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BIOCHEMICAL APPROACH TO THE ACTION OF PESTICIDES ON *CUCUMIS SATIVUS* IN VARIOUS STAGES OF DEVELOPMENT

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Abstract

The use of pesticides has become a major environmental problem against the background of the intensification of agriculture. Thus, this economic sector of major importance worldwide has become a source of soil and plant pollution. Recent specialized studies show that pesticides can easily reach cultivated plants. In this situation, pesticides can have direct negative effects, accumulating in their tissues and acting enzymatically.
In this context, the aim of this study was to evaluate by spectrophotometric techniques the enzymatic changes that occur in *Cucumis Sativus*, as a result of its treatment with Dithane M45, Topsin 70 WDG and Mospilan 20 SG.
The monitoring of biochemical characteristics of the analyzed samples showed that, after pesticides were added, all the physico-chemical and biochemical characteristics of the *Cucumis Sativus* changed. Thus, the peroxidase activity monitoring showed that it varied from 75.1±0.18 enzymatic units after the first determination (seedling phase of plants) to 81.7±0.84 enzymatic units after three weeks from planting. Finally, the peroxidase activity was 75±0.42 enzymatic units to the maturity phase of the plants. Also, the results indicated that the activity of polyphenoxidases increases throughout this study (to 35.9±0.74 enzymatic units) because the antioxidant defense mechanism of plant cells has been activated. In all the analysed situations, research indicated notable differences from the behaviour of the control plants.
This study should be followed by future research on the response of the soil microbial community through their enzymatic system to the cumulative action of pesticides.

Keywords: insecticides, fungicides, peroxidase, polyphenoloxidases, plant pollution.

1 INTRODUCTION

Due to the intensification of agriculture, one of the largest economic sectors in the world, as well as the need to increase productivity, the use of pesticides has become excessive, being specially designed to control plant diseases. In this context, the use of pesticides can be considered one of the main sources of soil and plant pollution. Ecologically, the use of persistent pesticides leads to higher toxicity [1]. In order to achieve more sustainable agricultural systems and to reduce the risks associated with the application of pesticides in agriculture, it seems that pesticide taxes are playing an increasingly important role in agriculture [2].

This dependence on the use of pesticides is the result of various causes. Of these, the control of pests and harmful organisms in the soil, the elimination of weeds, as well as the optimization of plant growth are of major importance. On the other hand, pesticides move to the environment, where they adversely affect soil biota, water and the atmosphere of ecosystems [3, 4].

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Moreover, due to a high degree of persistence and toxicity, pesticides have the ability to bioaccumulate and have a high risk of creating negative effects on plants, animals and the human body. Exposure of the population to the action of toxic substances from the misuse of pesticides can have serious consequences for human health [5]. World Health Organization (WHO) estimates that there are countless acute and chronic effects of pesticides every year worldwide [6]. Why is this happening? Because pesticides are mostly chemicals obtained by synthesis, and there is an extremely large number of types of pesticides on the market [7], respectively fungicides, insecticides, herbicides, defoliants, pheromones, etc. Because, although it cannot be generalized, according to the research carried out in the field [8] pesticides are mishandled by local farmers during their use.

Although their use leads to increased agricultural production, when pesticides are mishandled or mismanaged, they can have a negative impact on the environment and humans. Mahapatra et al. [9] showed that the impact of these pesticides, accumulated in excess on plants, results in damage to the physiological structure of plants thus causing phytotoxicity. Bragança et al. [10] highlighted the effect that certain pesticides have on the early development of cucumber plants, emphasizing the importance of parameters such as germination and seedling development, to know the phytotoxicity of pesticides and their metabolite [11].

It should be noted, however, that recent research [12] has shown that certain compounds, such as salicylic acid, may reduce the accumulation of pesticides in plants.

At the same time, Huang et al. [13] demonstrated, through robust quantitative analysis, the antioxidant response of plants to pesticide exposure, exemplified by cucumbers. These plants have recently been studied in detail [14] in terms of important traits, such as genetic resources, sexual expression, disease resistance, quality improvement, etc.

Although all these studies have shown the negative effects of pesticides in various situations, information on the inappropriate use of pesticides by farmers has been limited. The current study focuses on the response of plants to oxidative stress induced by the presence of misused pesticides by farmers, in terms of the concentrations used and the time between two consecutive sprays of cucumber plants.

In this context, here we have investigated the phytotoxic effects in the *Cucumis Sativus* plant of two commonly used fungicides (Topsin 79 WDG and Dithane M45) and an insecticide (Mospilan 20 SG), selected as the representatives.

2 METHODOLOGY

Certified pesticides of Dithane M45 and Topsin 79 WDG (two commonly used fungicides) and Mospilan 20 SG (insecticide) were purchased from a profile store. Pesticides have been used to spray cucumber plants as a mixture. Thus, the solution obtained from pesticides was made according to the recommendations of some local vegetable growers. 20g Topsin 79 WDG, 15g Mospilan 20 SG and 20g Dithane M45 were used for this purpose. These quantities were mixed in a container with 10L of water and then transferred to 2L container. The contents of each container for spraying were used. In addition, according to the same recommendations, the plants were sprayed at intervals of 7 days. Thus, the time between sprays in the case of the Dithane M45 and Topsin 79 WDG has been reduced. Usual laboratory utensils and reagents, electronic balance, centrifuge, 35kHz ultrasonic bath, microplate spectrophotometer, Tecane Infinite, UV-Vis Double Beam PC 8 Auto Scanning cell UVD-3200, Lobomed, INC spectrophotometer were used.

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Fresh seeds of *Cucumis Sativus* were purchased from the local supermarket. Also, the bio seeds were purchased from a vegetable grower (from cucumber plants untreated with pesticides).

Cucumber seedlings were grown for 86 days. The germination time was ten days. After germination, the seedlings were transferred. The cucumber plants were grown under conditions, similar to those used by local farmers under controlled conditions (20–30 °C during the day and 15–19 °C at night). The samples were collected after plant transfer.

Reagents: Pyrogalol, Pirocatechină from Fluka/Sigma–Aldrich Switzerland, deionized water (GFL Hanptschalter, Main Switch).

Peroxidase (POD) and polyphenoxidases (PPO) activity assays were performed following the methods described earlier [15] with some modification. To obtain the enzyme extract 0.5g of leaf tissue was cold homogenized in 2.5 mL 0.1 M Tris-HCl tampon (pH 7.2) and 2.5 mL EDTA (0.1 mM). The homogenates were ultrasonicated for 10 min. After that, the homogenates were centrifuged at 5000g (4 °C) for 15 min. The obtained supernatants for enzyme determinations were used. The POD activity was assayed using the rate of increase in absorbance (measured at 430 nm). In the case of PPO, the absorbance was measured at 495 nm.

Statistical analysis was performed using the SPSS 11.0 software. All statistical analyses were statistically significant at the 0.05 level [16].

3 RESULTS

Experimental data demonstrate that the response of plants to oxidative stress and the generation of reactive oxygen species is rapid. Plants achieve this by rapidly stimulating the enzymatic and non-enzymatic antioxidant defense components [9].

3.1 Evaluation of peroxidase activity

The action of pesticides on *Cucumis Sativus* was highlighted, first of all, by their effects on peroxidase activity (POD, EC 1.11.1.7) in the seedling phase of plants. Thus, figure 1 shows the enzymatic response of plants to the joint action of some fungicides and an insecticide, in the case of three types of plants in the seedling phase (Fig. 1).

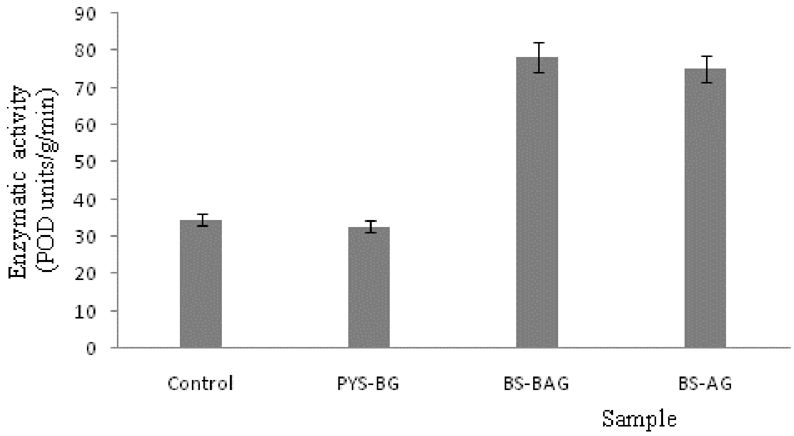

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Figure 1. POD activity in the seedling phase of the plants. Abbreviations: Control - untreated plants; PYS - BG - plants from untreated cucumber seeds grown in the previous year and treated in this study before germination; BS-BAG - plants from cucumber seeds - BIO treated before and after germination; BS - AG - cucumber seed plants - BIO treated after germination. Vertical bars represent the standard deviation of the mean (n = 3).

The data obtained showed a significant increase in peroxidase activity in two of the three plants compared to the plant used as a control. Thus, in the case of plants obtained from cucumber seeds considered BIO and which were treated with pesticides before and after germination (BS-BAG) the enzymatic activity increased by 126%.

At the same time, in the case of plants from seeds treated only after germination (BS - AG) the increase was about 120%. These results show that, at this stage of growth, the plants activated their cell-level defence mechanism against the oxidative stress induced by the presence of pesticides.

On the other hand, in the case of plants from cucumber seeds not treated with pesticides and grown in the previous year and which in the present study were treated with pesticides before germination (PYS - BG) the enzymatic activity was inhibited by 5.7%. This indicates that, at this stage of development, these plants have failed to develop at the cellular level an optimal antioxidant mechanism of defense against the aggressive effect of pesticides.

Also, as it can be seen from the analysis of data shown in Fig. 2 the peroxidase activity increased in the second stage of development of *Cucumis Sativus* plants, respectively three weeks after planting in the case of the same two types of plants.

The graphical data show an increase of approximately 133% (BS-BAG) and 94.5% (BS-AG) compared to the control and seedling phases respectively.

After three weeks from planting, the POD activity continues to decrease (9.5%) compared to the control in the case of PYS - BG samples.

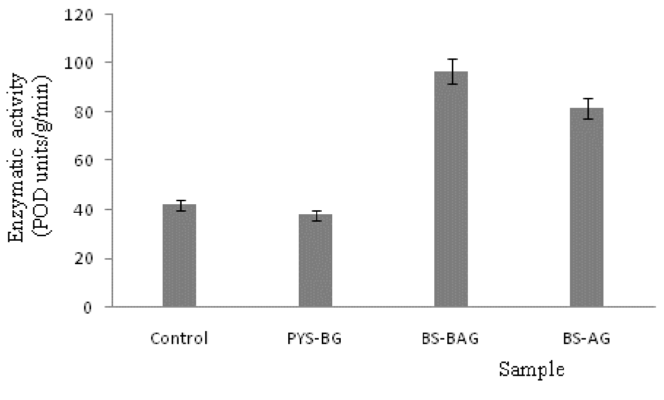

Figure 2. POD activity three weeks after planting. The abbreviations: as in figure 1.

Fig. 3 shows the peroxidase activity in the third phase of development (maturity phase) when the plant has flowered. In this case, it is found that the POD activity decreases compared to the situation in the second phase of plant development. There is the same variation in POD activity as in previous stages of plant development.

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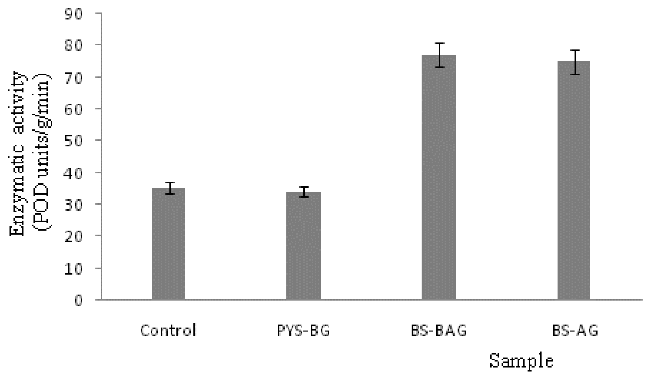

Figure 3. POD activity in the maturity phase.

However, the decrease in POD activity (3.1%) in the PYS - BG tests is much smaller than in the previous phase, which shows that, even in this case, although much later, transformations occur that lead to the activation of the defense mechanism. of cucumber plants to the action of pesticides. The same behaviour is observed (Fig. 4) in this case by reference to the control samples from the seedling phase.

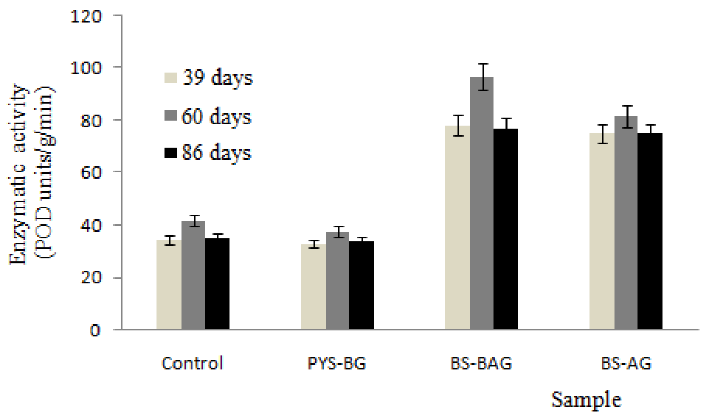

Figure 4. Time variation of peroxidase activity (39 days - germination time and the emergence of seedlings; 60 days – the second phase of development of cucumber plants, 89 days – the third phase of development of cucumber plants).

Our results show that, after longer exposure of plants to pesticides, after 86 days of pesticide treatment, the activity of POD shows an increase compared to the initial control samples in all the samples studied.

However, referring to each stage of development, the response of plants to the action of pesticides is found in an initial increase in POD activity with the stress factor followed by a slight decrease, probably after the optimal activation of the antioxidant mechanism.

It has also been proved that increased POD activity after pesticide administration is followed by decreased enzyme activity several days after pesticide treatment and other plant varieties, such as hot pepper plants [17].

Our results are evidence of the behaviour of cucumber plants to the action of some pesticides by their adaptive response given by activating the activity of POD, but also other antioxidant enzymes, thus stimulating and increasing resistance to stress induced by

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pesticides, which is correlated to an increased level of total anthocyanin and free proline [9, 12].

3.2 Evaluation of polyphenoloxidase activity

The results obtained regarding the level of polyphenoxidase activity (PPO, EC 1.14.18.1) proved to be important for the evaluation of the impact of pesticides on *Cucumis Sativus* in different stages of development.

The study of PPO activity in the seedling phase of the plants showed that the maximum enzyme activity was shown by BS-BAG (Fig. 5). This behaviour is similar to that obtained in the case of POD. However, in the case of plants from seeds treated after germination (BS - AG) the increase in PPO activity was less significant (approximately 7.75%) compared to the control.

Moreover, in the case of the PYS-BG sample, the enzymatic activity decreased by 19.98%. The possible cause of this behaviour of the plants can be attributed to the conditions in which the seeds were dried and the way in which they were stored.

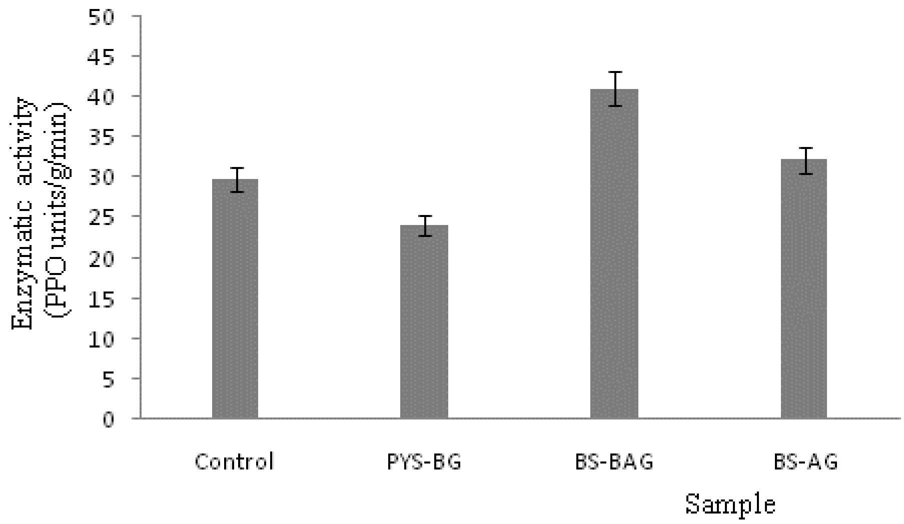

Figure 5. PPO activity in the seedling phase of the plants. The abbreviations: as in figure 1.

Also, the results obtained on the PPO activity showed that three weeks after planting, the plants behave similarly to the action of pesticides. In this context, the enzymatic inactivation of PYS-BG plants is noted (Fig. 6).

The study shows a decrease in PPO activity by 19.33% at this stage of development. This represents a smaller decrease (19.98%) compared to the control than in the first phase of development (Fig. 5). This response of plants due to the presence of pesticides is the activating result of the defense mechanism against oxidative stress.

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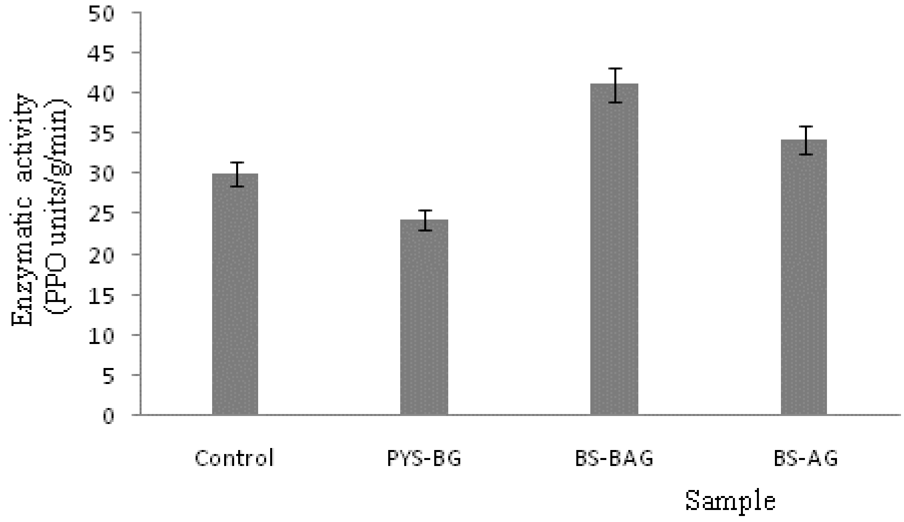

Figure 6. PPO activity three weeks after planting.

In what the enzymatic response of plants in the mature phase is concerned, the activity of polyphenoloxidases increased in the case of BS-BAG and BS-AG samples to approximately 42.1, respectively 35.9 units / g / min compared to the control (Fig. 7).

The results show that at this stage of plant development the activity of polyphenoloxidases had a slight increase compared to the second phase of development. Moreover, in the case of the BS-BAG sample, the maximum concentration of PPO in plants tended to stabilize after 86 days from the start of this study (Fig. 8).

On the other hand, as shown in Fig. 7, the activity of polyphenoloxidase in PYS-BG due to the inactivation of the enzyme by pesticides is significantly reduced.

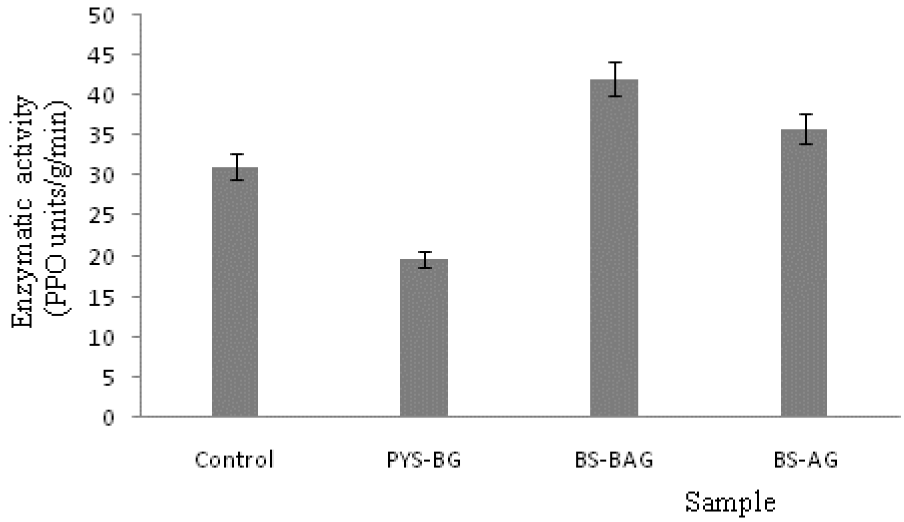

Figure 7. PPO activity in the maturity phase.

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The data presented in Fig. 8 show the effect of the pesticide treatment on the PPO activity of *Cucumis Sativus* until the maturity stage. It is found that, in terms of control plants, they did not undergo significant changes between the development phases, a fact also reported in the case of BS-BAG plants.

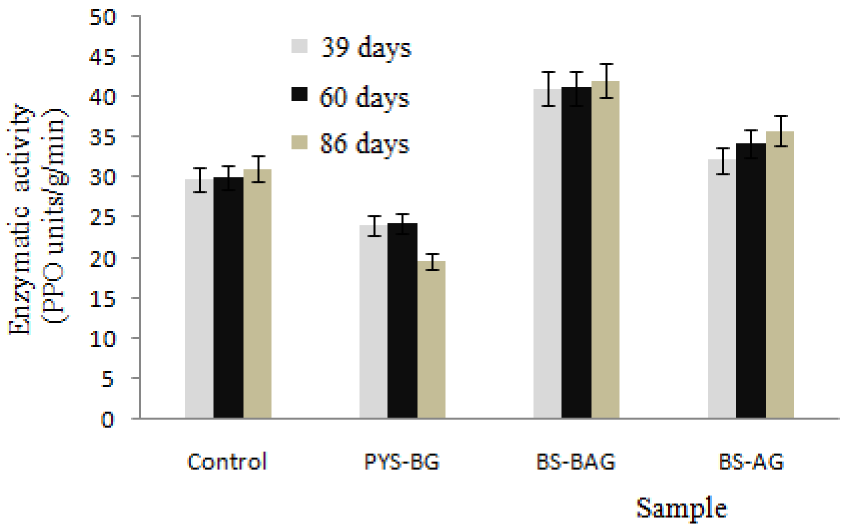

Figure 8. Time variation of polyphenoloxidase activity.

Based on the fact that the activity of PPO is considered an important activity of the metabolism of plants to defend against oxidative stress [11], the results of this study showed different responses of *Cucumis Sativus* to the action of pesticides. This is mainly the case of the PYS-BG plants, which showed a decrease in PPO levels even in the mature stage, which did not happen with the other plants. This behaviour indicates that polyphenoloxidases, similar to peroxidases, are directly influenced by the stress induced by excess and the improper use of pesticides in the treatment of *Cucumis Sativus*.

3.3 Spectrophotometric evaluation of *Cucumis Sativus* leaf extracts

Based on the impact of pesticides on the leaves, the absorption spectra in the visible range of the extracts obtained from the leaves of the plants reached the seedling stage were determined. Fig. 9 shows the high degree of absorption in the case of plants treated with pesticides before and after germination. This can be caused by pesticides that have been assimilated by the plant and therefore by their impact on the enzymatic system.

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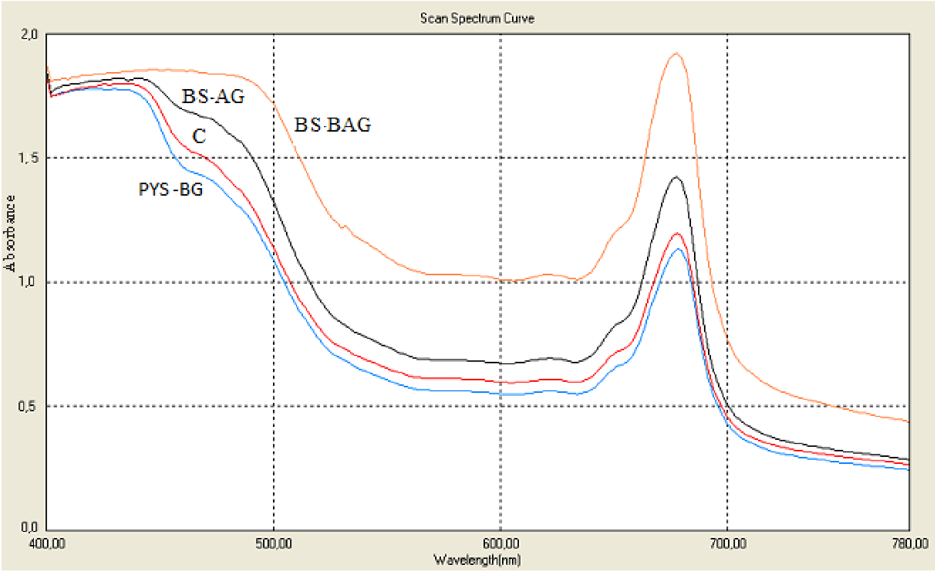

Figure 9. Vis absorption spectrum for seedlings.

In the case of extracts corresponding to PYS-BG plants, a decrease in the absorption degree is observed, which indicates a direct correlation with the variation of POD and PPO. This indicates the negative effect of pesticides on the metabolism of the plants analysed by affecting the photosynthesis process.

3.4 Pearson linear correlation coefficients

Statistical analysis, respectively Pearson linear correlation coefficients were estimated to show the relationship between quantitative parameters of the enzymatic activity [16, 18].

The correlation coefficients between the values of the biochemical parameters analysed in the case of cucumber plants treated with pesticides (Table 1) showed that there are significant correlations between them in the case of the analysed samples. However, the results show some insignificant correlations between the biochemical parameters in the case of control samples that were not subjected to pesticide treatment. This shows that the enzymatic response of plants is obtained according to the stage of their development.

Table 1. Pearson linear correlation coefficients between the values of POD and PPO in the case of the analysed sample. Marked correlations are significant at $p < 0.05$.

	POD-Control	POD-PYS-BG	POD-BS-BAG	POD-BS-AG	PPO-Control	PPO-PYS-BG	PPO-BS-BAG	PPO-BS-AG
POD-Control	1.000							
POD-PYS-BG	*0.986*	1.000						
POD-	*0.990*	*0.953*	1.000					

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BS-BAG								
POD-BS-AG	*0.995*	*0.964*	*0.999*	1.000				
PPO-Control	-0.269	-0.105	-0.401	-0.363	1.000			
PPO-PYS-BG	0.458	0.304	*0.578*	*0.544*	-0.979	1.000		
PPO-BS-BAG	-0.374	-0.214	-0.499	-0.464	*0.994*	*-0.996*	1.000	
PPO-BS-AG	0.156	0.318	0.017	0.058	*0.909*	*-0.806*	*0.858*	1.000

These results concerning the effect of studied pesticides on antioxidant enzymes activity, such as POD and PPO, from the leaves of cucumber plants showed usually a significant impact compared to the plants used as control.

The data in Table 1 show that the study of enzymatic activity by using advanced methods of statistical analysis can be effective and allows the optimal evaluation of the response of cucumber plants to the action of pesticides on their metabolism.

4 CONCLUSIONS

The analysed samples monitoring showed that, after the addition of pesticides, all the physicochemical and biochemical characteristics of *Cucumis Sativus* were modified. In all the analysed situations, the research indicated notable differences compared to the behaviour of the control plants. The excess of pesticides, as well as the decrease of the pause period between their use, is a real factor of oxidative stress on cucumber plants. The negative impact of pesticides on enzymatic activity proved to be the strongest in the case of the use of seeds obtained from cultivated cucumbers, in bio conditions, in the previous year of this study. Thus, in the context of mismanagement of pesticides on *Cucumis Sativus* by local farmers, we believe that there may be a risky exposure of the population to substances with real toxic potential.

This study should be followed by research on the response of the soil microbial community through their enzymatic system to the cumulative action of the pesticides and, of course, the development of strong strategies.

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