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ALLELOPATHIC EFFECTS AND INSECTICIDAL ACTIVITY OF *Salvia sclarea* L.

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ABSTRACT. Extensive use of synthetic pesticides has negative effects on the environment and on human and animal health. Knowledge of allelopathic interactions could provide powerful tools for a better exploitation of natural resources in the management of weeds and insects without using herbicides and insecticides. Therefore effect of two concentrations (0.1% and 0.2%) of *Salvia sclarea* L. aqueous extract on lipid peroxidation process, as well as the activity of the antioxidant enzymes (superoxid dismutase, guaiacol peroxidase, pyrogallol peroxidase and catalase) in leaves and roots of pepper (*Capsicum annum L.*) and black nightshade (*Solanum nigrum* L.) seedlings were examined. Our results showed that lower concentration of *S. sclarea* aqueous extract induced lipid peroxidation in black nightshade roots. The second aim was to evaluate effectiveness of aqueous extract as contact toxicant against whitefly (*Trialeurodes vaporariorum)*. It was observed that aqueous extract with concentration of 0.1% showed toxic effect with 56.66% mortality.

Keywords: *allelochemicals; antioxidative enzymes; biopesticide; phenolics*

INTRODUCTION

Allelopathy is defined as any direct or indirect, useful or harmful effects of one plant on other plants [1]. Although the term allelopathy is most commonly used to describe the chemical interaction between two plants, it has also been used to describe chemical communication between plants and other organisms, including bacteria, insects and mammals [2, 3]. Chemicals that impose allelopathic influences, called allelochemicals, are biomolecules produced in plant secondary metabolism [4, 5]. Phenolic acids were identified as the

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allelopathic agents more often than all other classes of secondary biomolecules [6]. Several studies have shown that plant defense against pathogens, nematodes and insects is based on the release and accumulation of phenolic compounds in soil. Phenolic compounds can offer an alternative to the chemical control of pathogens on agricultural crops [7]. Studies on the mechanisms and modes of action of allelopathic agents have revealed that phenolic compounds affect respiration, nutrient uptake, protein synthesis, photosynthesis, hormone levels, plant water potential and cell division and elongation [8, 9]. One of the main effects of allelochemicals on target plant is induced secondary oxidative stress manifested as uncontrolled production and accumulation of reactive oxygen species (ROS) [10]. Thus, phytotoxins caused disruption of membrane permeability, increased concentration of H_2O_2, enhanced malondialdehyde (MDA) level and alterations in activities of antioxidant enzymes [10, 11].

The cell can reduce the impact of ROS either by an endogenous and exogenous antioxidant defense [12]. They are classified in enzymatic and non-enzymatic systems [13]. Some of the antioxidant enzymes that are found to provide a protection against potentially deleterious effects of reactive oxygen species are superoxide dismutase, catalase, peroxidase, glutathione peroxidase [14]. These enzymes play important roles in protecting cell [15]. Superoxid dismutase (SOD) is the first enzyme in detoxifying process, which converts superoxide free radical anions ($O_2^{\cdot-}$) to H_2O_2 [16, 17]. Catalase (CAT) and peroxidases metabolize hydrogen peroxide. Peroxidases are a group of oxidoreductases that catalyse H_2O_2 dependent oxidation of a wide variety of substrates [18]. Catalases, in contrast to peroxidases, do not require a reducing substrate for their activity [19].

Salvia sclarea L. (fam. *Lamiaceae*), popularly known as Clary sage, is native to Southern Europe and cultivated worldwide [20, 21]. Clary sage is important source of essential oils and many other compounds derived from different parts of the plant [22, 23]. In aromatherapy, it is a good relaxant for stress, asthma, and digestive problems. *S. sclarea* has shown diverse biological activities manifested by different components that allowed for many medicinal and pharmaceutical applications of the plant materials and extracts [20, 21]. Aqueous extracts of *Salvia* species had inhibitory effect on germination of weed seeds [24].

Knowledge of allelopathic interactions could provide powerful tools for a better exploitation of natural resources in the management of weeds and insects without using synthetic herbicides and insecticides. These compounds are easily biodegradable, environmentally-friendly, and often cheaper than the synthetic ones [25]. Objectives of these experiments were to (i) compare and analyze the allelopathic effect of the aqueous extracts of *Salvia sclarea* L. in two concentrations on pepper and black nightshade antioxidant properties and to explore the properties of this species in the control of weeds and (ii) to evaluate effectiveness of aqueous extract as contact toxicant against greenhouse whitefly.

RESULTS AND DISCUSSION

Chemical composition

Plant phenolics constitute are one of the major groups of compounds which possess a broad spectrum of chemical and biological activities [26]. The total amount of phenols in *S. sclarea* aqueous extract was 22.22 ± 0.64 mg gallic acid (GA) equivalents g^{-1} dry weight (d.w.). Flavonoids as one of the most diverse group of natural compounds are probably the most important natural phenolics [26]. In *S. sclarea* aqueous extract flavonoids were found in amount of 0.35 ± 0.00 mg rutin equivalents g^{-1} d.w. According to Miliauskas et al. [26] the total amount of phenols and flavonoids in *S. sclarea* methanolic extract were 24.00 ± 1.10 mg GA equivalents g^{-1} plant extract and 4.80 ± 0.50 mg rutin equivalents g^{-1} plant extract, respectively.

Table 1. Effect of two concentrations of *Salvia sclarea* aqueous extracts on activities of antioxidant enzymes and on MDA content in leaves of black nightshade seedlings

Time	24h	72h	120h
CAT			
Control	10.34 ± 0.12^{a}	25.83 ± 1.77^{d}	15.28 ± 0.15^{b}
0.1 %	44.79 ± 1.40^{f}	33.66 ± 0.52^{e}	26.40 ± 1.00^{d}
0.2 %	25.61± 1.22^{d}	17.71 ± 0.78^{b}	20.76 ± 0.94^{c}
SOD			
Control	16.50 ± 0.04^{a}	16.28 ± 0.19^{a}	9.88 ± 0.11^{d}
0.1 %	21.58 ± 0.19^{f}	16.18 ± 0.05^{a,b}	15.88 ± 0.06^{b}
0.2 %	21.12 ± 0.13^{e}	19.37 ± 0.18^{c}	16.62 ± 0.06^{a}
GPX			
Control	99.26 ± 6.03^{a,b}	94.99 ± 7.02^{a}	87.99 ± 4.50^{a}
0.1 %	161.55 ±10.68^{c}	96.20 ± 8.78^{a}	109.40 ± 5.89^{a,b}
0.2 %	121.62 ± 6.45^{b}	196.30 ±11.87^{d}	110.84 ± 5.30^{a,b}
PPX			
Control	147.56 ± 4.92^{a}	148.25 ± 5.16^{a}	112.65 ± 3.88^{c}
0.1 %	196.78 ± 3.03^{d}	155.49 ± 3.56^{a}	106.92 ± 3.08^{c}
0.2 %	145.85 ± 7.40^{a}	242.52 ± 3.72^{e}	129.29 ± 1.20^{b}
LP			
Control	1.68 ± 0.04^{a}	2.15 ± 0.05^{c}	1.98 ± 0.07^{b}
0.1 %	2.52 ± 0.07^{e}	2.29 ± 0.07^{c,d}	2.19 ± 0.02^{c}
0.2 %	2.13 ± 0.04^{b,c}	2.42 ± 0.06^{d}	2.40 ± 0.06^{d,e}

The data are mean values ± standard error. $^{a-f}$ values without the same superscripts within each column differ significantly ($P < 0.05$)
CAT, catalase [U mg^{-1} protein]; SOD, superoxide dismutase [U mg^{-1} protein];
GPX, guaiacol peroxidase [U mg^{-1} protein]; PPX, pyrogallol peroxidase [U mg^{-1} protein];
LP, lipid peroxidation [nmol MDA mg^{-1} protein]

Effect of extracts on MDA content and antioxidant enzyme activity in the pepper and black nightshade seedlings

The response of plants to damaging adverse circumstances is closely related to their enzyme activity [27]. For this reason, activities of antioxidant enzymes are frequently used as indicators of oxidative stress in plants [28]. As it is shown in Table 1, the activity of CAT in the leaves of black nightshade plants was significantly increased by the 0.1% *S. sclarea* aqueous extract, 24h after the treatment. The significant increase in the SOD activity was detected after 120 h in plants treated with higher concentration of *S. sclarea* aqueous extract. The highest activities of peroxidases were observed in plants treated with higher concentration 72 h after the treatment. In the roots of black nightshade, the significant increase of CAT activity was detected in plants treated with higher concentration of *S. sclarea* aqueous extract 72 h after the treatment while the lower concentration have the same effect on the activity of SOD after 24 h (Table 2).

Table 2. Effect of two concentrations of *Salvia sclarea* aqueous extracts on activities of antioxidant enzymes and on MDA content in roots of black nightshade seedlings

Time	24h	72h	120h
CAT			
Control	3.61± 0.08^{a}	20.06 ± 0.60^{d}	9.36 ± 0.34^{b}
0.1 %	6.11 ± 0.51^{a,b}	15.24 ± 1.63^{c}	16.84 ± 1.51^{c,d}
0.2 %	7.92 ± 0.41^{b}	32.11 ± 2.67^{e}	7.16 ± 0.36^{a,b}
SOD			
Control	33.82 ± 0.08^{a}	25.90 ± 1.91^{c}	11.37 ± 0.68^{f}
0.1 %	68.07 ± 0.04^{d}	15.59 ± 1.39^{e}	32.42 ± 0.33^{a}
0.2 %	45.53 ± 0.44^{b}	23.60 ± 1.78^{c}	31.01 ± 0.65^{a}
GPX			
Control	1373.00 ± 99.75^{a,d,e}	1405.34 ± 110.34^{a,e}	1656.93 ± 196.1^{d}
0.1 %	2488.81 ± 100.99^{b}	1446.97 ± 99.36^{a,d,e}	1138.41 ± 46.40^{e}
0.2 %	1982.19 ± 65.68^{c}	1229.42 ± 44.20^{a,d,e}	1534.45 ± 29.03^{a,d}
PPX			
Control	1304.85 ± 24.37^{a}	1348.32 ± 20.98^{a}	1325.53 ± 56.60^{a}
0.1 %	1792.25 ± 55.88^{c}	1482.55 ± 106.05^{a}	899.80 ± 37.71^{b}
0.2 %	1379.10 ± 36.29^{a}	1474.83 ± 108.25^{a}	1064.56 ± 46.07^{b}
LP			
Control	2.38 ± 0.08^{a}	3.40 ± 0.07^{d}	1.88 ± 0.05^{c}
0.1 %	3.91 ± 0.21^{e}	4.02 ± 0.12^{e}	3.17 ± 0.12^{d}
0.2 %	3.64 ± 0.13^{d,e}	4.03 ± 0.14^{e}	2.77 ± 0.07^{b}

The data are mean values ± standard error. $^{a-f}$ values without the same superscripts within each column differ significantly ($P < 0.05$)
CAT, catalase [U mg^{-1} protein]; SOD, superoxide dismutase [U mg^{-1} protein];
GPX, guaiacol peroxidase [U mg^{-1} protein]; PPX, pyrogallol peroxidase [U mg^{-1} protein];
LP, lipid peroxidation [nmol MDA mg^{-1} protein]

The activities of peroxidases in the roots of black nightshade were significantly affected by the lower concentration of aqueous extracts, 24h after the treatment. After 120h, decrease of peroxidases activities were recorded. The activities of these enzymes showed downward trend with duration of the experiment. These observations are in agreement with earlier studies which reported up/down regulation in antioxidant enzyme activities under allelopathic stress of aqueous extracts from plants [29].

Table 3. Effect of two concentrations of *Salvia sclarea* aqueous extracts on activities of antioxidant enzymes and on MDA content in leaves of pepper seedlings

Time	24h	72h	120h
CAT			
Control	18.32 ± 0.48^{a}	16.84 ± 0.53^{a}	17.88 ± 0.77^{a}
0.1 %	17.35 ± 0.90^{a}	24.11 ± 1.03^{c}	20.52 ± 0.41^{b}
0.2 %	33.40 ± 0.52^{d}	22.28 ± 0.14^{b}	16.54 ± 0.24^{a}
SOD			
Control	11.23 ± 0.06^{a}	7.42 ± 0.04^{d}	5.96 ± 0.28^{e}
0.1 %	12.51 ± 0.03^{b}	7.17 ± 0.01^{d}	6.01 ± 0.01^{e}
0.2 %	10.24 ± 0.11^{c}	7.13 ± 0.02^{d}	4.76 ± 0.03^{f}
GPX			
Control	541.98 ± 11.57^{a}	486.07 ± 18.10^{b}	553.51 ± 22.27^{a}
0.1 %	379.29 ± 15.71^{c}	552.45 ± 11.73^{a}	252.86 ± 4.28^{e}
0.2 %	3339.09 ± 13.45^{d}	259.45 ± 10.47^{e}	317.26 ± 6.97^{d}
PPX			
Control	3551.93 ± 43.84^{a}	3854.00 ± 80.54^{c}	3348.29 ± 62.31^{b}
0.1 %	2198.78 ± 79.73^{e,f}	2298.00 ± 51.92^{e}	3026.71 ± 71.26^{d}
0.2 %	2255.68 ± 48.80^{e}	1746.62 ± 58.50^{g}	2051.72 ± 45.81^{f}
LP			
Control	2.52 ± 0.15^{a}	3.02 ± 0.11^{b}	3.29 ± 0.07^{b,c}
0.1 %	2.55 ± 0.08^{a}	3.47 ± 0.06^{c}	1.98 ± 0.03^{d}
0.2 %	3.28 ± 0.14^{b,c}	3.35 ± 0.14^{c}	1.78 ± 0.03^{d}

The data are mean values ± standard error. $^{a-g}$ values without the same superscripts within each column differ significantly ($P < 0.05$)
CAT, catalase[U mg^{-1} protein]; SOD, superoxide dismutase [U mg^{-1} protein];
GPX, guaiacol peroxidase [U mg^{-1} protein]; PPX, pyrogallol peroxidase [U mg^{-1} protein];
LP, lipid peroxidation [nmol MDA mg^{-1} protein]

An increase in the enzyme's activity in the first 72 h, probably occurs in response to stress [27]. For various plant species under oxidative stress, a significant increase of lipid peroxidation (LP) is observed. Oracz et al. [11] found that symptoms of membrane injuries occurred concomitantly to H_2O_2 accumulation and preceded an increase in the content of MDA, a compound that reveals the occurrence of measurable processes of lipid peroxidation. Thus, lipid peroxidation is a widely used stress indicator of plant membranes [30].

The significant increase in LP intensity was recorded in roots of black nightshade plants treated with lower concentration of *S. sclarea* aqueous extract 120h after the treatment. This could point to the fact that alelopathy provoked stress was strong enough that scavenging effects of SOD, CAT and peroxidases could not prevent oxidative burst and induction of LP. Higher production of MDA in the roots of black nightshade compared with MDA content in leaves showed that roots were more affected by allelochemicals than leaves.

Table 4. Effect of two concentrations of *Salvia sclarea* aqueous extracts on activities of antioxidant enzymes and on MDA content in roots of pepper seedlings

Time	24h	72h	120h
CAT			
Control	22.93± 0.67^{a}	20.64 ± 0.58^{b}	10.56 ± 0.22^{e}
0.1 %	18.88 ± 0.48^{b}	13.52 ± 0.86^{d}	5.50 ± 0.44^{f}
0.2 %	20.12 ± 1.22^{b}	15.79 ± 0.45^{c}	6.71± 0.32^{f}
SOD			
Control	7.27 ± 0.15^{a}	7.99 ± 0.07^{b}	9.34 ± 0.21^{e}
0.1 %	2.10 ± 0.06^{h}	5.12 ± 0.22^{c}	0.57 ± 0.06^{i}
0.2 %	3.33 ± 0.03^{f}	10.04 ± 0.05^{g}	4.55 ± 0.14^{d}
GPX			
Control	2655.56 ± 216.15^{a}	3589.58 ± 147.75^{c}	3315.58 ± 169.82^{b,c}
0.1 %	1709.12 ± 163.46^{d}	2530.02 ± 38.35^{a}	981.65 ± 20.54^{e}
0.2 %	3438.82 ± 122.31^{b,c}	3138.07 ± 172.15^{b}	1330.66 ± 21.66^{d,e}
PPX			
Control	2624.17 ± 25.56^{a}	2543.53 ± 51.18^{a}	1924.63 ± 52.26^{b}
0.1 %	1408.39 ± 51.03^{d}	1705.02 ± 31.87^{c}	587.81 ± 19.84^{e}
0.2 %	1637.99 ± 68.73^{c}	1934.68 ± 75.75^{b}	642.47 ± 15.01^{e}
LP			
Control	2.46 ± 0.07^{a}	2.92 ± 0.09^{b}	2.45 ± 0.08^{a}
0.1 %	2.42 ± 0.12^{a}	3.51 ± 0.30^{d}	1.96 ± 0.10^{c}
0.2 %	2.65 ± 0.11^{a,b}	2.50 ± 0.08^{a,b}	1.57 ± 0.12^{c}

The data are mean values ± standard error. $^{a-i}$ values without the same superscripts within each column differ significantly ($P < 0.05$)
CAT, catalase [U mg^{-1} protein]; SOD, superoxide dismutase [U mg^{-1} protein];
GPX, guaiacol peroxidase [U mg^{-1} protein]; PPX, pyrogallol peroxidase [U mg^{-1} protein];
LP, lipid peroxidation [nmol MDA mg^{-1} protein]

A significant decrease in activities of peroxidases in the leaves of the pepper plants were detected after treatment with *S. sclarea* aqueous extract, while activity of SOD was not affected by treatment (Table 3). An increase in activity of CAT was detected after 24 h in leaves of plants treated with higher

concentration of *S. sclarea* aqueous extract. In the roots, there was significant decrease in the activity of enzymes particularly of peroxidases and SOD at lower concentration of applied aqueous extracts (Table 4). Our results indicated that allelochemicals had both, inhibitory and stimulatory, effects on enzyme's activity. Similar findings have been reported by other authors [28].

There were no significant increase in LP intensity 24h and 72h after the treatment in leaves and roots of pepper plants treated with higher concentration. Accumulation of MDA was significantly lower in leaves and roots of pepper 120h after the treatment when compared with the control group. It indicated that effects of allelochemicals were low and defensive system of the plant was prevailed.

The phytotoxic effects of extracts were different between two understudy species, which points to the different sensitivity of species when facing allelochemicals [1]. Allelochemicals are selective in their actions and plants are selective in their responses [9]. Gilani et al. [31] found that the plant species have different allelopathic potentials and stimulatory effects of plants were decreased with increasing concentration. The sensitivity of plants dependent on concentration of applied extracts and duration of experiment as well [27, 32].

Toxicity test

Medicinal plants are rich sources of pesticides discovery, especially insecticides [25]. Natural products have low mammalian toxicity, high target specificity and biodegradability, and contain many active ingredients [33]. In the present work aqueous extract of *S. sclarea* was evaluated on greenhouse whitefly. The table 5 shows that mortality rate of greenhouse whiteflies after 96 h was above 50%. More effective formulation, with 56.66% mortality, was concentration of 0.1% *S. sclarea* aqueous extract. Furthemore, *S. sclarea* extracts had an overall excito-repellency against adult house flies [25], and offer effective bioactive compounds for growth inhibition of the fungi [34]. Zavala-Sánchez et al. [35] reported that chloroform extracts from *Salvia microphylla* and *Salvia connivens* had high insecticidal activity, and *Salvia keerlii* and *Salvia ballotiflora* had moderate insecticidal activity against armyworm, *Spodoptera frugiperda*. Tomczyk and Suszko [36] reported that Salvia officinalis extracts had toxic effect on larval stages and females of *Tetranychus urticae*. These reports have shown that different species of the genus Salvia have toxic effect on insects. Results of other authors indicate a potential use of aqueous plant extracts in pest management of greenhouse whiteflies [37].

Table 5. Mortality of *Trialeurodes vaporariorum* adult fed for 4 days with formulations containing a known concentration (0.1%, 0.2%) of *Salvia sclarea* aqueous extracts

	Mortality (%)		
Time	24h	72h	120h
Control	8.33^{c}	10.00^{c}	$16.66^{b,c}$
0.1 %	$6.66^{c,d}$	40.00^{a}	56.66^{a}
0.2 %	11.66^{c}	41.66^{a}	55.00^{a}
$^{a-d}$ values without the same superscripts within each column differ significantly ($P < 0.001$)			

CONCLUSIONS

The effects of *S. sclarea* aqueous extracts were different between pepper and black nightshade seedlings, which points to the different responses of species when facing allelochemicals. Two tested concentrations did not exhibit phytotoxic effect on the pepper or black nightshade seedlings. It was observed that aqueous extract with concentration of 0.1% showed toxic effect on greenhouse whitefly, an important insect pest of many plants including pepper, with 56.66% mortality. Therefore, we concluded that this plant may be explored in the development of bioinsecticides and use of natural substances could be an alternative method of insect control.

EXPERIMENTAL SECTION

Plant material and preparation of the aqueous extract

The aerial parts of the flowering plant *Salvia sclarea* L. were collected in the south of Serbia, in August. Voucher specimens of collected plant was confirmed and deposited at the Herbarium of The Department of Biology and Ecology, Faculty of Science, University of Novi Sad.

The air-dried plant material was ground into powder. The powdery material (10 g) was extracted with 100 ml distilled water (10% w/v). After 24 h, the extract was filtered through filter paper and kept at 4 °C until application.

Determination of total phenolic and flavonoid contents

The total phenolic content of *S. sclarea* aqueous extract was determined according to Folin-Ciocalteu's method [38]. Extract (0.02 ml) was mixed with 3.36 ml of deionized water, 0.4 ml of 20% sodium carbonate and 0.2 ml of 33% Folin–Ciocalteau reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 720 nm on a spectrophotometer. The data were expressed as mg gallic acid equivalents g^{-1} dry weight (mg GA equivalents g^{-1} d.w.).

Total flavonoids were estimated to the method described by Markham [39]. Extract (0.4 ml) was mixed with 1 ml of deionized water and 2.5 ml of aluminium chloride hexahydrate. After incubation at room temperature for 15 min, the reaction mixture absorbance was measured at 430 nm on a spectrophotometer. The data were expressed as mg rutin equivalents g^{-1} dry weight (mg rutin equivalents g^{-1} d.w.).

Seedling growth

The pepper (*Capsicum annuum* L.) cv. Anita and black nightshade (*Solanum nigrum* L.) seeds were grown in a controlled climate chamber at 28 ^{o}C, 60% relative humidity, a photoperiod of 18 h, and a light intensity of 175 µmol m^{-2} s^{-1}, in plastic pots containing sterile sand. After 30 days, the seedlings were transplanted in plastic pots containing 700 ml of Hoagland's solution, and 7 and 14 ml of *S. sclarea* aqueous extract, while pots of control contained the same volume of Hoagland's solution. Seedlings were harvested for further biochemical analyzes 24, 72 and 120 h after the treatments.

Enzyme extraction

Fresh leaves and roots (2 g each) were homogenized in 10 ml of phosphate buffer (0.1 M, pH 7.0). Homogenates were centrifuged for 20 min at 10.000 x *g* and filtered. The supernatants were used to test enzyme activity and to determine intensity of lipid peroxidation.

Membrane lipid peroxidation and protein content

Lipid peroxidation was measured at 532 nm using the thiobarbituric acid test (TBA) test [40]. The enzyme extract (0.5 ml) was incubated with 2 ml of 20% TCA containing 0.5% thiobarbituric acid for 40 min at 95 ^{o}C. The reaction was stopped by cooling on ice for 10 min and the product was centrifuged at 10.000 x *g* for 15 min. The total amount of TBA-reactive substances is given as nmol malondialdehyde (MDA) equivalents mg^{-1} protein.

Protein content in homogenates were determined according to the method of Bradford [41], using bovine serum albumin as a protein standard.

Assay of catalase activity

Catalase (CAT) (EC 1.11.1.6) activity was determined according to Sathya and Bjorn [42]. The decomposition of H_2O_2 was followed as a decrease in absorbance at 240 nm. The enzyme extract (0.02 ml extract of leaves and 0.1 ml extract of roots, separately) was added to the assay mixture containing 1 ml for leaves and 1.5 ml for roots 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2. The activity of the enzyme was expressed as U per 1 g of protein (U g^{-1} protein).

Assay of superoxide dismutase activity

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed according to the method of Mandal et al. [40] slightly modified by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) chloride. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM L-methionine, 75 μM NBT, 0.1 mM EDTA, 2 μM riboflavin and 0.02 ml of the enzyme extract. It was kept under a fluorescent lamp for 30 min, and then the absorbance was read at 560 nm. One unit of the SOD activity was defined as the amount of enzymes required to inhibit reduction of NBT by 50%.

The activity of the enzyme was expressed as U per 1 mg of protein (U mg^{-1} protein).

Assay of peroxidase activity

Peroxidase (EC 1.11.1.7) activity was measured using guaiacol (guaiacol peroxidase; GPX) and pyrogallol (pyrogallol peroxidase; PPX) as substrates according to Morkunas and Gmerek [43]. Peroxidase activity (GPX and PPX) was expressed as U per 1 mg of protein (U mg^{-1} protein).

Pyrogallol peroxidase activity: this method includes the measurement of the content of purpurogallin- a product of pyrogallol oxidation. The enzyme extract (0.02 ml) was added to the assay mixture containing 3 ml of 180 mM pyrogallol and 0.02 ml of 2 mM H_2O_2. Absorbance was recorded at 430 nm using a spectrophotometer.

Guaiacol peroxidase activity: this method consists of the assay of tetraguaiacol- a colored product of guaiacol oxidation in the investigated sample. The enzyme extract (0.04 ml) was added to the assay mixture containing 3 ml of 20 μM guaiacol and 0.02 ml of 3 mM H_2O_2. Absorbance was recorded at 436 nm using a spectrophotometer.

Insects and toxicity test

The experiment on the adult of whitefly, *Trialeurodes vaporariorum* (Westwood, 1856) (Homoptera: Aleyrodidae), collected in the greenhouse, was carried out at the Faculty of Agriculture, University of Novi Sad.

The bioassays were carried out using groups of 20 adult insects *T. vaporariorum*, kept in the transparent laboratory dishes (25 cm x 12 cm), fed on the pepper nursery plants containing a known concentration (0.1%, 0.2%) of aqueous extract. Aqueous extracts were applied together with adjuvant (Trend) for better adhesion to the leaf surface. Pepper plants with water, adjuvant and 20 insects for each were used as controls. The experiment was set in three replicates and control. A no-choice method, in which control and treated plants were placed individually in each dish, was adopted in this experiment. Mortality was checked after 24, 48 and 96 h.

Statistical analysis

Values of the biochemical parameters were expressed as means ± standard error of determinations made in triplicates and tested by ANOVA followed by comparison of the means by Duncan's multiple range test ($P<0.05$). Data were analyzed using STATISTICA for Windows version 11.0.

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