities observed in infected tomato seedlings did not cause a decrease in MDA levels. It has been stated that the high MDA content detected in Fusarium-infected seedlings reflects higher production of ROS and may be associated with increased SOD activity. It was determined that the CAT and SOD activities of the seedlings treated with aqueous extracts of A. indica and *S. babylonica* after infection were higher than those in the control group. It was stated that lipid peroxidation level and antioxidant enzyme activities (CAT, SOD) increased as a result of infection of tomato seedlings with *F. oxysporium*. As a result of the research, it was stated that aqueous extracts of *A. indica* and *S. babylonica* prevented disease development in *F. oxysporum*-infected plants and reduced the level of lipid peroxidation with a mechanism that enables the activation of antioxidant defense system enzymes [26]. Consistent with the results of this study, it was determined that the activation of antioxidant defense system enzymes increased and the lipid peroxidation content decreased as a result of the application of the extract and then the fungus in our research results.

It was determined that antioxidan enzyme activities were higher in tomato plants infected with *B. cinerea* [31, 6]. In line with these results, higher antioxidant enzyme activities were found in *V. faba* infected with *B. cinerea* compared to the negative control in our research results.

When apple fruit infected with *B. cinerea* was treated plant extracts such as *Azadirachta indica*, fennel, lavender, thyme, salvia, it was stated that these plant extracts could be used to control the disease as an alternative option to chemical fungicides [32].

It was stated that o-hydroxyethylorutin application increased the amount of antioxidant defense system enzymes in tomato leaves. In this way, it was determined that o-hydroxyethylorutin activates the antioxidant defense system in tomato leaves infected with *B. cinerea* [33].

It was treated different pepper cultivars infected with B. cinerea with salicylic acid (2-hydroxybenzoic acid), abscisic acid, methyl jasmonate, and calcium chloride as inorganic compounds in order to stimulate the defense resistance of different pepper cultivars. It has been determined that these defense system stimulating compounds increase the activity of defense system enzymes in pepper cultivars. Thus, it was stated that such compounds could explain the potential to increase pepper resistance to control *B. cinerea*, which causes gray mold in pepper [34].

It has been determined that chitosan stimulates the defense system in tomatoes and cucumbers [5]. It was stated that *Triticum aestivum* (wheat) leaves stimulated SOD, CAT and POD enzyme activities by oligochitosan treatment [35]. It was determined that SOD activity increased pretreatment of tomato plants with o-hydroxyethylrutin and then 2 and 6 hours after inoculation with *B.cinerea* compared to the control group and only fungus treatment group. It was assessed that SOD activity decreased in the group treated with o-hydroxyethylrutin and fungus, compared to the group treated with only fungus, 24 and 48 hours after inoculation with the fungus [36]. It was determined that CAT activity was significantly increased after interaction of C3 plants with *B. cinerea* or *Pseudomonas syringae.* Inoculation with *B. cinerea* or *P. syringae* has been shown to cause an increase in total SOD activity in C3 plants [37]. It was suggested that effective microorganisms and nanosilver are promising alternatives to fungicides to control chocolate spot disease in fava bean [38].

With increasing fungicide dodine concentration and treatment time, the total protein amount decreased significantly compared to the control, while the peroxidase activity increased. Obtained results showed that dodine stimulated the plant defense system [39]. Malondialdehyde accumulation was found to be less in a more drought tolerant maize cultivar [40].

It has been revealed that two new metal complexes have DPPH, hydroxyl and superoxide radical scavenging activities and total antioxidant activity [41]. In a study, the most salt sensitive and salt resistant barley genotypes were determined [42]. Larval total antioxidant activity was also decreased in *Drosophila melanogaster* fed with *Lupinus albus* (white lupine) [43]. It was stated that black radish seed extracts did not provide any meaningful results in terms of antioxidant activity [44]. It was determined that GR activity increased significantly in the livers of rainbow trout exposed to carboxin fungicide and caused oxidative stress [45]. In recent years, herbal medicines have become an important source of new treatment for various types of cancer [46]. It has been determined that while some amino acids increase the activity of POX and APOX enzymes, some decrease them [47]. Glutathione, produced by eukaryotes and prokaryotes, functions to protect the cell against adverse environmental conditions, including oxidative stress [48]. The polyphenol oxidase enzyme was extracted from the plant Trachystemon orientalis. It has been determined that some metals have activating, inhibitory and both activating

and inhibitory effects on this enzyme [49]. It was determined that the increase in the amount of MDA in crayfish fillets increased with time at different storage temperatures [50]. In metabolism, hexanal and acetone were defined as input and Butanol as output. It has been stated that it is a preliminary study to explore metabolism and may be a method for diagnosing diseases [51].

4. CONCLUSION

The use of secondary metabolites produced naturally in plants instead of synthetic plant chemicals is safer for consumers and environment. For this reason, the way has been opened for the use of secondary metabolites obtained from plants in order to activate the antioxidant defense system of plants against fungal infections. *R. sativus* contains various pharmaceutically important secondary metabolites whose different metabolites are soluble in different solvents. Therefore, different extracts of R. sativus were prepared by dissolving *R. sativus* tuber in different solvents (distilled water, ethanol, methanol) in the scope of this research. As a result of treatment of certain extracts of *R. sativus* tuber and *B. cinerea* inoculation to the leaves of *V. faba*, it was revealed that APOX, GR, CAT, SOD activities increased in the extract:fungus treatment groups compared to alone fungus treatment group. As a result of the study, it was evaluated that extract:fungus treatments generally increased the antioxidant enzyme activity (APOX, GR, CAT, SOD) levels and decreased the lipid peroxidation content when compared to the negative control (treated with distilled water only) and positive control (treated with *B. cinerea* only) groups. It can be stated that *R. sativus* tuber water, ethanol and methanol extracts generally give an increased antioxidant defense response when compared to the inoculation of alone *B. cinerea* in *V. faba* leaves. It can be stated that the antioxidant activity of *R. sativus* tuber is due to various bioactive compounds that can be found in thistuber.

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