

## Protective effects induced by growth regulators treatment on eggplant (*Solanum melongena* L.) seedlings

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### Abstract

High temperature is now considered to be one of the major abiotic stresses inducing negative effects on plant growth as results of increased production of toxic reactive oxygen species. Higher stress tolerance is correlated with higher activities of antioxidant defense enzymes which are activated to prevent oxidative damage. In the meantime, treatment with growth regulators appears to be involved in induction of tolerance to various abiotic stresses, besides their important role in regulating plant growth, development and fruiting. Influence of environmental conditions and of some growth regulators treatments (Razormin 0.1%, Spraygard 1%, BAC Foliar spray 0.3% and BIO Roots 0.2%) on the some biometric and biochemical parameters has been investigated in leaves of eggplant (*Solanum melongena* L.) seedlings. The researches performed led to conclusion that growth regulators treatment could attenuate the negative impact of heat stress on plant growth by protecting the photosynthetic apparatus and enhancing antioxidant enzyme systems.

**Key words:** antioxidants, heat, oxidases, stress, *Solanum melongena*

### 1. Introduction

Eggplant (*Solanum melongena* L.) is one of the most important vegetable species grown for its edible fruit. Originates from India, eggplant is now generally grown as a vegetable all over the world. Also in our country eggplant is an appreciated vegetable among consumers, therefore it has been cultivated on about 10,000 ha. The most representative part of the eggplant cultivation is performed as field crops, in summer-autumn crop system using seedlings. Eggplant seedlings are produced in protected areas from April to June. Most often heat occurs this time of year, so that high values of maximum and minimum temperature are recorded during days and nights, exceeding those required for optimal growth of vegetable seedlings prepared for summer-autumn crop establishment. It is already known that stressors caused a morphological and physiological response from eggplant seedlings. Warm climate plant species like eggplant, grown in the temperate climate zone using transplants, are subjected to environmental stress which limits seedlings growth, crop productivity and quality (SEKARA A. & al. [1]). Temperature stress induces negative effects on plant growth and metabolism, so high temperature is now considered to be one of the major abiotic stresses for yield reduction in crops (HASANUZZAMAN & al. [2]). High temperature stress is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development (WAHID [3]).

A common consequence of the biotic and abiotic stress is the increased production of toxic compounds, especially reactive oxygen species produced as result of oxidative metabolism in chloroplasts, mitochondria and peroxisomes. In response to high temperature, the reaction catalyzed by ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) can lead to the production of H<sub>2</sub>O<sub>2</sub> as a consequence of increases in its oxygenase reactions (KIM & al. [4]). Successive reduction of molecular oxygen to H<sub>2</sub>O produces the intermediates O<sub>2</sub><sup>•-</sup>, HO<sup>•</sup> and H<sub>2</sub>O<sub>2</sub>, potentially toxic in living cells. Accumulation of reactive oxygen species in cells may lead to some unspecific oxidation of

proteins and membrane lipids and may damage DNA, protein, chlorophyll and membrane function, the final results being an oxidative stress (ZAWOZNIK & al. [5]; MITTLER [6]; YIN & al. [7]).

Plants exposed to extreme temperatures activated the self-defense mechanisms including several non-enzymatic and enzymatic antioxidants to convert the reactive species in harmless constituents (VRANOVA & al. [8]; TORRES-BARCELÓ & al. [9]). Antioxidants ( $\alpha$ -tocopherol,  $\beta$ -carotene, glutathione, ascorbic acid) and related enzymes such as catalase, peroxidase, polyphenol peroxidase, superoxide dismutase are activated to prevent oxidative damage so that higher activities of antioxidant defense enzymes are correlated with higher stress tolerance (ALMESELMANI & al. [10]; BABU & al. [11]; ALMESELMANI & al. [12]).

The highly toxic  $H_2O_2$ , widespread molecule in many biological systems, is scavenged by catalase (EC1.11.1.6) and peroxidase (EC1.11.1.7).

Catalase is biosynthesized in living cells as required by various oxidation reactions and it can act as a signaling molecule that regulates plant development, stress adaptation and programmed cell death (APEL & al. [13]).

Peroxidases, found in almost all vegetables, consist of a family of haem-containing enzymes that degrades  $H_2O_2$  through the oxidation of co – substrates such as phenolic compounds and/or antioxidants, thus eliminating the harmful effects of  $H_2O_2$  in plants (ASADA [14]). The role of peroxidases played in the vegetables is not completely understood, although it was correlated with photosynthetic activity in plant (KRAVIĆ & al. [15]; GONCHARENKO & al. [16]) and also it was associated with major physiological processes as cell wall biosynthesis, response to injury, disease resistance and wound repair (PRESTAMO & al. [17]; MOORE & al. [18]; ALMAGRO & al. [19]).

In recent years, the researchers' interest is turning to the growth regulators. Besides their important role in improvement of nutritional quality of food crops and their efficiency to regulate plant growth, development, fruiting and senescence (EL-ROKIEK & al. [20]), it seems to be involved in induction of tolerance to various abiotic stresses (HORVA'TH & al. [21]; SALEHI & al. [22]).

Some treatments of vegetables with plant growth regulators showed positive effects on oxidases activities. For example, methyl jasmonate-treated raspberries, strawberries and blueberries showed higher activities of peroxidase and superoxide dismutase (CHANJIRAKUL & al. [23]). Also the use of N, P, K fertilizers has a positive effect by increasing the content of chlorophyll and soluble protein, enhanced photosynthetic rate and the activities of superoxide dismutase, peroxidase and catalase (ZHOU & al. [24]; GONCHARENKO & al. [16]).

The research reported in this study used different growth regulators (Razormin, Spraygard, BAC Foliar spray, Bio Roots) as foliar treatments on eggplant seedlings and the comparative results were studied.

The improvement of temperature stress tolerance is often related to increased activities of enzymes involved in antioxidant systems of plants (HASANUZZAMAN & al. [25]). The effect of the bioregulators on antioxidant enzymes and photosynthetic pigments in eggplants plant is less known, so that the influence of environmental conditions and of some growth regulators treatments on the biometric (plants height, root volume) and biochemical parameters (chlorophylls and carotenoids content, activity of peroxidases and catalase) has been investigated in leaves of eggplant seedlings.

## 2. Materials and methods

A monofactorial experiment with six variants was established, considering application of simple growth regulators Razormin 0.1%, Spraygard 1%, BAC Foliar spray 0.3% and BIO Roots 0.2% on heat stressed and unstressed eggplant seedlings (*Pana Corbului 36*) in two distinct stages: one week, respectively three weeks after transplant operation.

*Razormin* is a mixture of growth factors (amino acids, polysaccharides, macro and micro-nutrients), which induces development of the root system, stimulate the nutrients absorption, increases vegetative mass and finally an increase in the quantity and quality of production.

*Spraygard* is a complex product that acts as safener, penetrant, dispersant, creates adhesion of the treatment solutions on the leaves. *Spraygard* adjuvant has a unique formula in a single coating based on the synthetic resin that is "environmentally friendly" and the polymer di-1-p-menthene and ethoxylated alcohol by applying it on the plant and on its leaves forms a pellicle that persists 2 days up to 2 weeks, having as a side effect the reduction of perspiration, therefore a better water management within the plant. This fact causes the physiological chain reactions whose results are being expressed by increasing the plant resistance to stress factors such as the drought and the cold. The effect of reducing perspiration recommends the product application strictly on the leaves.

*BAC Foliar* is a foliar organic nutrient which stimulates chlorophyll production in the leaves.

*Bio Roots* is a natural root growth supplement which contains vitamins, enzymes, organic and humic acids that helps plants establish healthy and vibrant roots.

**Experimental variants** were noted:

C – unstressed control;

V1 – eggplant seedlings exposed at heat stress in absence of growth regulators treatment;

V2 – eggplant seedlings exposed at heat stress and treated with *Spraygard* 1%;

V3 – eggplant seedlings exposed at heat stress and treated with *Razormin* 0.1%;

V4 – eggplant seedlings exposed at heat stress and treated with *BAC Foliar* spray 0.3%;

V5 – eggplant seedlings exposed at heat stress and treated with *BIO Roots* 0.2%.

The experiment was installed into an experimental greenhouse of the Hortinvest Research Centre–USAMV Bucharest. It was used the block method in linear alignment with 4 repetitions. The total number of plants in the experiment was 360, each variant containing 60 plants, with 15 plants per repetition.

Sowing was made into an sowing case on April, 9. Because heat and water were optimal provided, mass emergence of seedlings occurred after 8 days. The transplant operation was done at April, 22 in two phases: I–in alveolar blades (of 100 ml volume) for two weeks; II–in plastic pots of 300 ml volume in order to protect plant roots against water or nutritional stress. For cultivation was used a professional-type substrate *Kekkila BP peat* characterized by a high content of mineral nitrogen and phosphorous and optimum content in potassium (the N:P:K ratio was 1:0.6:0.7); the pH value was situated at the inferior limits of the optimum interval. The content in total forms (%) of the N (2.17%), P (0.271%), K (0.18%) can be considered as normal.

Specific agrotechnics for transplant nursery was applied during the growth period: daily ventilation, watering, weeding weeds. The unstressed control variant was maintained at the temperature of 22–26 °C at day and 18–20 °C at night. For the heat stressed variants, the temperature was not controlled and recorded the following variations:

- the maximum effective temperature average for April in the greenhouse was 31.8°C and the minimum effective temperature average at 24.6 °C;
- the maximum effective temperature average for May in the greenhouse was 37.4°C and the minimum effective temperature average at 25.2 °C;
- the maximum effective temperature average for June in the greenhouse was 42.8°C and the minimum effective temperature average at 28.6 °C.

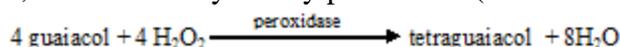
During the development of experiments observations and measurements of plant growth were made in different stages: weekly **biometric parameters of seedlings** (plant height; root volume) were measured, while **biochemical determinations** in the active leaves were performed at the end of the experiment (after 54 days), when the seedlings have reached the optimum for a successful planting (June, 17).

#### **Biochemical analysis methods**

In order to estimate the oxidative stress occurred on cell level, characteristic parameters were analyzed, such as proteins content, specific activities of catalase and of peroxidase, assimilatory pigments content using appropriate methods of analysis.

- **The proteins content** was determined by Lowry method, which is based on the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteu phosphomolybdic-phosphotungstic acid to heteropoly molybdenum blue by the copper-catalyzed oxidation of aromatic aminoacids (LOWRY & al. [26]). The results were expressed in g/100 g fresh weight.

- **The activity of peroxidase** was determined by spectrophotometric measuring of the speed of colour achievement at 436 nm and 25°C in the decomposition reaction of hydrogen peroxide with guaiacol as hydrogen donor, which is catalyzed by peroxidase (BERGMAYER [27]).



*The enzymatic unit:* the amount of enzyme which catalyzed the transformation of one micromole of hydrogen peroxide/minute at 25°C.

- **The activity of catalase** was determined with method essentially described by BEERS and SIZER [28], in which the disappearance of peroxide is followed spectrophotometrically at 240 nm.

*The enzymatic unit:* one unit decomposes one micromole of H<sub>2</sub>O<sub>2</sub> per minute at 25°C and pH 7.0 under the specified conditions.

- **Determinations of the assimilatory pigments content** in the active leaves: *chlorophyll* and *carotenoid pigments* were extracted in 80% acetone and determined spectrophotometrically (wavelengths 663 nm, 647 nm and 480 nm) using the extinction coefficients and equations described by SCHOPFER [29]. The results were expressed in mg/100 g fresh weight.

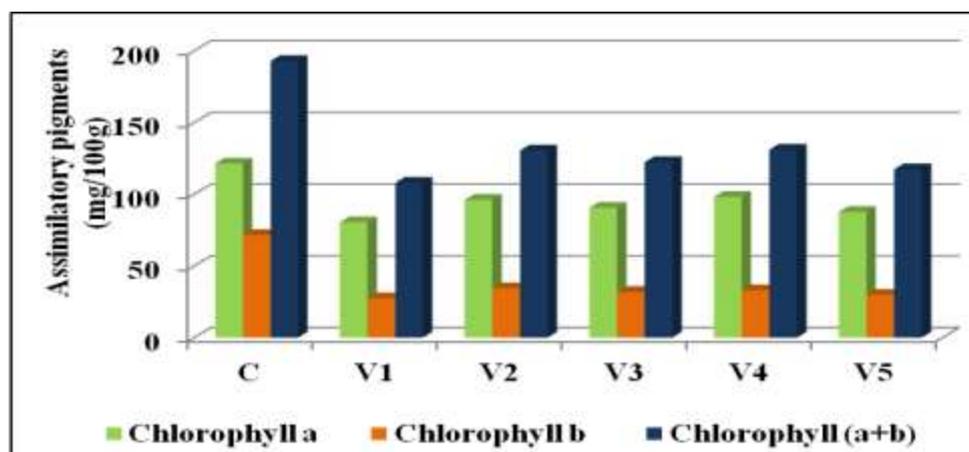
### 3. Results and discussions

The increase of environmental temperature up to a certain value increases plant growth, photosynthesis, respiration and enzyme activity, but after that these parameters tend to decline because most enzymes are irreversibly denatured and inactivated at high temperatures (generally up to 60°C) (HASANUZZAMAN & al. [25]). Consequently, changes in environmental temperature affect the physiological processes in plants, but their efficiency to manage the high temperature depends on the species, being genetically determinate. On the other hand, the application of some bioregulators is expected to increase tolerance to high temperature stress, so analyze of assimilatory pigments and peroxidase and catalase activity as oxidative stress markers was performed.

#### Determination of assimilatory pigments content

Assimilatory pigments, such as *chlorophylls*, play an important role in the photochemical reactions of photosynthesis (TAIZ & al. [30]), therefore changes in the chlorophyll content will influence photosynthetic efficiency.

The obtained data indicate that high temperature significantly affected the chlorophyll content in the eggplants seedlings: a decrease of 44.03% was observed in the untreated plants exposed to heat stress (V1) compared to the unexposed control plants (C) (figure 1).



**Figure 1.** Chlorophyll content in the experimental variants

Similar results regarding the effect of heat stress exposure on chlorophylls content were reported at various plants as *Triticum aestivum* (TEWARI & al. [31]; ALMESELMANI & al. [32]), *Festuca arudinacea* (CUI & al. [33]), *Solanum ssp.* (AIEN & al. [34]), *Lycopersicum esculentum* (BEROVA & al. [35]).

Also an increase in the chlorophyll *a/b* ratio occurred in the stressed plants compared with control plants, caused mainly by a higher reducing in chlorophyll *b* content compared to chlorophyll *a* content (table 1) in according to the data reported by other authors (CUI & al. [33]; ZHU & al. [36]).

**Table 1.** Values and ratio of assimilatory pigments in the experimental variants

Variants	Carotenoids (mg/100g)	Chlorophyll a/b	Chlorophyll (a+b)/Carotenoids
C (unstressed control)	2.45	2.52	78.71
V1(untreated stressed control)	2.58	3.13	41.82
V2 (treated with Spraygard)	2.55	2.78	51.14
V3 (treated with Razormin)	3.51	2.84	34.84
V4 (treated with BAC foliar)	3.73	2.98	35.12
V5 (treated with BIORoot)	3.62	2.97	32.4

Under growth regulators treatment a smaller diminution of total chlorophyll content in stressed eggplants was noticed: only 32% in the plants treated with BAC Foliar (V4) and 36.37% in Razormin variant (V3). Generally, the growth regulators treatment induced a better accumulation of chlorophylls in the eggplants leaves.

Scientific studies report that a 10-15°C increase over normal growth temperature results in degradation of chlorophyll and thus affecting photosynthesis process. The reasons for decreasing in photosynthetic pigments under high temperature may be attributed to the inhibition of biosynthesis, changes in ultrastructure of chloroplast and photo-deterioration (TEWARI & al. [31]; REDA & al. [37]).

**Carotenoids** are an important class of antioxidants which play a major role in the protection of plants against photo-oxidative processes (STAHL & al. [38]; GRAMZA-MICHALOWSKA & al. [39]); their destruction through oxidation may reduce efficiency of the antioxidant defense system (CHEDEA & al. [40]).

Unlike chlorophylls, carotenoids registered an increase as result of heat exposure (table 1): 2.58 mg/100 g carotenoids determined in stressed control in absence of treatment (V1) compared to 2.45 mg/100 g carotenoids in unstressed control. Also a decrease of *chlorophyll (a+b)/carotenoids* ratio was registered in the stressed plants (table 1). Previous studies reported lower *chlorophyll (a+b)/carotenoid* ratio in two heat stressed cultivars of *Festuca arudinacea* in relation to the control plants (CUI & al. [33]) and carotenoids amounts increased in *Populus cathayana* cuttings exposed under moderate stress conditions (XIAO & al. [41]).

However, the increase of carotenoids amounts was more pronounced in the stressed eggplants under growth regulators treatment (between 2.55 mg/100 g in Spraygard treated variant and 3.73 mg/100 g in BAC Foliar treated variant) compared with untreated control (2.58 mg/100 g at V1). Similar observations of bioregulators treatment increasing carotenoids content were reported in some species of pepper plants (*Capsicum annuum* var. *grossum*, *Capsicum annuum* var. *accuminatum*), while higher bioregulator concentrations impacted negatively on carotenoid content in *Capsicum chinense* plants (OLAYA & al. [42]), also in *Triticum aestivum* plants (SAHU & al. [43]). This suggests that the response to treatments with the bioregulators is genetically adjusted.

Given that carotenoids act as antioxidant compounds involved in protection of photosynthetic systems, it is well documented that a higher level of total carotenoids support the plant to tolerate

the stressful condition. These results are in agreement with previous studies related to plant acclimation to stress (LOGAN & al. [44]; LOGGINI & al. [45]; RUBAN & al. [46]).

### Determination of content in proteins

Analysis of proteins content in the leaves of eggplant seedlings revealed that heat stress induced an increase, so that higher values of this parameter were registered in the stressed plants (1.49 g%) in absence of bioregulators treatment compared with the unstressed control (1.31 g%) (figure 2). However, the increase of protein content was more pronounced in the leaves of the eggplants treated with growth regulators (between 1.35-2.52 g%) in comparison to untreated stressed control (1.49 g%), Razormin treated variant (V3) reaching the highest value of proteins amount (2.52 g%). The increase in protein content might suggest a change in the gene expression that would be associated with a possible thermotolerance and acclimation to stress condition (CAMEJO & al. [47]).

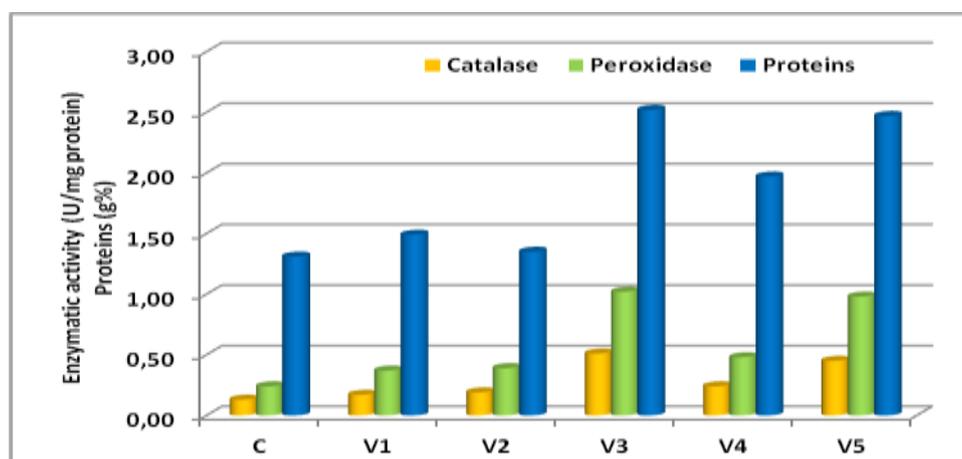


Figure 2. Enzymatic activity and proteins content in the experimental variants

### Determination of enzymatic activity in the seedlings leaves

There are numerous plant studies which indicate that the tolerance to temperature stress in plants is positively correlated with an increase in antioxidants (HASANUZZAMAN & al. [2]; BABU & al. [11], ALMESELMANI & al. [12]).

It is known that *peroxidase* plays a special role in the total metabolism of plant due to its multifunctional activity (CHKHUBIANISHVILI & al. [48]). Higher activity of peroxidase was registered in the untreated eggplants under heat stress (0.37 U/mg protein) compared with control (0.24 U/mg protein), but response to heat stress was amplified in the eggplants under growth regulators treatment by enhancing the peroxidase activity (between 0.39 U/mg protein in Spraygard variant (V3) and 1.02 U/mg in Razormin variant (V4).

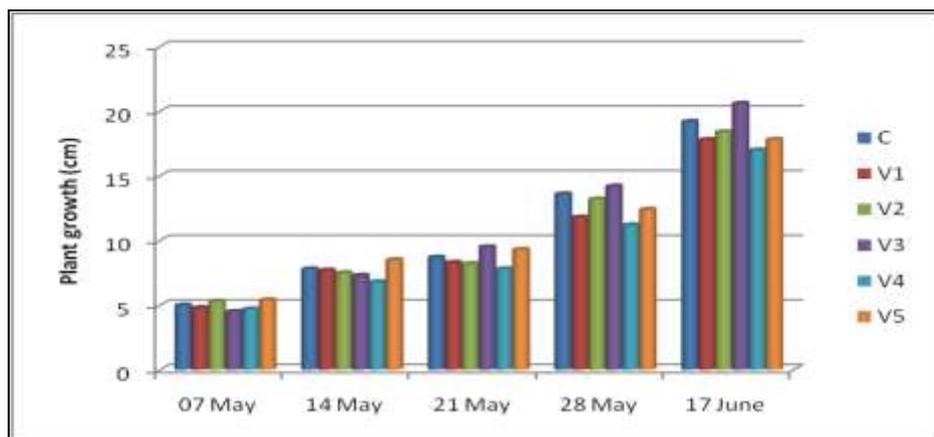
*Catalase activity* follows the same dynamics as peroxidase, only the measured values were lower: 0.13 U/mg protein in control eggplants, which increased at 0.17 U/mg protein in absence of bioregulators. Also the catalasic activity increased in the stressed plants treated with growth regulators. Higher catalase activities were registered in the eggplants under treatment with roots stimulators (0.51 U/mg protein in Razormin variant and 0.45 U/mg protein in BIO Root variant).

CHKHUBIANISHVILI & al. [48] reported a regularity observed as results of studying the enzymatic activity of catalase and peroxidase in some herbaceous plants: decrease in the activity of one enzyme was accompanied by activation of the other one.

The obtained results are in according with some authors who concluded that the activation of protein synthesis in plants, in combination with increase of oxidases activity under stress conditions may be the result of metabolism conversion in order to achievement of the plants adaptability under stressful conditions of life (TUCIC & al. [49]; CHKHUBIANISHVILI & al. [48]; WU & al. [50]).

### Determination of some biometric parameters of seedlings

The dynamic of seedlings growth was estimated by measurement of *plant height*. The obtained data regarding the growth of eggplant seedlings during the experiments (figure 3) indicated a plant distress occurred as effects of heat stress in most of the studied variants (V1, V2, V4, V5). Thus, reduced values of the plants size were noticed in the stressed variants compared with control variant unstressed (19.2 cm). However, the reduction of height plants was lower in the variants treated with Spraygard (18.4 cm) and Razormin (20.6 cm) compared with the untreated variant (V1), which achieved only 17.8 cm. It seems that in the case of V3 variant, Razormin 0.1% treatments led to an increased plant capacity to overcome the effects of heat stress, therefore the plants of this variant achieved the highest plant height (20.6 cm).



**Figure 3.** The dynamic of eggplant seedlings growth

Root system is significantly involved in plant adaptation to heat stress due to their role in water and nutrient uptake. Maintaining an active root growth is essential for plant development and productivity during periods of high temperatures.

Measurements of the *root volume* (table 2) indicated that the heat stressed eggplant seedlings (V1 variant) developed a strong root system to cope with heat stress: a volume of 5.6 cm<sup>3</sup>, meaning 1.75 times higher than root volume measured for control plants (unstressed variant C).

**Table 2.** Root volume measured in the experimental variants

Variants	Root volume (cm <sup>3</sup> )
C (unstressed control)	3.2
V1(untreated stressed control)	5.6
V2 (treated with Spraygard)	3.4
V3 (treated with Razormin)	4
V4 (treated with BAC foliar)	3.2
V5 (treated with BIO Root)	3.4

It is expected that the bioregulators treatment to maintain viability and functionality of the root system during heat stress periods. Razormin treatment induced a less pronounced increase in the root volume (4 cm<sup>3</sup>), probably because this growth regulator mainly stimulates root function of water and minerals absorption, therefore the plant development even under stressful conditions.

#### 4. Conclusions

High temperature significantly affected the chlorophyll content in the eggplants seedlings, but under growth regulators treatment a smaller diminution of total chlorophyll content in stressed eggplants was noticed.

Unlike chlorophyll, as result of heat exposure carotenoids registered an increase, which was more pronounced in the stressed eggplants under growth regulators treatment.

Also the protein content and the oxidases activity were enhanced under high temperature conditions, mostly in the eggplant seedlings treated with growth regulators.

A distress of growth plant occurred as effects of heat stress, as reduction of height plants and increase of root volume were recorded in the heat exposed variant in the absence of bioregulators treatment. Razormin 0.1% treatments led to an increased plant capacity to cope with the effects of heat stress.

An overview of the researches performed on the eggplant seedlings can led to conclusion that growth regulators treatment could attenuate the negative impact of heat stress on plant growth by protecting the photosynthetic apparatus and enhancing antioxidant enzyme systems.

Addition of Razormin and BAC Foliar spray had the best ameliorative effect against high temperature, while the addition of BIO Roots and Sprayguard had no significant effects.

However, further studies should be considered in order to asses possible combined treatments with different bioregulators to achieve optimum effects for improving the plants growth and productivity under stressful conditions triggered by the global climate changes occurred lately.

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