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EFFECT OF APPLICATION OF BIOSTIMULANT PROTIFERT LN 6.5 ON THE EPIPHYTIC AND RHIZOSPHERE BACTERIA OF PEPPER SEEDLINGS

Katya DIMITROVA, Yordanka KARTALSKA, Nikolay PANAYOTOV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: katia_dimitrova@au-plovdiv.ng

Abstract

The study estimated the effect of biostimulant Protifert LN 6.5, with either foliar or soil treatment at three different doses - 1%, 2%, and 3% on the epiphytic and rhizosphere microflora of pepper seedlings of two varieties - Kurtovska kapia 1619 (KK) and Bulgarski rotund (BR). Irrespectively of the type of treatment, the higher number of epiphytic and rhizosphere bacteria was observed when the biostimulant was applied at dose of 2% but the effect was dependent on the pepper variety. Analysis showed that the type of treatment significantly affected only the microflora in the area of application. The doses of 1% and 3% did not affect the number of bacteria and in general, the estimated values was lower than those for control plants. The beneficial effect of biostimulants based on amino acids is related to improvement of nutrients absorption and the mineralization of organic matter which can increase soil microorganisms abundance. Further research can focus on mechanism of action of biostimulants and their specific effects on plant growth and productivity and on microflora.

***Key words**: biostimulant, epiphytic, pepper, rhizosphere microflora.*

INTRODUCTION

The modern agricultural practices aim not only an increase of plant productivity and yield but also preserving the soil fertility. However, the integral part of most practices is application of chemical fertilizers that according to the aim set by the EU should be reduced with 20 % until 2050. A number of studies have indicated that the soil microorganisms are the most dynamic indicators for agricultural systems and are important indicators of soil fertility (Mamilov & Dilly, 2002; Anderson, 2003). One of the possible alternatives to the widely applied chemical fertilizers are plant biostimulants which are defined as useful natural substances that can improve plant growth and development even at minimal doses (Luta et al., 2024). The category of plant biostimulants is comprised of various bioactive natural substances, such as hydrolysates of animal and plant proteins, which possess the potential to improve crop performance. Chemically, the protein hydrolysates are defined as “mixtures of polypeptides, oligopeptides, and amino acids that are produced from protein sources by partial hydrolysis” (Schaafsma, 2009). According to Calvo et al. (2014) the protein-based plant biostimulants are two types: 1) protein hydrolysates consisting of a mixture of peptides and amino acids of animal or plant origin, and 2) a single amino acids formulation of glutamate, glutamine, proline, etc. The biostimulant Protifert LN 6, which was used in the current study, belongs to the former of the abovementioned groups of protein hydrolysates. Furthermore, the use of protein hydrolysates obtained by animal residues processing can be used as an eco-friendly approach for waste reduction (Colla et al., 2015). The biostimulants have already been applied not only for staple crops aiming to achieve various effects, including stress alleviation, but also for vegetable crops (Salmani and Rezaei, 2023). The pepper is the second most important vegetable crop in Bulgaria, and it is widely grown across the country and is used for different purposes – as fresh, dry, canned food or for spice preparation. A large part of the vegetable crop is grown with pre-produced seedlings which is the main agrotechnological practice in vegetable production. It has been reported that the seedlings germination and plant growth were stimulated by the application of biostimulants and the effects were explained by the action of signaling bioactive molecules that participate in the primary and secondary plant metabolism (Calvo et al., 2014). Positive effects of application of biostimulant Protifert on potatoes have been reported by Ghemam et al. (2016) and on peach by Laita et al. (2022). The studies about the use of biostimulants on vegetable crops and especially about their effects on epiphytic and soil rhizosphere microflora are insufficient. Due to variety of available biostimulant and their versatile effects, there are still many uncertainties about the application times, methods and doses (Shahrajabian et al., 2021). The growing interest about the use of biostimulants demands for more information about their properties and usage.

The objective of the current study was to assess the effect of biostimulant Protifert LN 6.5 on the epiphytic and rhizosphere bacteria during cultivation of pepper seedlings.

MATERIALS AND METHODS

The experiments were conducted in the Experimental Field of the Agricultural University of Plovdiv, Bulgaria and analyses in the laboratories of the Departments of "Horticulture" and "Microbiology and ecological biotechnologies". Seeds from pepper variety Kurtovska Kapia 1619 (KK) and Bulgarian Rotund (BR) were sown in 176-hole styrofoam trays during the first ten days of March at each experimental year. In phase two true leaves, the seedlings were transplanted into pots (number 8) and were grown in an unheated plastic greenhouse. Each of the tested variants has three replications with 15 plants each. The experiments and analysis were done in the period 2021-2023. As soil substrate was used Peat Domoflor Mix 4 UAB Domoflor, Vilnius, Lithuania, with the following characteristics: 100% white peat with particle size 0-10 mm, pH 5.5-6.5, EC 0.6-0.7 mCm/cm, N:P:K - 14:16:18kg.m^3 and perlite in a ratio of 3:1. At the development of four leaves, the foliar fertilizer Protifert LN 6.5 (manufacturer SICIT 2000, Italy) at doses of 1%, 2% and 3% was applied on the leaves - 6-8 ml per plant, and on the soil - by watering each plant with 30 ml of solution of each dose. Control plants, depending on the type of application, were either sprayed or watered with the equal quantity of tap water. For determination of epiphytic microflora, a weighed averaged sample comprised of 5g of leaves per each variant was placed in a flask with 45ml of sterile distilled water. Soil samples were taken from the rhizosphere zone at a depth of 0 to 5 cm. The sample preparation for epiphytic microflora used the same approach. Petri dishes with nutrient agar (HiMedia, India) were inoculated with 100 µl of 10^{-4} dilution for each variant following the viable plate count standard procedure.

The presented data are averaged values from two year`s experiment (n = 4). Preliminary calculations, log transformation and graphs were done with Excel. The graphs columns presented the mean and error bars - standard deviation. Statistical analysis was done on log-transformed data with SPSS program (IBM, ver. 26). The analysis of skewness and kurtosis of data showed that z-values that exceeded the acceptable range of -1.96, +1.96 (Ghasemi et al., 2012). Additionally, the Levene`s test showed a statistical significance which violated the assumption about the homogeneity of variances and the data normal distribution. Since this assumption, as one of the main prerequisites for conducting the three-way analysis of variance was not met the comparison between variants was done by nonparametric Kruskal-Wallis H-test (Ostertagová et al., 2014).

RESULTS AND DISCUSSIONS

The current study was an integral part of two-year experiment that also assessed the effect of biostimulant Protifert LN 6.5 on several plant characteristics. The details of experimental design and results can be seen in Panayotov & Kartalska (2023). In brief, the results showed that application of Protifert LN 6.5 has a positive effect on the vegetative development of pepper seedlings. The analyzed root and leaves morphological parameters showed the highest values when biostimulant was applied at dose of 2%. The authors considered that the treated pepper seedlings have maintained a harmonious development without excessive growth or significant changes in the weight ratio of plant organs. Such development is a prerequisite for better seedlings adaptation and growth after planting. Furthermore, it was observed that the biostimulant has a positive effect on flower buds since the number of flower buds have increased

in both studied varieties (Panayotov & Kartalska, 2023).
The type of application of biostimulant affected both epiphytic and rhizosphere bacteria. The highest number of epiphytic bacteria was observed after foliar application of Protifert LN 6.5 at dose of 2% with 6.11 (± 0.13) and 5.89 (± 0.09) CFU/g log for variety KK and BR, respectively (Figure 1). The similar trend was observed on the rhizosphere bacteria but to a lower extend. The quantity of rhizosphere bacteria did not exceed 5.59 (± 0.82) and 5.17 (± 0.17) CFU/g for KK and BR variety, respectively.

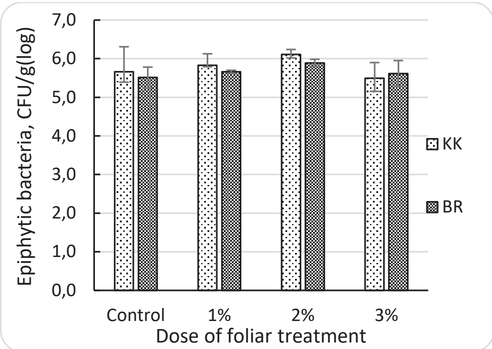

Figure 1. Epiphytic microflora (CFU/g, log) after foliar treatment

The foliar application of biostimulant at a lowest dose of 1% still has some stimulatory effect on the epiphytic bacteria in comparison to the control plants. The effect was more pronounced at dose of 1% for KK variety with value of 5.83 CFU/g (±0.30) than for the BR variety - 5.66 CFU/g (±0.04) with the corresponding control values of 5.66 (±0.65) and 5.51 CFU/g (±0.27), respectively. It appeared that the foliar application of biostimulant also have effect on rhizosphere bacteria. Despite that the differences were relatively small the number of rhizosphere bacteria in both varieties exceeded the control values but the effect was dose dependent since the dose of 2% provided higher values. The effect of foliar application of biostimulant on rhizosphere bacteria at either 1% or 3% for KK variety (5.15 and 5.00 CFU/g) showed that number of bacteria that did not exceed the control value – 5.46 (± 0.88). The same was true for BR variety but only for dose of 1% - 4.89 CFU/g (± 0.19) since the corresponding control was 5.11 CFU/g (± 0.17) and at 3% dose the number of rhizosphere bacteria was higher - 5.19 CFU/g (± 0.11) (Figure 2). It was not expected that the foliar treatment would affect significantly the rhizosphere microflora despite there are some suggestions that due to nutrients` transport in the plants the leaf treatment could affect the composition of root exudates and thus the soil microflora (Tekaya et al., 2021). In the current study, the biostimulant foliar treatment at a dose of 2% showed the higher stimulatory effect on rhizosphere microflora at KK variety (Figure 2).

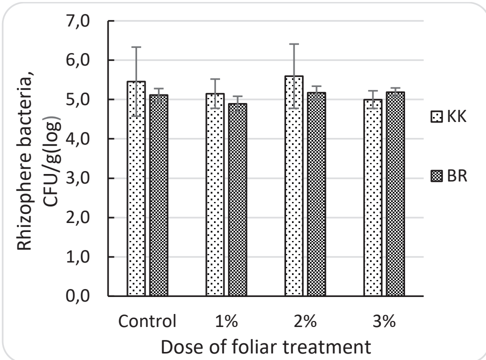

Figure 2. Rhizosphere bacteria (CFU/g, log) after foliar treatment

The soil application has insignificant effect on the number of epiphytic bacteria and the estimated values across the two pepper varieties were very similar. At dose of 2% the two pepper varieties showed the highest values - 5.12 (± 0.09) and 5.11 (± 0.11) CFU/g for KK and BR, respectively. Much alike were the estimated values at 1% and 3% - 4.49 and 4.35 for KK variety and 5.09 and 4.83 for BR variety. None of the values at 1% and 3% exceeded the corresponding controls - 5.05 (± 0.62) and 4.79 CFU/g (± 0.47) for the KK and BR varieties, respectively (Figure 3). The higher value of rhizosphere bacteria after soil application was estimated for KK variety at dose of 2% - 6.27 CFU/g (± 0.27). The same trend was not so noticeable for BR variety which rhizosphere bacteria reached 5.49 CFU/g (± 0.21). The use of amino acids-based biostimulants could improve the assimilation of nutrients and the mineralization of organic matter, which affect the amount of soil microorganisms and their activity (Wadduwage et al., 2024).

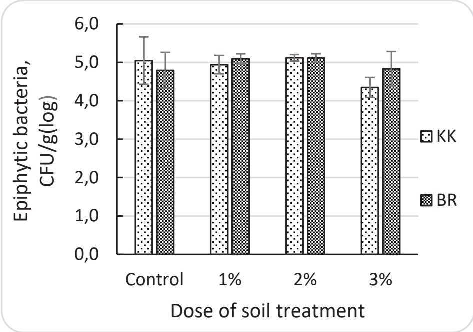

Figure 3. Epiphytic bacteria (CFU/g, log) after soil treatment

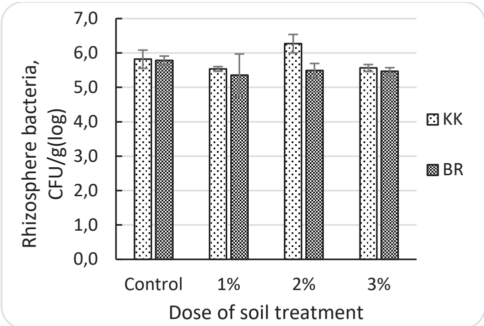

Figure 4. Rhizosphere bacteria (CFU/g, log) after soil treatment

The analysis revealed that application of biostimulant at dose of 1% and 3% did not stimulate the number of rhizosphere microorganisms and their quantity was lower in compareson to the control. For KK variety the two values were very similar - 5.54 and 5.56 at 1% and 3% dose and the corresponding control value was 5.84 CFU/g (± 0.27). The BR variety showed a better correspondence between the doses of application and the number of rhizosphere bacteria since at the dose of 1% the value was 5.35 CFU/g (± 0.62) and at 3% - 5.47 (± 0.11) CFU/g despite that the both was lower than the control 5.78 CFU/g (± 0.13). One possible explanation of the observed trend could be related to the fact that higher doses of amino acids could negatively affect the soil microflora. Kajikawa et al. (2002) reported both stimulatory and inhibitory effect of several amino acids. The microorganism responded differently to single amino acids and to their specific combinations.

In general, when applied at optimal doses organic biostimulants positively affect plant growth and productivity through changes in epiphytic and soil microorganisms (Mahnert et al., 2018). According to Sivojiene et al. (2021) the soil microorganisms are one of the main indicators for assessing stability of a given soil ecosystem and its fertility. The fertilizers can affect the microorganisms and as a result their influence on the soil processes, exchange of substances in the soil-colloid solution and plants mineral absorption (Jacoby et al., 2017, Jannson & Hofmockel, 2018). The use of plant biostimulants can increase the amount of soil microbiota involved in the organic matter decomposition (Sun et al., 2014; Daquiado et al., 2016). According to Hole et al. (2005) the changes in microflora abundance can be used as an indicator for assessment of different organo-minerals applied in the agricultural practice and for determination of potential fertility of soil.

Table 1. The mean rank for effect of dose of biostimulant and type of treatment

Variety/ Treatment	**Factor/ *p* value**	**Rank**			
Foliar treatment	***Dose***[1]	**Control**	**1%**	**2%**	**3%**
KK	p = .219	16.94	15.38	21.69	12
BR	p = .481	15	13.44	20.38	17.19
Type of treatment[2]		E	R		
KK	p < .00	19.94*	13.06		
BR	p < .03	23.91*	9.09		
Soil treatment	***Dose***[1]	**Control**	**1%**	**2%**	**3%**
KK	p = .201	19.13	14.56	20.63	11.69
BR	p = .858	18.69	14.75	16.75	15.81
Type of treatment[2]		E	R		
KK	p < .00	9.16*	23.84		
BR	p < .00	8.5*	24.5		

Legend: [1] - dose, df. 3, n = 8, [2] - type of treatment, df. 1, n = 16, abbr. E - epiphytic, R - rhizosphere bacteria

A Kruskal-Wallis H test did not show a statistically significant difference between the studied doses on the microflora of two pepper varieties (Table 1). However, the variety KK showed higher ranks of microflora both at foliar and soil treatment at dose of 2% but this was true for variety BR only after foliar treatment. The effect of soil treatment on microflora of BR variety at dose of 2% showed a mean rank (16.75) which exceeded the other doses of application but not the control one (18.69). In both pepper varieties, the type of treatment significantly affected the number of cultivable bacteria of the corresponding microflora`s category in an objective and consistent manner.

CONCLUSIONS

The main purpose of application of amino acid-based plant biostimulants is towards increase plant growth and development and alleviation of plant stress. Due to immense role of microorganisms in soil processes and plant productivity the contemporary studies have focused on revealing the effect of biostimulants both on epiphytic or rhizosphere microflora. The results from the current study showed that the application of biostimulant can affect both epiphytic and rhizosphere microflora irrespectively to the type of application. However, the observed effect was dose dependent and the soil application at dose of 3% indicated a slight negative effect on rhizosphere microflora. The observed stimulatory effect on microorganisms could be related either directly to the provided amino acids or to the alteration of some nutrients or bioactive substances. The response of microflora to different doses of applied biostimulant was not statistically significant but despite the relatively similar values, there was a consistent difference between the two pepper varieties. This imply that application of biostimulant should take into account some specifics of crop varieties and their interactions with the microorganisms in order to fulfill its potential as environmental-friendly approach in agricultural practices for vegetable crops.

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