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Proceeding Paper

Effect of Spirotetramat Application on Salicylic Acid, Antioxidative Enzymes, Amino Acids, Mineral Elements, and Soluble Carbohydrates in Cucumber (*Cucumis sativus* L.) †

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Abstract: Pesticide application may have adverse effects on crop plants. This study provides initial evidence on the effect of spirotetramat, an insecticide, on plant physiological characteristics as a non-target organism. Cucumber plants (*Cucumis sativus* L.) exposed to spirotetramat were studied 10 days after treatment. There was an increase in the activity of antioxidant enzymes including superoxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase, and phenylalanine ammonia-lyase. The amounts of malondialdehyde, total chlorophyll, and hydrogen peroxide in addition to electrolyte leakage index were not affected by spirotetramat. Further biochemical analyses revealed an increase in the content of some amino acids, as well as sucrose, glucose, and fructose. The concentration of salicylic acid and also minerals like calcium, manganese, copper, zinc, iron, nitrogen, and magnesium were elevated in spirotetramat-treated plants. Results have shown that spirotetramat can manipulate cucumber plant physiology by inducing biochemical responses that are reflected in changes in antioxidative enzymes, amino acids, soluble carbohydrates, salicylic acid, and mineral elements. Contrary to previous documents suggesting that plants are less influenced by insecticides in conducted conditions, our results show that cucumber plants can be affected by spirotetramat at the recommended rate in different biochemical aspects in greenhouses.

Keywords: antioxidative system; biochemical changes; host plant; oxidative stress; physiological responses

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1. Introduction

Herbivorous insects attack plants during their growth cycle, which results in crop yield reduction. Thus, control of crop pests depends on pesticide utilization [1]. Many pesticides are absorbed by plants through the leaf surface and may influence plant defense systems such as antioxidative and detoxification systems [2].

Cucumber (*Cucumis sativus* L.) is one of the major greenhouse vegetables in the world [3]. It is vulnerable to some herbivorous insects such as tobacco whitefly (*Bemisia tabaci*), for which the use of insecticides is mainly unavoidable for their control [4]. Spirotetramat, a spirocyclic tetramic acid derivative, is effective against sucking pests such as whiteflies because of the insecticide's physicochemical properties, efficacy, and compatibility with plants [5]. This two-way systemic (ambimobile) insecticide, which translocates in xylem and phloem, inhibits lipid biosynthesis in the herbivorous insect's body [6]. Like other

insecticides, spirotetramat is designed based on insect physiology and has no known recognized site of action in plants. When absorbed by the plant, the active ingredient is transformed into a biologically active form in which spiroteramat-enol is the most prominent compound. This derivative, produced through hydrolytic cleavage of the parent compound, is a weak acid with low lipophilicity. These two important properties of spirotetramat-enol have led to it moving in both the phloem and xylem of the plant, which means ambimobile insecticide [7].

Plants are persistently confronted with a range of (a)biotic stresses in their natural habitats. Pesticides, as important inducers of abiotic stress, have profound effects on the physiology and biochemistry of plants in agricultural ecosystems [8]. Plants have evolved efficient immune responses, which enable them to survive and reproduce. The response of plants to abiotic stresses triggers a wide range of defense reactions that provide the regulatory potential to conserve host plant fitness [9]. The impact of abiotic stress on plant fitness has been extensively explored at many levels, including the physiological responses of plants [10].

Enzymatic and non-enzymatic antioxidants are well known for their role in protecting plants from (a)biotic stresses. Exposure to some pesticides results in the accumulation of reactive oxygen species (ROS) in plants that have evolved different defense mechanisms to protect themselves against these xenobiotics [11]. Few studies have elucidated the plant defense systems by following oxidative stress in response to insecticide application. We have hypothesized that an insecticide with an unknown site of action in plants cannot cause profound effects on plant biochemical systems because insecticides have been produced based on insects' physiological characteristics. Accordingly, the aim of this study was the evaluation of different enzymatic and non-enzymatic fluctuation in cucumber plants in response to spirotetramat. Investigation into the metabolic pathways of plants treated with insecticides can be useful for future insecticide synthesis and their application in pest management programs.

2. Materials and Methods

2.1. Plant Growth and Insecticide Treatment

The seeds of greenhouse cucumber (cultivar hybrid super N3) were planted in sterilized soil composed of 1:1:2 cocopeat:peat moss:perlite and were then grown in a greenhouse under controlled conditions of a 16:8 h (L:D) photoperiod, light intensity of 5100 Lux, temperature of 26 ± 2 °C, and 30–40% relative humidity. They were irrigated every three days up to the soil capacity with distilled water. Spirotetramat (Movento® SC 100, Bayer CropScience, Monheim am Rhein, Germany) was sprayed at the recommended dose for the tobacco whitefly in the greenhouse (50 mg a.i. L^{-1}) on 30-day-old cucumber seedlings (6–8 true leaves stage). Simultaneously, control plants were sprayed with distilled water. After spraying the whole cucumber leaf surfaces to the point of runoff, all of the leaves were harvested at 10 days after treatment, as the most efficient time for whitefly control. The harvested leaves were ground in liquid nitrogen and stored at −80 °C for subsequent analysis.

2.2. Assay of Enzyme Activity

For superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPX) activity, the method of Homayoonzadeh et al. [12] was used. The SOD activity, which measures inhibition of the photochemical reduction of nitro blue tetrazolium spectrophotometrically at 560 nm, was carried out for 20 min at 25 °C both under a fluorescent light (40 W) and in the dark. CAT activity was assayed using hydrogen peroxide (H_2O_2) as a substrate and measurement at 240 nm (ε = 0.000394 mM^{-1} cm^{-1}). For GPX activity, absorbance of the formed tetraguaiacol was determined at 470 nm using guaiacol as an electron donor group and H_2O_2 as a substrate (ε = 26.6 mM^{-1} cm^{-1}). Assessment of ascorbate peroxidase (APX), glutathione reductase (GR), and phenylalanine ammonia-lyase (PAL) activity was performed based on the method of Homayoonzadeh et al. [13]. For APX activity,

H_2O_2 and ascorbic acid were used as substrate and reductant, respectively. Decrease in absorbance at 290 nm was recorded due to oxidation of ascorbic acid (ε = 2.8 mM^{-1} cm^{-1}). GR activity was carried out with recording absorbance at 412 nm, due to formation of 5′-thio-2-nitrobenzoic acid (as a reaction product) resulting from the reaction of 5,5′-dithiobis(2-nitrobenzoic acid) (as a substrate) with reduced glutathione (ε = 14.15 mM^{-1} cm^{-1}). PAL activity was determined based on the rate of production of cinnamic acid (CA) by using phenylalanine as a substrate after incubation at 37 °C. The extraction of CA product by ethyl acetate was performed and then resuspended in sodium hydroxide and measured at 290 nm (ε = 9500 M^{-1} cm^{-1}).

2.3. Contents of Plant Health Indices and Salicylic Acid

Contents of malondialdehyde (MDA), total chlorophyll (Chl), and salicylic acid (SA) were measured using the method as claimed by Homayoonzadeh et al. [14]. MDA content was quantified using the thiobarbituric acid test and products were analyzed colorimetrically at 532 and 600 nm. Chl was extracted using 100% acetone and quantified with read of absorbance at 662 nm for Chl *a*, 645 nm for Chl *b*, and 470 nm for Car. SA extraction was carried out using methanol and subsequently quantified with a Shimadzu LC-9A HPLC system equipped with a UV/VIS detector (SPD-6AV) (Shimadzu, Kyoto, Japan). The separation was performed on a GLC-ODS C18, 150 mm × 6 mm i.d. column, at 40 °C and 24 psi internal pressure with a 10 min run time. Separation was carried out isocratically with a mobile phase composed of methanol:water (70:30) with a flow rate of 1 mL min^{-1}. The detection wavelength was 235 nm. The concentration of H_2O_2 as well as electrolyte leakage index (ELI) was estimated according to the method as described by Homayoonzadeh et al. [15]. H_2O_2 content measurement is based on potassium iodide oxidation by H_2O_2 in acidic medium (trichloroacetic acid). The absorbance of the reaction mixture was measured at 390 nm. Assessment of ELI was performed using a platinum electrode, and then the percentages of initial to final conductivity were recorded.

2.4. Concentration of Amino Acids

Extraction of amino acids (AAs) was carried out based on the method of Fish [16] using sterile deionized water. After extraction, analysis was carried out by HPLC with orthophthalaldehyde precolumn derivatization, separation on a ProntoSIL 120-3-C18 H column (250 mm × 3 mm i.d.) (Knauer, Berlin, Germany) and monitoring with a fluorescence detector. The solutions were injected on to the column then separated and detected using the following conditions: 30 min run time, 0.6 mL min^{-1} flow rate, column temperature 30 °C, $\lambda_{excitation}$ at 330 nm, and $\lambda_{emission}$ at 450 nm. Separation was carried out using a linear gradient from solvent A (20 mM sodium acetate in water:acetonitrile, 97:3) to solvent B (20 mM sodium acetate in water:acetonitrile, 50:50).

2.5. Amount of Soluble Carbohydrates

Individual sugars including sucrose, glucose, and fructose were extracted based on the method of Meyer and Terry [17] with 62.5% methanol. Amounts of sucrose, glucose, and fructose were measured using an HPLC system. The samples were injected into a Eurokat H column of 300 mm × 8 mm i.d. (Knauer, Berlin, Germany). The mobile phase was sulfuric acid 0.01 N (100%) at a flow rate of 0.5 mL min^{-1}. The column temperature was held at 30 °C. Monitoring was performed over 40 min using a refractive index detector.

2.6. Titer of Mineral Elements

Elemental analysis was carried out following the method of Zafar et al. [18]. According to this method, samples were chemically digested using a mixture of nitric acid, sulfuric acid, and perchloric acid (5:1:0.5) and then analyzed for the elements of interest utilizing an atomic absorption spectrophotometer AA-680 with suitable hollow cathode lamps (Shimadzu, Kyoto, Japan). Contents of different elements were determined using calibration curves constructed using standard solutions of the elements calcium (Ca), manganese

(Mn), copper (Cu), zinc (Zn), iron (Fe), and magnesium (Mg). Nitrogen (N) content was determined by the Kjeldahl method [19]. Accordingly, chemical digestion (using sulfuric acid 1 N), fractional distillation, and titration (using hydrochloric acid 0.1 N) were performed to obtain the final concentration of ammonium.

2.7. Statistical Analysis

Experiments were consigned to a completely randomized design with three independent biological replicates. After the data passed Shapiro–Wilk's test for normality and Levene's test for equality of variances, an unpaired *t*-test was used to analyze the data. All analyses were carried out in GraphPad Prism version 8.2.0 (La Jolla, CA, USA).

3. Results

3.1. Enzymatic Parameters

Based on the results, the activity of antioxidant enzymes increased in response to spirotetramat treatment. Specific activities of SOD, CAT, and GPX were higher in treated plants compared to control counterparts, at 1.61-, 1.21-, and 1.33-fold, respectively. In addition, results for APX, GR, and PAL specific activities showed a similar increasing trend with 1.43-, 1.29-, 1.44-fold, respectively (Table 1).

Table 1. Mean (±SE) specific activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), and phenylalanine ammonia-lyase (PAL) (μmol min^{-1} mg^{-1} protein) in cucumber seedlings after exposure to recommended dose of spirotetramat (Movento®). Asterisks are used to show statistically significant differences between treated and non-treated plants (n = 3 replications per treatment; *t*-test analysis; $p < 0.05$).

Enzymes	Control	Treated	*t*-Value	*p*-Value
SOD	0.359 ± 0.026	0.578 ± 0.027 *	4.656	0.014
CAT	0.046 ± 0.002	0.056 ± 0.003 *	3.753	0.039
GPX	0.266 ± 0.018	0.354 ± 0.024 *	2.509	0.045
APX	0.359 ± 0.025	0.516 ± 0.041 *	2.107	0.032
GR	0.561 ± 0.021	0.724 ± 0.032 *	3.076	0.013
PAL	0.207 ± 0.003	0.299 ± 0.004 *	4.082	0.026

3.2. Plant Health Indices and SA

There were no significant changes in contents of plant health indices consisting of MDA, Chl, and H_2O_2 in response to treatment. In contrast, the SA amount in treated plants increased 1.37-fold (Table 2).

Table 2. Mean (±SE) contents of malondialdehyde (MDA), total chlorophyll (Chl), hydrogen peroxide (H_2O_2), salicylic acid (SA) (μg g^{-1} fresh weight), and electrolyte leakage index (ELI) (%) in cucumber seedlings after exposure to recommended dose of spirotetramat (Movento®). Asterisks are used to show statistically significant differences between treated and non-treated plants (n = 3 replications per treatment; *t*-test analysis; $p < 0.05$).

Parameters	Control	Treated	*t*-Value	*p*-Value
MDA	0.469 ± 0.026	0.458 ± 0.037	0.656	0.095
Chl	45.14 ± 5.021	49.56 ± 6.013	0.753	0.088
H_2O_2	9.376 ± 0.518	8.964 ± 0.424	0.819	0.076
SA	0.357 ± 0.029	0.491 ± 0.041 *	4.852	0.049
ELI	15.31 ± 0.413	14.78 ± 0.524	0.922	0.066

3.3. Soluble Carbohydrates

Further analyses on soluble sugar also showed accumulation of more sucrose (2.54-fold), glucose (2.51-fold), and fructose (2.59-fold) (Table 3) in treated plants.

Table 3. Mean (±SE) concentration of sucrose, glucose, and fructose (mg g^{-1} fresh weight) in cucumber seedlings after exposure to recommended dose of spirotetramat (Movento®). Asterisks are used to show statistically significant differences between treated and non-treated plants (n = 3 replications per treatment; *t*-test analysis; $p < 0.05$).

Parameters	Control	Treated	*t*-Value	*p*-Value
Sucrose	1.22 ± 0.261	3.11 ± 0.581 *	5.513	0.039
Glucose	0.91 ± 0.101	2.29 ± 0.011 *	4.413	0.024
Fructose	0.32 ± 0.008	0.83 ± 0.003 *	3.844	0.016

3.4. Mineral Elements

In addition, treated plants had higher contents of Ca (1.05-fold), Mn (1.63-fold), Cu (1.58-fold), Zn (1.71-fold), Fe (1.48-fold), Mg (1.05-fold), and N (1.19-fold) than the control (Table 4).

Table 4. Mean (±SE) contents of minerals (mg g^{-1} fresh weight) in cucumber seedlings after exposure to recommended dose of spirotetramat (Movento®). Asterisks are used to show statistically significant differences between treated and non-treated plants (n = 3 replications per treatment; *t*-test analysis; $p < 0.05$).

Parameters	Control	Treated	*t*-Value	*p*-Value
Calcium	23.73 ± 0.161	25.11 ± 0.181 *	5.623	0.045
Manganese	0.058 ± 0.015	0.095 ± 0.011 *	4.523	0.035
Copper	0.012 ± 0.008	0.019 ± 0.003 *	3.954	0.027
Zinc	0.007 ± 0.001	0.012 ± 0.002 *	2.921	0.017
Iron	0.138 ± 0.003	0.205 ± 0.005 *	5.489	0.048
Magnesium	13.97 ± 0.19	14.68 ± 0.11 *	4.952	0.031
Nitrogen	0.031 ± 0.002	0.037 ± 0.003 *	3.101	0.025

3.5. AAs

Measurement of AA concentration demonstrated that spirotetramat-treated plants had more arginine (2.47-fold), cysteine (2.23-fold), GABA (1.91-fold), glutamic acid (2.38-fold), glutamine (3.49-fold), glycine (2.47-fold), isoleucine (1.63-fold), lysine (3.06-fold), methionine (2.07-fold), ornithine (5-fold), phenylalanine (2.89-fold), tryptophan (2.57-fold), and tyrosine (2.2-fold) than the controls. However, concentrations of alanine, asparagine, aspartic acid, histidine, leucine, serine, threonine, and valine did not differ between treatment and control (Table 5).

Table 5. Mean (±SE) contents of amino acids (AAs) (mg g^{-1} fresh weight) in cucumber seedlings after exposure to recommended dose of spirotetramat (Movento®). Asterisks are used to show statistically significant differences between treated and non-treated plants (n = 3 replications per treatment; *t*-test analysis; $p < 0.05$).

AAs	Control	Treated	*t*-Value	*p*-Value
Alanine	15.91 ± 0.64	15.74 ± 0.68	0.946	0.870
Arginine	2.76 ± 0.44	6.82 ± 0.29 *	2.302	0.015
Asparagine	5.17 ± 0.45	5.66 ± 0.81	0.529	0.623
Aspartic acid	44.92 ± 2.77	43.92 ± 2.14	0.662	0.789
Cysteine	10.96 ± 0.30	24.41 ± 0.33 *	3.569	0.019
GABA	0.34 ± 0.02	0.65 ± 0.06 *	4.629	0.025
Glutamic acid	68.68 ± 4.85	163.6 ± 4.67 *	5.364	0.037

Table 5. *Cont.*

AAs	Control	Treated	*t*-Value	*p*-Value
Glutamine	19.20 ± 0.35	67.13 ± 4.81 *	2.159	0.047
Glycine	4.02 ± 0.26	9.94 ± 0.84 *	3.179	0.011
Histidine	6.09 ± 1.12	9.42 ± 3.13	0.715	0.373
Isoleucine	2.28 ± 0.07	3.73 ± 0.21 *	4.801	0.026
Leucine	1.36 ± 0.06	1.18 ± 0.05	0.805	0.101
Lysine	3.00 ± 0.14	9.18 ± 0.28 *	5.521	0.036
Methionine	1.35 ± 0.25	2.80 ± 0.34 *	4.582	0.026
Ornithine	0.05 ± 0.01	0.021 ± 0.03 *	2.360	0.019
Phenylalanine	2.21 ± 0.08	6.39 ± 0.24 *	3.892	0.0402
Serine	22.10 ± 0.36	21.94 ± 1.03	0.922	0.820
Threonine	14.07 ± 0.36	14.19 ± 0.52	0.452	0.866
Tryptophan	3.92 ± 0.96	10.11 ± 1.75 *	5.520	0.036
Tyrosine	2.65 ± 0.09	5.83 ± 0.02 *	4.852	0.015
Valine	2.38 ± 0.08	2.66 ± 0.07	0.850	0.752

4. Discussion

Treatment with spirotetramat, Movento® SC 100, induces physiological and biochemical responses in cucumber plants at 10 days after treatment and that is reflected in changes in the metabolism of antioxidant enzymes, salicylic acid, amino acids, mineral elements, and soluble carbohydrates.

The first line of defense against oxidative stress is the metalloenzyme SOD, which dismutates $O_2^{\bullet -}$ to O_2 and H_2O_2. CAT, a tetrameric heme-containing enzyme, catalyzes the dismutation of two molecules of H_2O_2 into H_2O and O_2. This enzyme is unique and does not require a reducing equivalent. It also has a considerably fast turnover rate in peroxisomes [20]. APX is a central component of the ascorbate-glutathione cycle with a higher affinity for H_2O_2 as compared to CAT. It is responsible for the degradation of H_2O_2 in the cytosol and chloroplasts and uses two molecules of ascorbic acid as reductants. GPX, a heme-containing enzyme, removes H_2O_2 by oxidizing aromatic electron donors, such as guaiacol [21]. GR is also one of the main enzymes in the ascorbate-glutathione cycle, which sustains the reduced status of glutathione. It also plays a vital role in the maintenance of sulfhydryl groups, as a substrate for glutathione *S*-transferase [22].

The activity of SOD, CAT, APX, GPX, and GR (Table 1) was elevated in spirotetramat-treated seedlings. As with our findings, Homayoonzadeh et al. [15] demonstrated that application of insecticides imidacloprid and dichlorvos at the recommended rate in cucumber plants enhanced SOD-, CAT-, APX-, and GPX-specific activities. Shakir et al. [23] reported enhanced activities of SOD, CAT, APX, GPX, and GR in tomato shoots subjected to different levels of the insecticides emamectin, cypermethrin, and imidacloprid. According to Homayoonzadeh et al. [13], the application of imidacloprid and phosalone as two different insecticides at recommended doses enhanced levels of SOD, CAT, APX, GPX, and GR in *Pistachio vera* seedlings. Parween et al. [24] also showed that GR activity was stimulated in *Vigna radiata* when exposed to chlorpyrifos, as an organophosphorus insecticide. It was also suggested that the activity of SOD was stimulated by insecticide-derived $O_2^{\bullet -}$ to protect plants from the toxicity of insecticides [24]. Additionally, CAT, GPX, and APX have protective roles in plants exposed to insecticide toxicity due to their pronounced ability to detoxify H_2O_2 [23].

PAL is the key enzyme in the plant secondary metabolism, which catalyzes the first step in the phenylpropanoid pathway leading to the synthesis of the phenolic compounds [25]. In the present investigation, spirotetramat increased the activity of PAL (Table 1) in cucumber plants. In agreement with our findings, Homayoonzadeh et al. [15] demonstrated that the application of insecticides imidacloprid and dichlorvos at the recommended rate in cucumber plants elevated the activity of PAL. Meanwhile, earlier studies indicated an increase in the levels of PAL activity in pistachio seedlings exposed to imidacloprid and phosalone insecticides [13].

Soluble carbohydrates contribute significantly to plant responses to stresses and therefore are commonly recognized as giving rise to the concept of sweet immunity due to their crucial functions in the plant defense system. In addition, they act as signaling molecules and a ROS scavengers at low and high concentrations, respectively [26]. Cucumber plants showed an increase in soluble carbohydrate amounts in response to spirotetramat treatment (Table 3). The findings of our study corroborate the findings of Homayoonzadeh et al. [13] who demonstrated that the application of insecticides imidacloprid and phosalone increased the content of soluble carbohydrates in the pistachio seedlings. In addition, Homayoonzadeh et al. [15] reported an enhanced level of soluble carbohydrate in cucumber plants in response to insecticides imidacloprid and dichlorvos at the recommended dose.

Essential elements are required by plants to ensure normal growth, development, and maintenance. In this study, the contents of Ca, Mn, Cu, Zn, Fe, Mg, and N were elevated upon exposure to spirotetramat (Table 4). Ca is essential for strengthening cell walls and plays a fundamental role in membrane stability [27] as well as functioning as a secondary messenger when plants are exposed to (a)biotic stresses [28]. Mg plays a vital role in the photosynthesis process in several ways; however, its main role is to provide a metal ion in the porphyrin ring of Chl [29]. Mn plays a primary role as a cofactor in several enzyme reactions as well as participating in plant defense systems against oxidative stress by scavenging ROS [30]. Cu has various fundamental roles in the plant immune system, including ROS metabolism, cell wall remodeling, and as an enzyme cofactor [31]. Zn is involved in a wide variety of plant metabolism processes, including as an enzyme cofactor [32] and protein synthesis process [33]. Fe acts as a cofactor in many enzymatic reactions [34] as well as being a constituent of the heme portion of antioxidant enzymes [35]. The primary function of N is to provide amino groups in AAs, which also increased upon treatment, and its availability has a direct effect on protein amount [36]. The toxic effects of pesticides can be mitigated by nutrients [37]. The rise observed in elements may result from increased uptake and transport of nutrients from the environment through manipulation of rhizosphere chemical properties [38].

AAs are building blocks for proteins involved in enzyme activity and redox homeostasis [39]. They can also be modified in plants exposed to stress to produce specific secondary metabolites [40]. Cucumber seedlings exposed to spirotetramat showed an increase in the amount of some AAs (Table 5). AAs play a central role in the detoxification of xenobiotics in plants by conjugation reactions. Conjugation reaction results in a conjugate with a higher molecular weight that is more water-soluble and is usually more susceptible to further removing processes in the plants [41].

SA, a small phenolic compound, makes a substantial contribution in the multiple physiological processes and activation of the plant defense system against (a)biotic stress, which in turn could cause systemic resistance occurrence [42]. The metabolism of signaling molecules, such as SA, can also be regulated by xenobiotics [43]. In the present study, SA level increased in cucumber seedlings exposed to spirotetramat (Table 2). According to Szczepaniec et al. [44], the application of insecticide imidacloprid on tomato seedlings as well as the insecticide thiamethoxam on cotton plants results in increased levels of SA. Ford et al. [45] also observed that the content of SA increased in *Arabidopsis thaliana* in response to the application of the insecticide clothianidin.

H_2O_2 is a signaling molecule with high diffusibility and a relatively long life. Its concentration must be maintained in a delicate balance between H_2O_2 production and scavenging systems [46]. MDA, the byproduct of peroxidation of polyunsaturated fatty acids in phospholipids, is recognized as the biochemical marker of toxic lipid peroxidation [47]. Photosynthetic pigments are indicators of photosynthesis efficiency [48] and ELI is used as a membrane damage index because of the phase transition of membrane lipids [49]. Thus, detecting H_2O_2, MDA, Chl, and ELI can be helpful as plant health indices in comparison to the amount of damage created by (a)biotic stresses.

The contents of H_2O_2, MDA, Chl, and ELI in cucumber seedlings subjected to spirotetramat did not significantly change with respect to controls (Table 2). The lack of an MDA

and ELI response may be related to inhibition of lipid peroxidation by elevated levels of SA [42]. The absence of an effect on photosynthetic pigments may be accounted for by increases in sucrose content as this can suppress Chl biosynthesis by inhibition of the activity of the Chl biosynthetic enzymes [50]. Biosynthetic pathways of photosynthetic pigments can be affected by SA [51]. In addition, non-changeability in H_2O_2 concentration may be related to the induction of H_2O_2 scavenger enzymes like CAT, APX, and GPX. Consequently, we propose that H_2O_2 acts as a signaling molecule in response to treatment with spirotetramat. Indicative parameters of health such as photosynthetic pigments, MDA, and ELI were not affected by spirotetramat, suggesting that changes to H_2O_2 levels were inducing the host plant antioxidant system and were not an indication of a toxic effect.

Observed biochemical changes of cucumber seedlings in response to spirotetramat with probable molecular mechanisms were prepared entitled a metabolic pathways network (Figure S1). In this figure, correlation among all of the parameters was illustrated, which leads to activation of possible physiological cycles.

The effect of spirotetramat, Movento® SC 100, on the cucumber plant, a non-target organism, is complex. Treatment with Movento® shows the interaction of the product components with biochemical and physiological pathways of cucumber plants as a side effect. Consequently, this interaction leads to a complex network of metabolic changes. Many biochemical pathways are activated in cucumber plants in response to spirotetramat, which may play crucial roles in plant defense against herbivore pests. The information gained from this study may be useful for developing new pest management programs. In addition, the observed responses may be directly attributed to spirotetramat; however, it cannot be ruled out that other components, including adjuvants and auxiliary compounds present in the formulation, also had an effect on the plant biochemistry.

In conclusion, this study focused on observed physiological changes in plants exposed to hazardous substances present in the environment, such as insecticides. The results of this study can add to our understanding of the underlying consequences associated with the toxic state of xenobiotics on plants. There were metabolic changes in cucumber plants in response to spirotetramat application.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/IECPS2021-11921/s1, Figure S1: The metabolic pathway of physiological changes of cucumber seedlings in response to spirotetramat is demonstrated. The supposable pathways are shown in the dotted arrow, while the line arrow is used for doubtless pathways. The bold font is utilized to represent the assayed metabolites and enzymes.

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