

Investigations on testing the treatments with inhibitory effects over phytopathogenic bacteria

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Abstract

Research on the role of some bio-products in the biological control of phytopathogenic agents remains a challenge for modern biotechnologies used in sustainable agriculture.

*The investigations carried out in the laboratory based on testing of treatments with inhibitory effects on phytopathogenic bacteria led to experimental results confirming the viability of using BSP + BSV and B4 bio-products for the biological control of some phytopathogenic bacteria (*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato* and *Erwinia carotovora*), both for *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple*.*

Keywords: bio-product, phytopathogen bacterium, *Lycopersicon esculentum* L. var. *Marmade*, *Solanum melongena* L. var. *Long Purple*.

Introduction

For the vegetables cultures the chemical protection method was imposed as a fundamental method, but which lead in time to the production and materialization of a series of inconvenients, such as: lack of ecological balance; the appearance of a pathogen agent strain resistant to chemicals; toxic wastes in the soil, on the plants, in vegetal products and even in animal products; diseases of the human body [9, 10 and 14]. In addition, the modern requirements of practiced vegetable growing requires the identification of certain solving modalities which allow the obtaining of superior quality plants at minimum costs [5, 9]. Thus, the identification of a viable solution for the solving of these problems at the highest performance constitutes a true competition for the research directions involved in sustainable agriculture.

Of the solutions offered by the modern biotechnologies, the use of the treatments based on the interaction between the phytopathogenics microorganisms and those used in their biological control generate a sum of advantages, both for the nutritional layer in which the vegetable growing takes place, and for the final consumers of the respective crop.

Therefore, the purpose of our investigations has been the laboratory testing of the efficiency of the bio-products (BSP+BSV and B4) obtained from a new batch produced by INCDCF-ICCF Bucharest, for the biological control of some phytopathogenic bacteria (*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato* and *Erwinia carotovora*), both for *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple*.

Material and methods

The inocula source for the laboratory testing of the efficiency of the bio-products (BSP+BSV and B4) obtained from a new batch produced by INCDCF-ICCF Bucharest, for the biological control of some phytopathogenic bacteria (*Xanthomonas campestris* pv.

vesicatoria, *Pseudomonas syringae* pv. *tomato* and *Erwinia carotovora*), both for *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple*.

The experiments aiming at the *in vitro* regeneration of *Lycopersicon* L. and *Solanum* L. plantlets used as a main inocula source the seeds found on the market in the phase of vegetative rest and obtained *ex vitro* by means of conventional horticultural methods.

The tomato (*Lycopersicon esculentum* L. var. *Marmade*) and eggplant (*Solanum melongena* L. var. *Long Purple*) seeds were of varieties that has as main distinctive element, compared to the varieties used in the previous experimental stage, the possibility to be grown semi-early (used preponderantly for consumption in fresh state) and not late (used especially for consumption in preserved condition). Practically, the tomato (*Lycopersicon esculentum* L.) seeds were of the *Marmade* variety, characterized by: semi-early cultivation; determined growth; large, uniformly coloured fruit, round, flattened, slightly costed. While the eggplant (*Solanum melongena* L. var. *Long Purple*) seeds were of the *Long Purple* variety, characterized by: semi-early cultivation; oval-elongated fruit, colour dark violet with black reflexes.

The *Lycopersicon esculentum* L. var. *Marmade* and the *Solanum melongena* L. var. *Long Purple* plants obtained by laboratory germination and found in the stage with 2-3 real leaves were used as vegetal starting structures for the stage of inducing the contamination with pathogen microorganisms for testing the efficiency of the bio-products produced by INCDCF - ICCF.

The phytopathogenic microorganisms have been isolated from plants belonging to the *Solanaceae* family. Thereby, the following types of microorganisms have been studied: *Xanthomonas campestris* pv. *vesicatoria*, which induces leaf maculation and blister; *Pseudomonas syringae* pv. *tomato* which induces the pustular maculation of fruits and *Erwinia carotovora*.

The bio-products from a new batch selected in the laboratory, within a previous experiment, for the action of inhibition of the development of bacteria, found the following work variants: BSP+BSV and B4. The toxic action of the bio-products obtained from the *Bacillus subtilis* stems of different origins (BSP+BSV – isolated from couch grass and medicinal plants and B4 – isolated from leaves of leaf-bearing trees in the woods) was tested in the laboratory. The concentration at which the bio-product has the best inhibitory action over the phytopathogenic bacteria was determined by means of the dilution method. The fertilization of the bacteria studied was realized by including the bio-products in the culture environment (peptone – glucose – agar). The inhibition percentage against a control (culture environment without a bio-product included) was checked after six days [4, 11]. The working materials and methods applied for the isolation, inoculation, cultivation, identification, testing of the virulence and the processing of the microorganisms used in this laboratory testing of some bio-products intended for the control of the phytopathogenic bacteria, complied with the specific working conditions for the microbiological field [3, 8, 12, 13].

The nutritive substrate used for the germination of seeds and for obtaining the plants of *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple*, was peat type supplemented with sand (4:1). The nutritive substrat asepsitisation was made by autoclaving at the $t^{\circ}=180^{\circ}\text{C}$, for 3h [7].

The culture conditions used for the germination of seeds and for obtaining the plants of *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple*. The seeded seeds were incubated at the temperature of $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ during the lighting period and $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$ during the darkness period, with a photoperiod of 16 h light and a luminous intensity of 2500 luxes [6].

The preparation of plants for inoculation was made by transferring each plant into a plastic cup with nutritive substrate consisting of the peat and sand (4:1). In order to create the conditions necessary for the contamination (high humidity), the plastic cups with plants and nutritive substrate were included in a plastic container provided with sterilized distilled water.

The inoculation of the plants obtained in laboratory was made by applying a cotton-wool wad impregnated with bacterial cells suspension (titre 10^7 ufc/ml). The bacterial cells suspension was administrated before the appliance of a bio-product solution (BSP+ BSV or B4) with a concentration of 4%, of antibiotic or copper sulphate ($\text{CuSO}_4 \times 5\text{H}_2\text{O}$) 1%. Three experimental alternatives have been carried out for the biological control, as the efficiency degree of the bio-product based treatments may be confirmed only on the basis of parallel comparisons with other control agents used in the horticultural crops. Thus, the experimental alternatives noted V3, V4 and V5 have had a control role. Practically, the experimental alternatives used in this process have been the following:

- V1 – infected plants + B4 (4%);
- V2 – infected plants + BSP+ BSV 4%;
- V3 – infected plants + antibiotic (cephotaxime);
- V4 – infected plants + copper sulphate 1%;
- V5 – infected and not treated plants.

For each experimental variant there were made 3 repetitions that consisted of 10 plants. The above mentioned experimental variant was performed for each vegetal species and bacterial stem (2x3).

The experimental observations obtained in the versions treated with bacterial products were compared with a control treated with a known biological product (infected plants treated with antibiotic – cephotaxime) and with a chemically treated control (infected plants treated with copper sulphate 1%), based on the $\text{GA}\% = (\text{F} \times \text{I}):100$ formula, with the following abbreviations: $\text{GA}\%$ = the attack degree calculated from the product between the ill plants frequency and multiplied with the intensity affecting a plant; $\text{F}\%$ = the number of plants from the total of 100 plants and $\text{I}\%$ = the affected percentage of a plant [1, 2, 11].

Results and discussions

The results obtained in the case of treatments applied for the protection of plants from the phytopathogen bacteria of the *Xanthomonas campestris* pv. *vesicatoria* strain (Fig. 1.)

The tomato plants (*Lycopersicon esculentum* L. var. *Marmade*) contaminated with phytopathogen bacteria *Xanthomonas campestris* pv. *vesicatoria* strain have benefited of superior phytosanitary protection by applying the treatment with antibiotic ($\text{E}\% = 89.7\%$). The treatments made on the basis of bio-products B4 (4%) and BSP+BSV (4%), have generated a good efficiency (87.6% and, respectively, 86.2%), because it was at a level close to the efficiency generated by the antibiotic and at a level higher than the efficiency generated by the chemically treated control ($\text{E}\% = 76.6\%$).

The treatments made for the protection of eggplant plants (*Solanum melongena* L. var. *Long Purple*) contaminated with phytopathogen bacteria *Xanthomonas campestris* pv. *vesicatoria* strain have registered the best protection by using bio-product B4 (4%) - $\text{E}\% = 82.1\%$. Through the treatments made on the basis of the antibiotic or the bio-product BSP+BSV (4%) very good values of efficiency were registered ($\text{E}\% = 80.9\%$, respectively $\text{E}\% = 79.5\%$). The treatment made on the basis of copper sulphate, usually recommended in vegetable growing, has generated an efficiency value of only 71.9%.

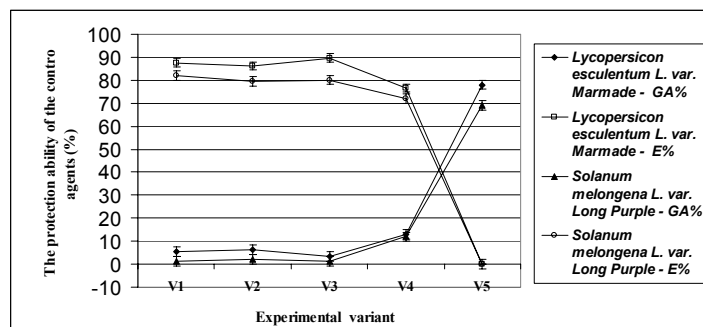


Fig. 1. The protection ability of the control agents (bio-product, antibiotic or copper sulphate) from the treatments used against the contamination with *Xanthomonas campestris* pv. *vesicatoria* phytopathogen bacteria on *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple* plants

The results obtained in the case of treatments applied for the protection of plants from the phytopathogen bacteria *Pseudomonas syringae* pv. *tomato* strain (Fig. 2.)

The tomato plants (*Lycopersicon esculentum* L. var. *Marmade*) contaminated with phytopathogen bacteria of the *Pseudomonas syringae* pv. *tomato* strain had a very good protection of over 85.0% compared with the treatments made on the basis of antibiotic (E% = 86.4%) or on the basis of biological products, respectively bio-product B4 (4%) with E% = 82.1% and bio-product BSP+BSV (4%) with E% = 81.7%. The tomato plants (*Lycopersicon esculentum* L. var. *Marmade*) used as chemically treated control registered inferior efficiency (E% = 84.1%), compared to the values obtained for the treatments on the basis of antibiotic or of bio-products.

For the eggplant plants (*Solanum melongena* L. var. *Long Purple*) contaminated with phytopathogen bacteria *Pseudomonas syringae* pv. *tomato* strain the best protection was registered as a consequence of applying the treatments based on bio-products. Thus, the treatments made on the basis of bio-products BSP+BSV (4%) and B4 (4%) generated the best values of efficiency (E% = 90.6%, respectively 89.6%). while the treatments on the basis of antibiotic or copper sulphate generated inferior efficiency values (E% = 81.6%, respectively 77.0%).

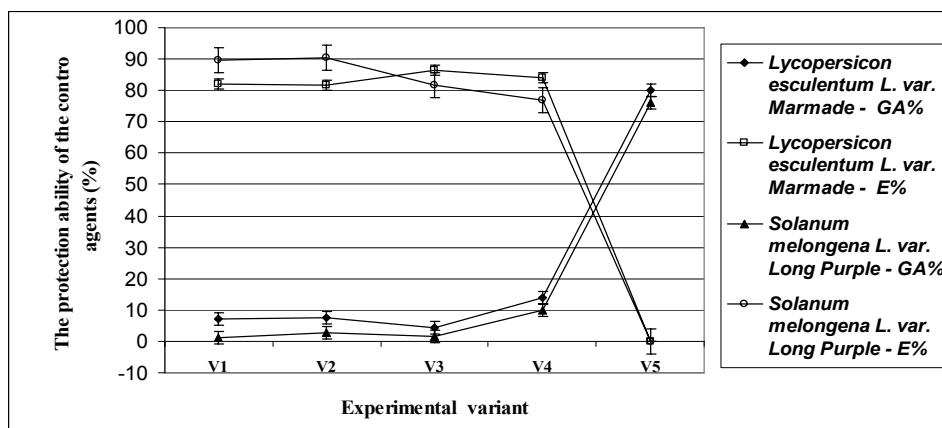


Fig. 2. The protection ability of the control agents (bio-product, antibiotic or copper sulphate) from the treatments used against the contamination with *Pseudomonas syringae* pv. *tomato* phytopathogen bacteria on *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple* plants.

The results obtained in the case of treatments applied for the protection of plants against the phytopathogen bacteria *Erwinia carotovora* strain (Fig. 3.)

The treatments made for the protection of tomato plants (*Lycopersicon esculentum* L. var. *Marmade*) contaminated with phytopathogen bacteria *Erwinia carotovora* strain have

generated superior experimental results, in case of bio-products B4 (4%) - E% = 87.5% - and BSP+BSV (4%) - E% = 82.8% - compared to the antibiotic (E% = 80.5%) or the chemical agent (E% = 78.8%).

The eggplant plants (*Solanum melongena* L. var. *Long Purple*) contaminated with phytopathogen bacteria of the *Erwinia carotovora* strain has a similar protection level for three of the treatments applied. Practically, the treatments applied generated experimental results which had similar values, both in case of using bio-products B4 (4%) and BSP+BSV (4%) - E% = 91.4%, respectively E% = 90.9% - and in case of using the antibiotic (E% = 89.5%). The treatment variant which used copper sulphate generated the lowest protection level for the eggplant plants (*Solanum melongena* L. var. *Long Purple*), having an efficiency of 85.0%.

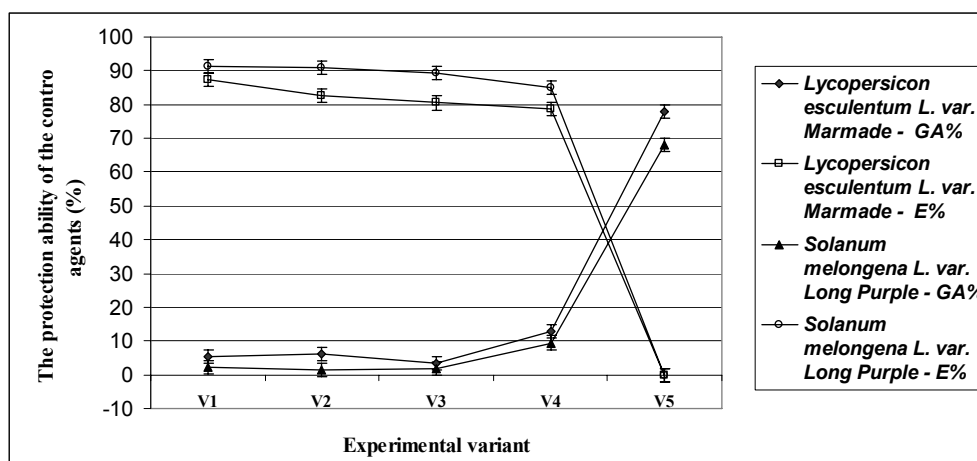


Fig. 3. The protection ability of the control agents (bio-product, antibiotic or copper sulphate) from the treatments used against the contamination with *Erwinia carotovora* phytopathogen bacteria on *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple* plants.

Based on the experimental data registered (Fig. 1-3), it can be noted that: the efficiency of the treatments based on the two bio-products (BSP+BSV and B4) – V1 and V2 – is situated at similar or even slightly superior levels as compared with those of the control treatments based on antibiotic V3 – or copper sulphate – V4. Based on what we know from the specialty literature in general, that the classical control agents (such as antibiotic or copper sulphate) have a much higher efficiency than that of the bio-products, it is obvious that the use of the two bio-products manufactured by INCDCF – ICCF based on some bacterial strains of *Bacillus subtilis* is a real success. It practically confirms once more the superior capacity of the bacterial strains of *Bacillus subtilis* to act as biotic control agents over the bacterial phytopathogenic agents.

As well, based on the experimental data registered (Fig. 1-3), it can be noted that the infected and untreated plants register attack degrees with values comprised between 60% and 80% (V5), which confirm the need to apply treatments with the purpose of avoiding the loss of vegetal biological material.

Conclusions

The investigations regarding the laboratory testing of the efficiency of the bio-products (BSP+BSV and B4) resulted from a new batch produced by INCDCF-ICCF Bucharest for the biological control of some phytopathogenic bacteria (*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato* and *Erwinia carotovora*), concerning both *Lycopersicon esculentum* L. var. *Marmade* and *Solanum melongena* L. var. *Long Purple*, led to the experimental results based on which we can conclude that:

- the efficiency of the treatments based on the use of the BSP+BSV bio-product at plants of the *Lycopersicon esculentum* L. var. Marmade reached the best value for the biological control of the phytopathogenic bacteria belonging to *Erwinia carotovora* strain because it registered a medium value higher than the similar values registered for the treatments based on the use of the chemical agent of treatment (copper sulphate) or of a classic antibiotic (cephotaxime);
- the efficiency of the treatments based on the use of the BSP+BSV bio-product at plants of the *Solanum melongena* L. var. Long Purple type reached the best value for the biological control of the phytopathogenic bacteria belonging to *Pseudomonas syringae* pv. *Tomato* strain because it registered a medium value higher than the similar values registered for the other treatments;
- the efficiency of the treatments based on the use of the B4 bio-product at plants of *Lycopersicon esculentum* L. var. Marmade reached the best value for the biological control of the phytopathogenic bacteria belonging to *Erwinia carotovora* strain because it registered a medium value higher than the similar values registered for the other treatments;
- the efficiency of the treatments based on the use of the B4 bio-product upon plants of *Solanum melongena* L. var. Long Purple reached the best value for the biological control of the phytopathogenic bacteria belonging to *Xanthomonas campestris* pv. *Vesicatoria* strain because it registered a medium value higher than the similar values registered for the other treatments;

In case of using, bio-products resulted from strains of the *Bacillus subtilis* for preventing the plant infections it has been noted that the preinfection with experimental stems induced protection at a satisfying and comparable level, both through the level produced with the help of the chemical agent of treatment (copper sulphate) and with the level realized by applying a classic antibiotic (cephotaxime).

The performed experimental investigations recommend the application of treatments based on the two bio-products manufactured by INCDCF-ICCF Bucharest for the biological control of the phytopatogenic bacteria (*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato* and *Erwinia carotovora*), concerning both *Lycopersicon esculentum* L. var. Marmade and *Solanum melongena* L. var. Long Purple.

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