Effect of benzotiadiazole on phytopathological and physiological processes in tomato

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The modern plant protection system has involved the new means that act not directly on fungi and bacteria, but activate the natural defense mechanisms of the plant and enhance the same physiological and biochemical changes in plants as does the biological systemic activated resistance. In 1999–2000, the role of the plant activator Bion (benzothiadiazole) in the resistance of tomato against early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) was investigated at the Lithuanian Institute of Horticulture. Physiological and phytopathological observations were carried out on greenhouse tomato *Aušriai* exposed to Bion. The data showed that the plant activator Bion and the fungicide Euparen (tolylfluanid) reduced early blight incidence by 10.35% and late blight by 34.5% in comparison with untreated plants. Application of the plant activator and the fungicide increased tomato yield by 4.5 kg/m^2 and exhibited a rather high biological efficiency (42–63%).

Tomato sprayed with Bion accumulated insignificantly more pigments in leaves at a normal temperature. Under these conditions other physiological processes were similar. At a high temperature the physiological processes were more intensive in tomatoes sprayed with Bion than in non-sprayed ones.

Key words: *Solanum lycopersicum* L., *Alternaria solani, Phytophthora infestans,* benzothiadiazole, tolylfluanid, pigments, phytomonitoring, photosynthesis

INTRODUCTION

Early blight caused by *Alternaria solani* Ellis et Martin and late blight caused by *Phytophthora infestans* (Mont.) de Bary are harmful diseases affecting field and glasshouse tomato production around the world [6]. Disease control relies mainly on fungicides, but a combination of high inoculums pressure, glasshouse conditions that favor fungal growth, and exacting consumer standards for unblemished produce necessitates frequent pesticide applications. The application of fungicides often produces a harmful effect on ecosystems. Concerns about the environmental effects of fungicides have also led to increased restriction of their use, and as a result, other forms of control are urgently being sought. A promising approach in this regard is the use of prophylactic compounds such as benzothiadiazole (Bion).

Bion, a novel chemical activator of plant disease resistance [2], has no known direct antifungal effect and is thought to play a role similar to that of salicylic acid in the signal transducion pathway that leads to systemic acquired resistance (SAR) [7]. Bion has no direct effect on fungi and bacteria such as

Alternaria brassicae, Alternaria brassicola, Botrytis cinerea, Cladosporium cucumerinum, Fusarium culmorum, Helminthosporium oryzae, Helminthosporium teres, Penicillium digitatum, Penicillium expansum, Penicillium italicum, Pyricularia oryzae, Verticillium dahliae, Septoria nodorum, Ceratocystis ulmi, Phytophthora infestans, Rhizoctonia solani, Ustilago maydis, Mucor hiernalis, Pseudomonas lachrymans (from technical characteristics). Bion activates the same physiological and biochemical changes in plants as does the biological activated SAR.

Bion is commonly considered as the best protector against *Erysiphe graminis* and as a moderate protector against *Septoria* spp. and *Puccinia* spp. in cereal crops [4, 9, 10]. Application of benzothiadiazole to rockmelon foliage before flowering had a major effect on the incidence and extent of postharvest diseases such as *Alternaria, Fusarium* and *Rhizopus* rots [3]. It should be noted that Bion activates defense mechanisms in plants: tomato and cyclamen were successfully protected against *Fusarium* spp. [12, 14].

In another case high doses of benzothiadiazole induced occasional cell wall thickening and accumulation of a compound that stained purple with toluidine blue O, but the defense response was weak, sporadic, and insufficient to reduce powdery mildew infection on cucumber [18].

It is possible to determine the impact of abiotic factors on a plant according to various physiological parameters. One of them is the content of chlorophylls and other pigments in plant leaves. Chlorophyll content is an important factor, which predetermines photosynthesis intensity and indicates optimal environmental conditions for a plant [1, 5, 17]. Another method to estimate the physiological state of a plant is phytomonitoring. It is a complex investigation of plant state and function using information-measuring systems allowing long-term and non-invasive measurements, to combine the processing of several parameters and the use of a trend of the characteristics instead of their instant absolute values [15, 20, 21].

The objectives of this study were (I) to compare the efficacy of Bion 50 WG and Euparen 50 WP for controlling tomato early blight and late blight, (II) to investigate the inducing effects of Bion on physiological processes in tomato plants.

MATERIALS AND METHODS

In 1999–2000, vegetative trials were carried out at the Laboratories of Plant Protection and Plant Physiology of the Lithuanian Institute of Horticulture. Tomatoes 'Aušriai' (determinant type) for phytopathological investigations were grown in unheated greenhouses according to the tomato growing technology in soil and for physiological investigations on compost substrate in 58 × 36 × 27 cm plastic boxes. At the time of physiological investigations first truss tomatoes flowered and began to grow fruits.

Bion 50 WG (benzo (1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester) was obtained from Novartis Crop Protection AG, Switzerland. The impact of Bion on the development of early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) infection was studied according to the following scheme: 1) untreated (control); 2) two applications of Euparen 50 WP (tolylfluanid), 0.15% at the time when the first symptoms of the disease showed up; 3) one spraying with Bion, 0.05% before the incidence of infection, and after 14 days two applications with Euparen (0.15%). The infection was natural.

The early blight and late blight incidence was evaluated according to the scale: 0 – healthy leaves; 1 – spots covered up to 5% of leaf area; 2 – 10%; 3 – 20%; 4 – 50%; 5 – 75% and more of leaf area.

The content of chlorophylls and carotenoids in green tomato leaves was established in 100% acetone extract according to Wetshtein [19] by with a Spekol 11 spectrophotometer. The phytometric systems Ecoplant-011 (Biopribor, Kishinev, Moldova) and LPS-03 (PhyTech Ltd., Israel) were used for the phytomonitoring. They allow observing physiological processes in a plant, including parameters of environmental factors, in dynamics, for a long-term and non-invasively. The Ecoplant-011 system consisted of a measuring equipment module, gas analyzer, a set of sensors and leaf chambers for measuring CO_2 metabolism, telescopic stands with a joining cable and personal computer. The LPS-03 phytomonitor has no gas analyzer and leaf chambers. The following sensors were used for investigations: leaf temperature, air temperature, and stem and fruit diameter evolution. The gas analyzer measured photosynthesis intensity in the Ecoplant-011 system. Data on CO_2 metabolism were obtained from 0.2 dm^2 of leaf area. Leaf chambers were placed on leaves below and above the first truss and leaf temperature sensors on the same leaves. In investigations using the LPS-03 phytomonitor the position of sensors was chosen according to PhyTech Ltd. instruction [8, 16]. The monitotoring lasted three days in 1999 and seven days in 2000.

RESULTS AND DISCUSSION

The distribution of the foliar diseases depended on weather conditions, because the microclimate in the greenhouse was not regulated. In both years of investigations climatic conditions of the vegetation period were medium favorable for fungal diseases to develop. The most harmful diseases of tomato, such as early blight (*Alternaria solani* Ellis et Martin) and late blight (*Phytophthora infestans* (Mont.) de Bary) were observed.

In 1999, first symptoms of early blight in untreated plants appeared in the middle of July, in 2000 – at the end of July and developed until harvesting. The first visible symptoms of early blight were circular dark-brown to black spots of various size on the oldest leaves of control plants. Later the agent of the disease affected the upper leaves, stems and fruits. The spots, about 1 cm in diameter, were readily recognized as concentric rings.

In 1999–2000, initial infection of late blight in untreated plants appeared in the middle of August. At first, irregular greenish-black water-soaked areas appeared on the oldest leaves. The spots increased rapidly, and in humid weather a bluish grey growth of fungi sometimes developed on the lower leaf surfaces. Brownish cankers were often found on the stems and leaf petioles. Fruit were infected at all stages of growth.

Symptoms of diseases in the treatment where Euparen and Bion plus Euparen were applied appeared 10–14 days later.

Table 1. **The effect of Bion 50 WG and Euparen 50 WP on *Alternaria solani* and yield of tomato 'Aušriai'**

Treatment	Rate, %	Early blight incidence, %		Biological efficiency, %	
		1999	2000	1999	2000
Untreated		26.2	23.1	–	–
Euparen 50 WP	0.12	16.2	14.7	38.2	36.4
Bion 50 WG + Euparen 50 WP	0.005 + 0.12	14.9	13.7	43.2	40.7
LSD_{05}		4.1	3.9		

Table 2. **The effect of Bion 50 WG and Euparen 50 WP on *Phytophthora infestans* and yield of tomato 'Aušriai'**

Treatment	Rate, %	Late blight incidence, %		Biological efficiency, %	
		1999	2000	1999	2000
Untreated		58.3	50.6	–	–
Euparen 50 WP	0.12	25.1	21.7	56.9	57.1
Bion 50 WG + Euparen 50 WP	0.005 + 0.12	20.7	19.2	64.5	62.1
LSD_{05}		12.8	8.9		

Results of the study showed that all the chemical means tested reduced disease incidence, increased tomato yield and exhibited a high biological efficiency. In 1999 the incidence of *Alternaria solani* ranged from 14.9–16.2% (data for Euparen and Bion, respectively) to 26.2% (untreated) (Table 1). Analogous results were obtained in 2000. Treatment with the plant activator Bion decreased disease incidence by 9.4–11.3% and showed a rather sufficient biological efficiency (40.7–43.2%) in both years of investigations. The use of Euparen alone was less efficient in reducing the incidence of early blight less (about 5%).

In this experiment, the fungicide and Bion suppressed the process of *Phytophthora infestans* from 19.2–20.7% to 58.3% in comparison with control plants. The biological efficiency of the study preparations reached 62.1–64.5%. Only the fungicide Euparen suppressed disease incidence by 57% (Table 2).

Summarizing the obtained data on disease incidence, it is possible to predicate that Bion was more active to induce a certain systemic activated resistance in tomatoes. In both years of studies, both the incidence and the infection process of early blight and late blight, the biological efficiency of the test compounds were similar. The mean severity of *Alternaria solani* reached 9.2% in the treatment with Euparen and 10.35% with Bion and Euparen. It accordingly signified 37.2% and 41.95% of biological efficiency. The ability of Bion and Euparen to control the infection process of *Phytophthora infestans* on tomato was similar. The efficacy of the study compounds was from 57% to 63.3%. The best results were obtained from the treatment with Bion and Euparen, where the yield of tomato increased by 4.5 kg/m² (Fig. 1).

Tomato leaves spayed with Bion, accumulated a slightly higher content of chlorophylls and carotenoids (Fig. 2). This difference was not significant. The tendency was observed in both years of investigation.

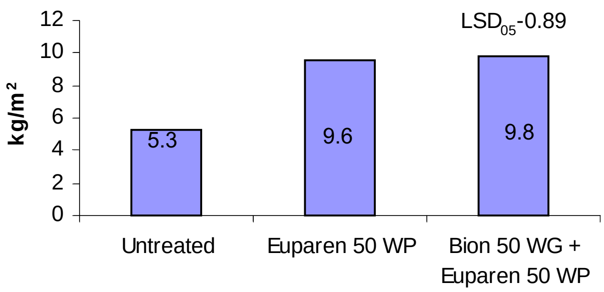

Fig. 1. The effect of plant activator Bion on tomato yield in 1999–2000

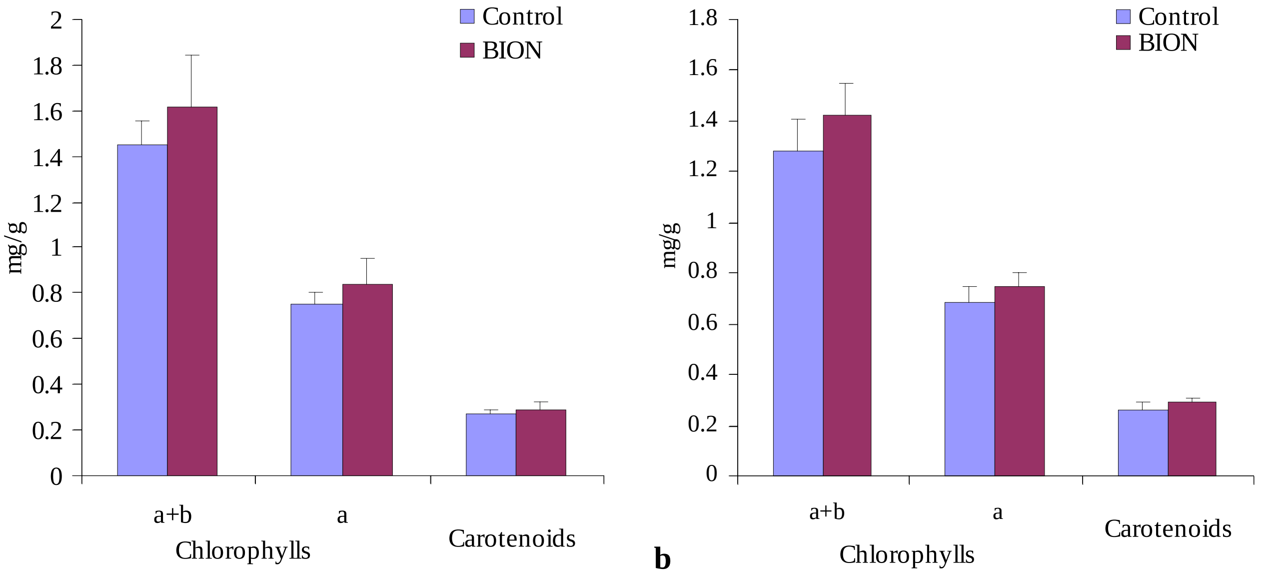

Fig. 2. The content of pigments in tomato leaves: *a* – 1999, *b* – 2000

Photosynthesis intensity of tomato leaves was measured with an Ecoplant-011 phytometric system in 1999. From data presented in Fig. 3, c, we can see that the air temperature was very high (~35 °C). Meanwhile, photosynthesis in tomato was intensive. It was more intensive in leaves below the first truss in tomato sprayed with Bion (Fig. 3, a). Photosynthesis slowed down in the middle of the day when the temperature rose above 35 °C. Photosynthesis intensity was lower in leaves above the first truss and was similar in both variants, but the temperature of these leaves was higher than of the air (Fig. 3, d), and this could predetermine attenuation of photosynthesis in both variants. Leaf–air temperature difference is an indirect of transpiration. These data show that transpiration intensity was insufficient to cool leaves of higher storey. Meanwhile, in leaves of tomato sprayed with Bion below the first truss transpiration, according to leave temperature lower than air, was more intensive and when temperature rose till 37 °C they did not overheat (Fig. 3, c).

At 25 °C (Fig. 4) photosynthesis in tomato was similar in both variants (Fig. 4, a, b). Transpiration intensity was similar in lower leaves (Fig. 4, c). In the leaves that grew above the first truss, because of the leaf temperature lower than that of the air transpiration of tomato exposed to Bion was more intensive (Fig. 4, d).

Changes of water uptake in tomatoes were measured with an LPS-03 phytometric system. An indication of these changes is evolution of stem and fruit diameters. In 1999, during the experiment the air temperature was rather high, but it did not suppress the increment of stem diameter during the day (Fig. 5, a). It was positive both in tomato sprayed and not sprayed with Bion. Negative stem increment during the day shows water deficit in plants [5, 16]. The stem of tomato sprayed with Bion thickened slowly, but it could be caused by a more intensive transpiration, as proved by the temperature which was lower in the leaves than in the air (Figs. 3, 4). Meanwhile, fruits grew more intensively in tomato sprayed with Bion (Fig. 5, c). Fruit increment should be equal in normal conditions [5, 16]. High temperature slowed down the growth of all fruits, but in tomatoes sprayed with Bion growth suppression was less pronounced.

In 2000, air temperature was above 30 °C at the beginning of treatment. Stem diameter increment during the day was slight and similar both in tomato affected and non-affected by Bion. When the temperature decreased, tomato sprayed with Bion grew more intensively (Fig. 6, a). Fruits of these tomatoes grew uniformly and more intensively. Meanwhile, fruit growth of non-sprayed tomato slowed down at a high temperature in the middle of the day.

Summarizing the obtained data it is possible to conclude that Bion enhances pigment biosynthesis in tomatoes, thought insignificantly. We obtained similar results in an earlier treatment with onions [13]. According to the findings of other authors [11], Bion improves physiological processes of nitrogen uptake. It is known that the amount of chlorophyll content in plant leaves is known to be highly dependent on mineral nutrients. Therefore a better uptake of nitrogen could predetermine a higher content of chlorophyll content in leaves of tomato sprayed with Bion. We did not find in the literature other data on how Bion influenced pigment synthesis or photosynthesis. In our experiments tomato sprayed

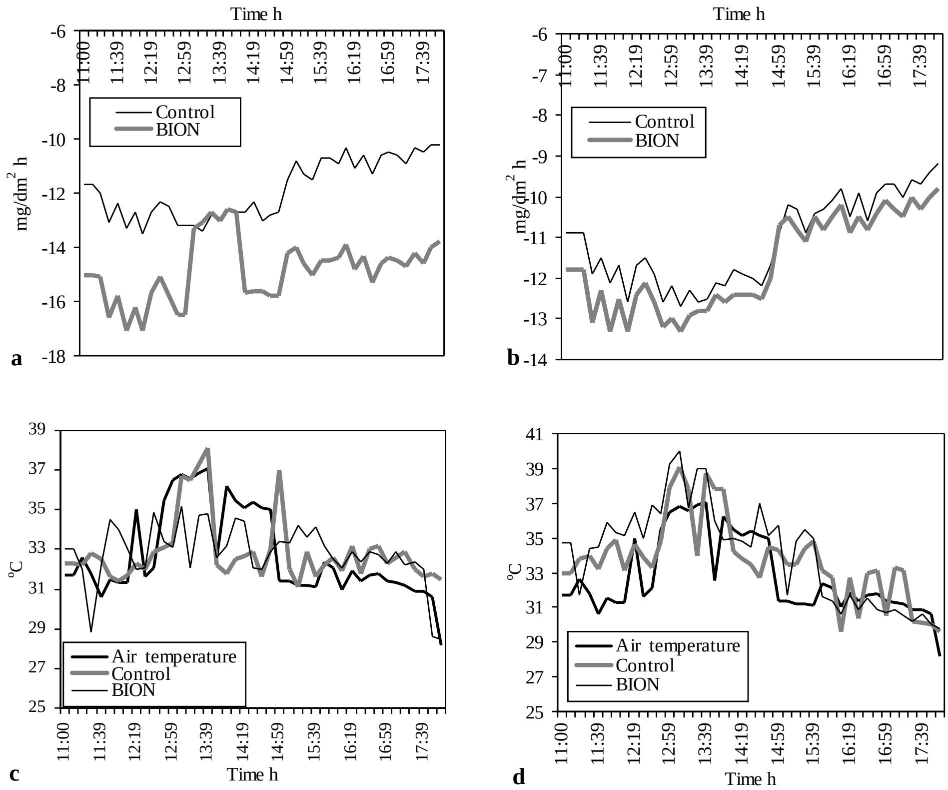

Fig. 3. Intensity of tomato photosynthesis at ~35 °C. *a* – photosynthesis intensity in leaf grown under the first truss; *b* – photosynthesis intensity in leaf grown above the first truss; *c* – air temperature and leaf grown under the first truss; *d* – air temperature and leaf grown above the first truss

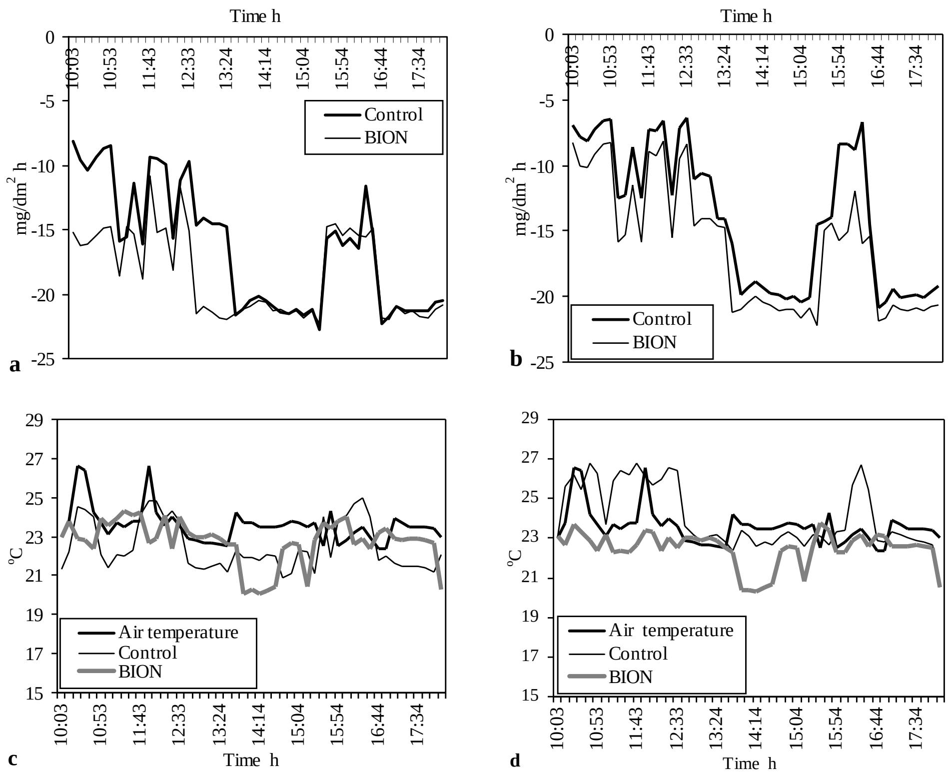

Fig. 4. Intensity of tomato photosynthesis at ~25 °C. *a* – photosynthesis intensity in leaf grown under the first truss; *b* – photosynthesis intensity in leaf grown above the first truss; *c* – air temperature and leaf grown under the first truss; *d* – air temperature and leaf grown above the first truss

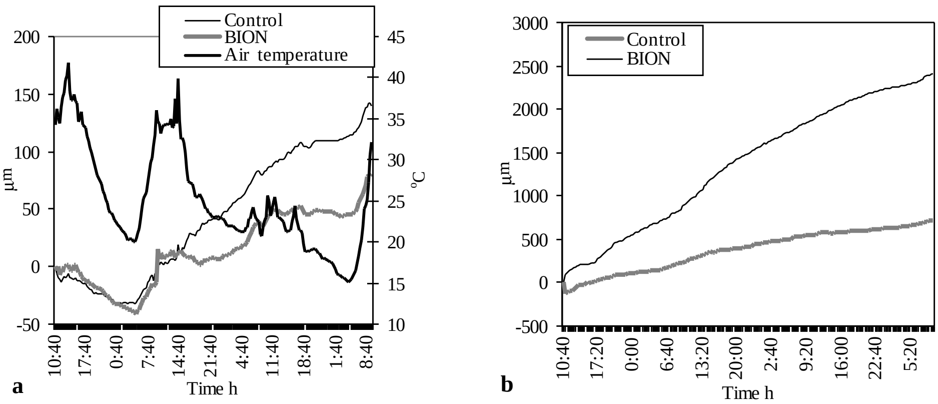

Fig. 5. Dynamics of stem (a) and fruit (b) diameter of tomato in 1999

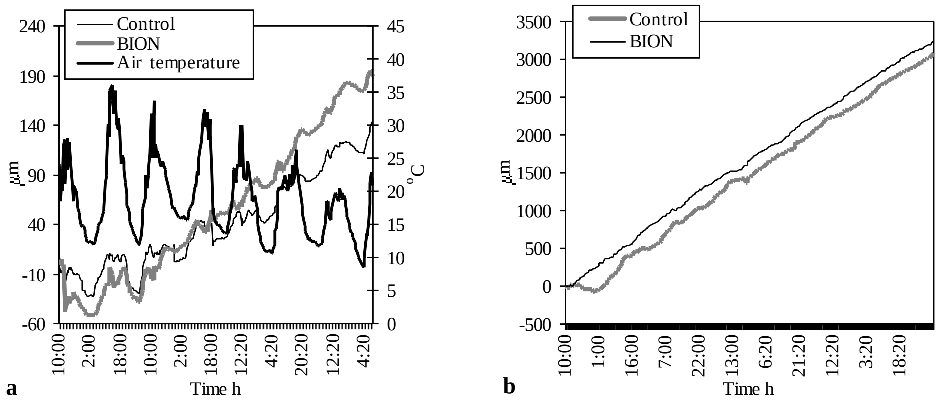

Fig. 6. Dynamics of stem (a) and fruit (b) diameter of tomato in 2000

with Bion showed more intensive photosynthesis, especially at high temperatures. In these conditions, transpiration also was more intensive in tomatoes exposed to Bion. High temperature exerted less influence on the fruit growth of these tomatoes.

CONCLUSIONS

1. Bion activates natural defense mechanisms of the tomato. Plants exposed to Bion are more resistant against causal agents of early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*), thus inhibiting the process of infection.

2. Tomatoes sprayed with Bion accumulate insignificantly more pigments in leaves at a normal temperature. In these conditions other physiological processes are similar. At a high temperature physiological processes were more intensive in tomato sprayed with Bion than in non-sprayed ones.

Received
24 October 2002

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BENZOTIADIAZOLO POVEIKIS FITOPATOLOGINIAMS IR FIZIOLOGINIAMS PROCESAMS POMIDORUOSE

S a n t r a u k a

Šiuolaikinei augalų apsaugai sukuriama vis naujų efektyvių cheminių junginių, kurie neturi tiesioginės įtakos ligų sukėlėjams, bet veikia fiziologinius ir biocheminius procesus augaluose, didina augalų atsparumą patogeniniams grybams, bakterijoms ir virusams. 1999–2000 metais Lietuvos sodininkystės ir daržininkystės institute buvo ištirtas augalų aktyvatoriaus Bion 50 WG (benzotiadiazolas) poveikis pomidorų atsparumui sausligei (*Alternaria solani*) ir marui (*Phytophthora infestans*). Gauti duomenys parodė, kad bionu ir euparenu (tolylfluanidas) purkštuose pomidoruose sausligės plitimas sumažėjo vidutiniškai 10,35% (tirtų priemonių biologinis efektyvumas – beveik 42%), maro – 34,5% (biologinis efektyvumas – 63%). Vaisių derlius padidėjo 4,5 kg/m^2.

Normalioje temperatūroje bionu paveiktų pomidorų pigmentų kiekis padidėjo nežymiai, o aukštoje temperatūroje fiziologiniai procesai vyko daug intensyviau.

Raktažodžiai: *Solanum lycopersicum* L., *Alternaria solani, Phytophthora infestans,* benzotiadiazolas, tolylfluanidas, pigmentai, fitomonitoringas, fotosintezė

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ВЛИЯНИЕ БЕНЗОТИАДИАЗОЛА НА ФИТОПАТОЛОГИЧЕСКИЕ И ФИЗИОЛОГИЧЕСКИЕ ПРОЦЕССЫ ТОМАТОВ

Р е з ю м е

В современной защите растений употребляются новые эффективные химические соединения, которые, не имея прямого действия на возбудителей болезней, влияют на физиологические и биохимические процессы растений, повышая при этом их устойчивость против патогенных грибов, бактерий и вирусов. В 1999–2000 гг. в Литовском институте садоводства и овощеводства проведены опыты по изучению влияния активатора растений Бион 50 WG (бензотиадиазол) на устойчивость томатов против бурой пятнистости (*Alternaria solani*) и фитофтороза (*Phytophthora infestans*). Результаты показали, что обработка томатов Бионом и Эупареном 50 WP (толилфлуанид) снизила распространение бурой пятнистости в среднем до 10,35% (биологическая эффективность препаратов достигала почти 42%), а распространение фитофтороза – до 34,5% (эффективность 63%). Была получена прибыль урожая до 4,5 кг/м^2.

Под влиянием Биона количество пигментов в листьях томатов увеличилось незначительно. При более высокой температуре физиологические процессы проходили интенсивнее.

Ключевые слова: *Solanum lycopersicum* L., *Alternaria solani, Phytophthora infestans,* бензотиадиазол, толилфлуанид, пигменты, фитомониторинг, фотосинтез