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The Use of Air Induction Nozzles for Application of Fertilizing Preparations Containing Beneficial Microorganisms

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Abstract: The work presents the structure and characteristics of field sprayer nozzles, as well as their impact on the survival of beneficial organisms in the selected fertilizing preparations. EŻK and EŻKT nozzles, (EŻK and EŻKT are trade names of single and twin jet air induction nozzles, respectively), that are available on the market have shown low efficiency in the discussed characteristics. Survival of microorganisms under initial conditions at 13.6×10^6 cfu/mL and pressure of 0 MPa, under critical conditions dropped to 1.7×10^6 cfu/mL for EŻK02 and 1.2×10^6 cfu/mL for EŻKT02, in both variants at a pressure of 0.5 MPa. When increasing the flow rate of the components, i.e., the size of the outlet orifices, it was observed that the survival of microorganisms increased by about 11.3% compared to the previously tested component. This resulted from the negative impact of the following: the pressure generated by the application device, number of outlet orifices, and size of an outlet orifice. The results of survival of microorganisms are given in the colony-forming unit (CFU). In addition to providing guidelines useful in the creation of a prototype sprayer intended for use in the application of microbiological preparations, the presented characteristics are a source of information for the end user as regards the proper conditions for the application of these preparations.

Keywords: beneficial microorganisms; air induction nozzles; fertilizing preparations; survival characteristics; sustainable agriculture

1. Introduction

In the current scenario where increasing trend in the numbers of sales and production of preparations consisting of i.a. beneficial microorganisms occurrs, the popularity of ecological products can be observable. This favours the emergence of new possibilities in the field of biological plant protection and, consequently, increases the availability of related agricultural plant products [1–3]. For example: in 2007, 225 preparations with beneficial microorganisms were registered in the European Union countries, while in 2015 their number amounted to 1417 [4,5]. It is often noticeable that the more products are on the market, the better their quality is. However, as the following literature review shows, this tendency is not reflected.

Substances containing i.a. microorganisms are referred to as preparations with beneficial organisms. These are natural products based on carefully selected compositions of non-genetically modified strains of microorganisms contained in a fermented mixture of natural ingredients. The products are characterised by probiotic, antioxidant, and bactericidal properties against unwanted pathogens [6].

The measurable properties affecting the environment mainly include: the formation of a lumpy structure and regulation of air-water conditions of the soil, soil and plant conditioning, restoring hardly accessible components to circulation, activation of manure fermentation, optimization of water management, intensification of microbiological processes in soil, acceleration of organic mass decomposition, and improvement of the composting process [7]. They are supposed to have a positive effect on the crops of the cultivated plant. When trying to determine the exact composition of the substances offered on the domestic market by seeking information from manufacturers, one can usually find out that a given composition is properly selected and it is protected by patent law. According to [8–10], they usually contain *Bacillus* and *Pseudomonas* bacteria and the following genera of fungi: *Trichoderma*, *Baeuveria*, *Coniothyrium*, *Matharhizium*, *Pythium*. Biopreparations which composition is based on viruses and microscopic nematodes *Heterorahabditis* and *Steinernema* can also be found on the market.

The registration process for biological plant protection products is as stringent as that of conventional plant protection products [11]. Manufacturers are required to provide a lot of information: the name and characteristics of the microorganism used, data showing the usefulness of the preparation, shelf life of the product, quantitative composition of the preparation, and all information related its use; however, there is no requirement of confirming the effectiveness of the product in field tests [3]. The purpose of these restrictions is to ensure that the substances are environmentally safe. This gives these products an unquestionable advantage over chemical plant protection products. This is conducive to ongoing attempts in European Union countries aimed at facilitating the possibility of registering and manufacturing biological plant protection products [12]. The process of production of a substance begins with the preparation of the stock culture which is subsequently proliferated in specially prepared devices called fermenters, and then gradually provided with increasing amounts of properly selected liquid nutrient medium. The number of such stages depends on the cycles chosen for a given final product, which in turn depend on the requirements of the strain meant for production [13].

The aforementioned registration procedure for preparations containing beneficial microorganisms does not require manufacturers to submit any detailed studies on the efficacy of the products. Their low efficacy is also mentioned in numerous publications. According to one of the national manufacturers of substances with beneficial organisms [7], the recommended form of application of the product is spraying it with the use of classic field sprayers, without the need to use any additional spraying components or their modification. In addition, there are no recommendations as to the value of suggested pressure set in the sprayer manifold. This method of application is criticised in [14], where the authors state that natural bacteria have proliferated in the soil during the centuries-old process. It was found that the amounts of beneficial organisms that are sprayed in accordance with the recommendations, as presented in the study [15], after application to the soil will be eliminated by the bacteria originally residing there [16]. It results from quantitative proportions. 1 millilitre of microbiological preparations contains 10^6–10^9 bacteria (cfu/mL), while 1 g of unmodified soil contains up to 10^{11} bacteria (cfu/mL) [9,14]. When analysing the amount of the preparation applied, it is observable that the number of bacteria is scant in comparison to the natural resources. It was found that there are fewer bacteria in 1 litre of the preparation than in 100 g of soil [13,15].

Research on the composition of biomass in soil conducted for several years in Switzerland showed that there were no changes in the soil after using preparations containing beneficial microorganisms [17–21]. The research consisted in a comparative analysis of the composition of soil after the application of microbiological preparations and without such application. Soil samples were taken every 3 months during 4 years of the study. Subsequently, the soil composition was compared. The researchers found no significant changes in biomass composition of the soil, nor any

effects on dehydrogenase accelerating the oxidation of organic matter or improvement in soil air exchange capacity or soil microbial activity [4,18,22–25]. What is more, no positive effect of the microbiological preparation on the composition and properties of the soil was found when it was used in the climate of Central Europe.

The components of field sprayers that affect the application process of fertilizing preparations are jet nozzles. In the authors' opinion, which is based on divagations, the low efficacy of the discussed substances results from the construction of the spraying components. The most common ones are classic nozzles as well as compact air-induction nozzles [26–29]. For almost half a century, conventional spray nozzles dominated the market of sprayer parts and were popular among farmers due to the simplicity of installation and possible repairs. However, the downside of this component was its lack of resistance to adverse wind conditions, as the application of liquid with the use of a conventional nozzle was possible only at the wind speed of up to approx. 2 m/s [28]. This was the reason for looking for more effective solutions. In turn, single and twin jet air induction nozzle (Figure 1a–d) makes it possible to spray the liquid at wind speeds of up to 7 m/s [29]. When analysing the cross-section of the construction of air induction nozzles (Figure 1a, b), the authors found numerous obstacles that inhibit the flow of the liquid, which, hypothetically, can be the reason for the low survival of microorganisms. The said obstacles as well as the pressure applied by the field sprayer manifold are the reasons for the low efficacy of fertilizers. The tested nozzle is the most effective of the available spraying parts, which was presented in detail in [16].

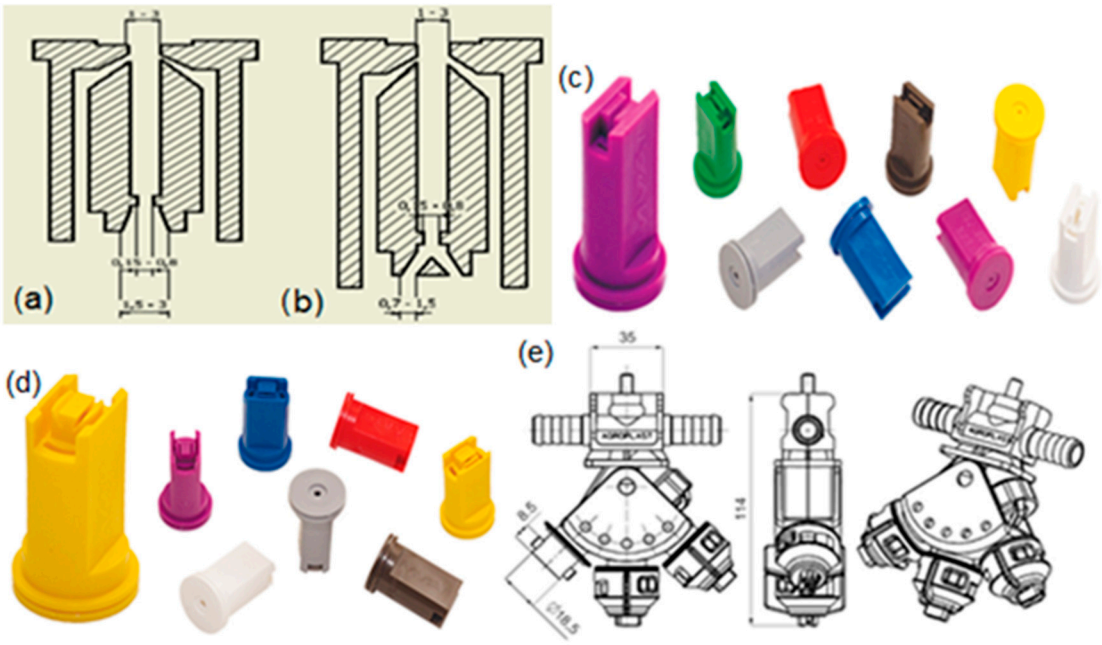

Figure 1. Overview picture: (**a**) cross-section of the EŻK compact air induction nozzle, (**b**) cross-section of the EŻKT compact twin jet air induction nozzle, (**c**) EŻK compact single jet air induction nozzle, (**d**) EŻKT compact twin jet air induction nozzle, (**e**) structure of the nozzle body [16].

The adopted method of application of the biological preparation is based solely on the manufacturer's recommendations [16]. The literature review conducted by the authors suggests that the low efficacy of the discussed preparations is caused by its improper application and, in particular, by the impact of the pressure applied by the field sprayer manifold on the survival of microorganism. The aim of this study is to present the characteristics of survival of beneficial microorganisms by way of examining the method of applying fertilizing preparations containing beneficial microorganisms with the use of air induction spray nozzles.

The scope of this study includes an experiment conducted by the authors in order to determine the parameters of devices for the application of preparations containing beneficial microorganisms.

2. Materials and Methods

The research was conducted on a sprayer [30] equipped with: a 3000 L tank, 3 hydraulic agitators, the most important component of which was a Venturi tube, an induction bowl, a radial diaphragm piston pump, an electrohydraulic manifold used to control the amount of the working liquid, a filter system, a boom, 42 spray heads and 2 sets of air induction spray nozzles, whose parameters' influence on the survival of microorganisms is the subject of this study: EŻK compact air induction spray nozzle (6 components) (Figure 1a,c), size: 02; 025; 03; 04; 05; 06 and EŻKT compact air induction twin jet spray nozzle (Figure 1b,d), size: 02; 025; 03; 04; 05; 06; [16,31]. According to the authors, it is also necessary to determine the impact of other sprayer parts, such as manifold, pump, and agitators, on the survival of microorganisms contained in the selected fertilizing preparation. Chojnacki [32] partially undertook this issue in his research, where he showed that the losses in microorganisms occur already at the stage of the mixing process with the use of hydraulic agitators and an internal sieve. By way of extrapolating to the aforementioned studies, it was decided to disconnect the internal agitator which generated loss [33], assess the negligible influence of the pump and manifold on the survival of microorganisms.

The survival of microorganisms was tested in EmFarma Plus biological preparation containing beneficial microorganisms [7]. The product consists of lactic acid bacteria, photosynthetic bacteria, fermenting fungi, yeast, organic sugar cane treacle, wine vinegar, ethyl alcohol, and revitalized, non-chlorinated water [7]. The stock culture includes the following bacteria: *Bifidobacterium Animals*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactococcus diacetylactis*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Bacillus subtilis var. natto.*, *Saccharomyces cerevisiae*, *Rhodopseudomonas palustris*, however, according to the manufacturer, the dominant culture in the substance is *Bifidobacterium bifidum* [16].

The authors used in the study a digital microscope (Levenhuk D70L), which was equipped with an improved Neubauer chamber which makes it possible to count the number of organisms in 1 mL of substance (cfu/mL) [16,34–37]. The collected samples were applied on a lawn culture at the concentration of 2% agar and 2% yeast. The method of proliferation of the bacteria was taken from Andrews' research [14]. The authors used also the following laboratory tools: an electronic pipette, 8 mL Petri dishes, and sterile plastic containers for taking samples from the sprayer.

At the beginning, the appropriate concentrations of the water solution and the preparation tested were determined. According to the manufacturer of the fertilizer [15], the possible proportions vary from 1:20 to 1:65 for use in plantation areas. The microscopic amount of bacteria in selected concentrations was analysed and it was found by way of an experiment that in case of the concentration of 1:65 there is a lower risk of error in the procedure of counting of microorganisms. This is due to the fact that there are fewer objects that are observed under the microscope.

200 L of the solution at the concentration of 1:65 was poured into the previously disinfected tank of the field sprayer. The tested pressure was applied by a throttling valve, read with the use of a mechanical manometer and controlled with an electronic manometer.

Samples of the liquid were taken from 10 nozzles located across the entire boom, 2 nozzles in each boom section. Such method of sampling was employed to obtain accurate results, which could be affected by the length of the liquid supply lines feeding the liquid to the nozzles. Each sample (in the volume of 2 mL) was taken separately by means of an electronic pipette, applied to a solid lawn culture and incubated. After 72 h of incubation, individual samples were counted in the Neubauer chamber using the Equation (2) by determining the number of cfu/mL, and the mean value was calculated for them according to the Equation (1).

Sampling, incubation, calculating, selection of concentration, and pressure setting in the sprayer manifold are presented in more detail in [16], due to the lack of space in this study.

$$x_{sr} = \frac{x_1 + \cdots + x_{10}}{10} \quad (1)$$

where:

$x_{śr}$-mean value of measurements,
$x_{1–10}$-value of individual measurements,

The process of calculating the values (cfu/mL) was based on the Equation (2):

$$\text{cfu/mL} = \frac{x}{S \cdot h} \times \frac{V_a}{V} \times 1000 \quad (2)$$

where:

cfu/mL-colony-forming units in one millilitre (pcs/mL),
x-the number of counted microorganisms in the Neubauer chamber (pcs),
S-total area of the half-chamber where living organisms were counted (mm^2),
h-depth of the Neubauer chamber (mm),
$\frac{V_a}{V}$-concentration of the tested preparation $\left(\frac{65}{1}\right)$.

For the purpose of statistical analysis, descriptive statistics needed to perform normality tests for each variant were prepared for the results obtained. Based on the tests, it was assumed that this is a normal distribution, and therefore a test determining the Pearson correlation coefficient (*r)* was employed for further statistical analysis. The conducted analysis determined the coefficient r and r^2 (coefficient of determination) for all the discussed sizes. The statistical analysis was performed in the Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) using the equations presented above.

3. Results and Discussion

Due to the employed research methodology, it was possible to obtain characteristics of the influence of the pressure and the size of the nozzle outlet orifices in a field sprayer on the number of colony-forming units in one millilitre of liquid (cfu/mL). On the charts below, the mean values of 10 measurements constituting 1 sample were marked with black points, while the mean values of 3 samples constituting the final value were marked in red.

Figures 2–7 present detailed characteristics of survival of colony-forming units in the preparation [7] for EŻK compact single jet air induction nozzle at the flow rate 02–06.

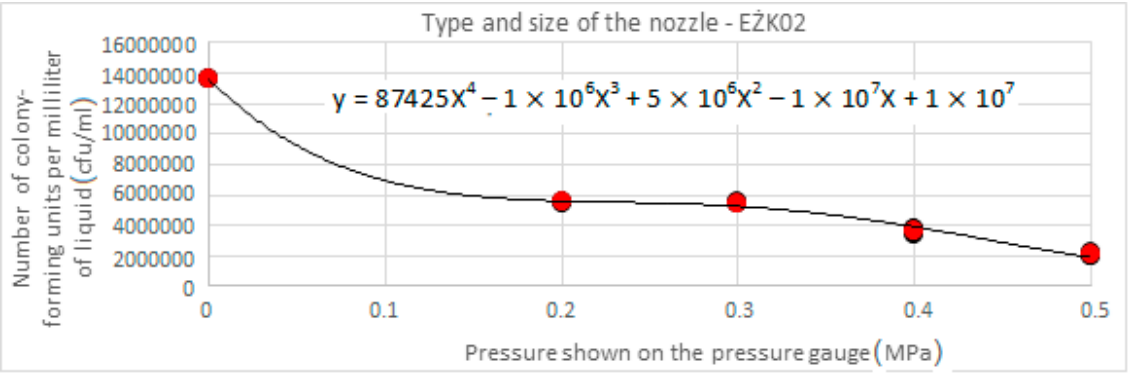

Figure 2. The influence of pressure on survival of cfu/mL of liquid for EŻK02. The vertical axis shows the cfu/mL values, while the horizontal axis shows the pressure (MPa) values set in the throttling valve and read in manometers.

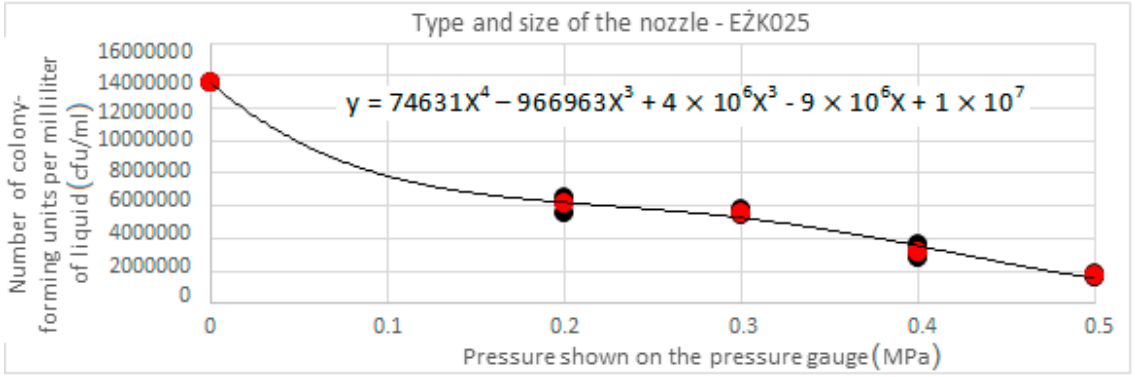

Figure 3. The influence of pressure on survival of cfu/mL of liquid for EŻK025.

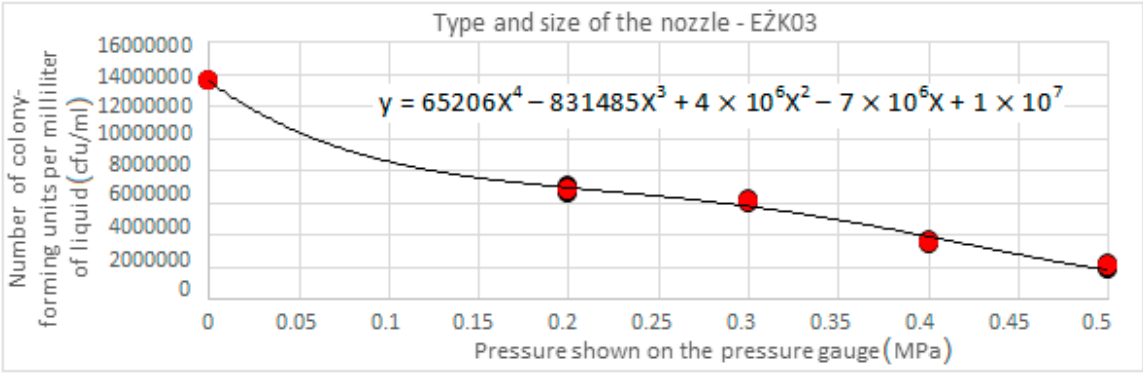

Figure 4. The influence of pressure on survival of cfu/mL of liquid for EŻK03.

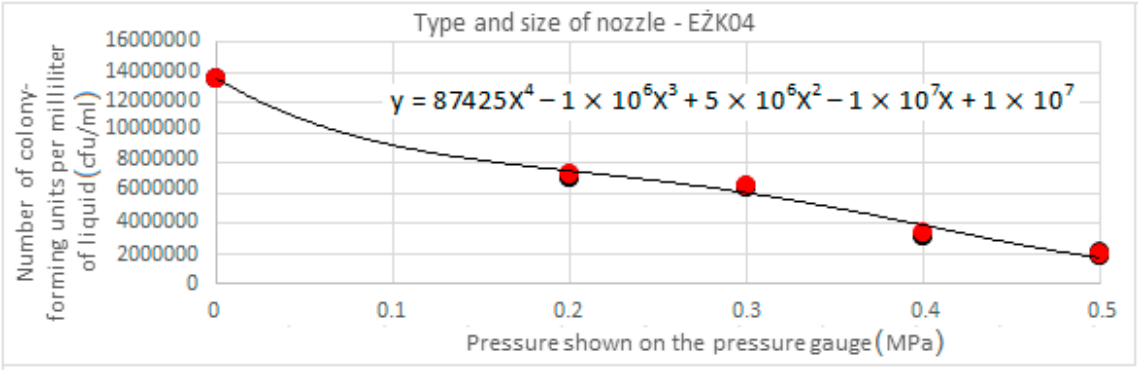

Figure 5. The influence of pressure on survival of cfu/mL of liquid for EŻK04.

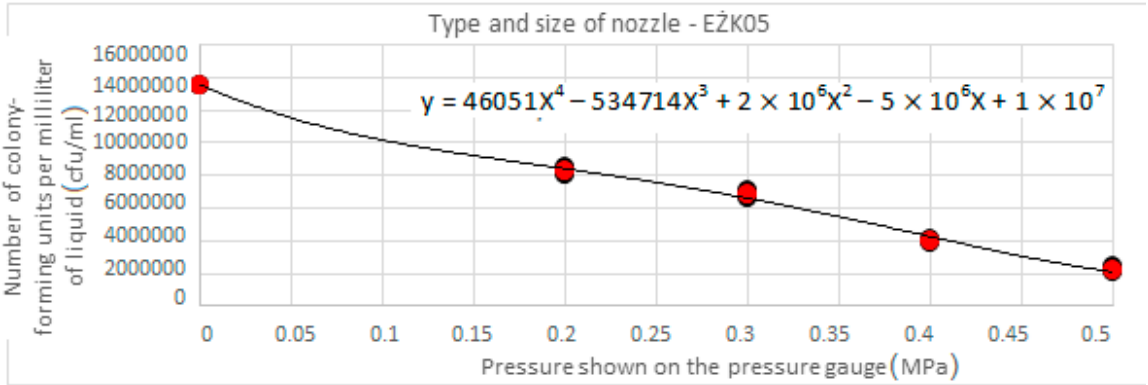

Figure 6. The influence of pressure on survival of cfu/mL of liquid for EŻK05.

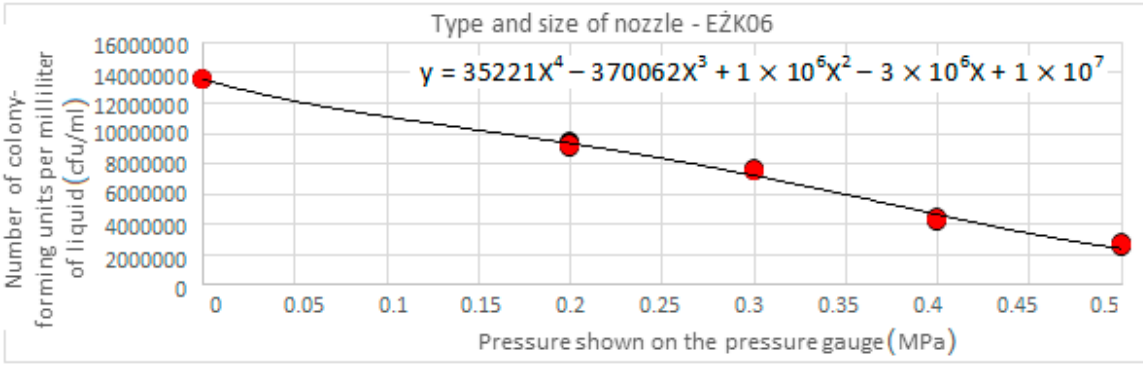

Figure 7. The influence of pressure on survival of cfu/mL of liquid for EŻK06.

Figures 8–13 present detailed characteristics of survival of colony-forming units in the preparation [7] for EŻKT compact twin jet air induction nozzle at the flow rate of 02–06.

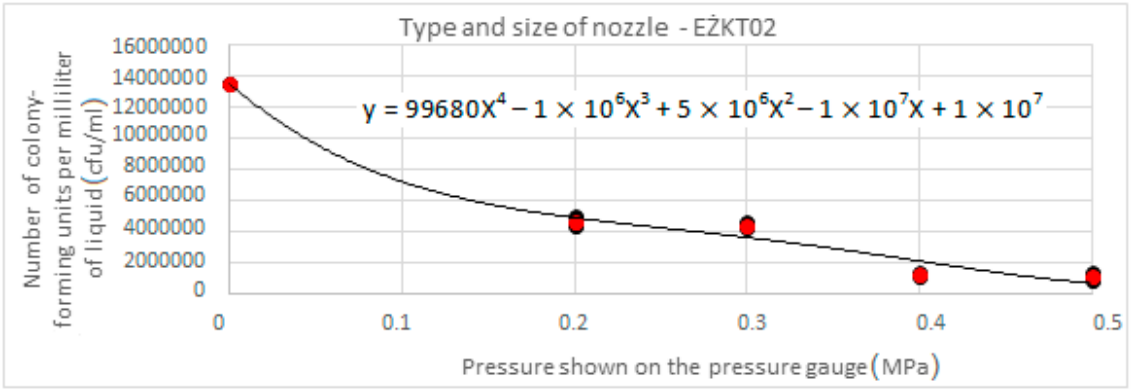

Figure 8. The influence of pressure on survival of cfu/mL of liquid for EŻKT02. The vertical axis shows the cfu/mL values, while the horizontal axis shows the pressure (MPa) values set in the throttling valve and read in manometers.

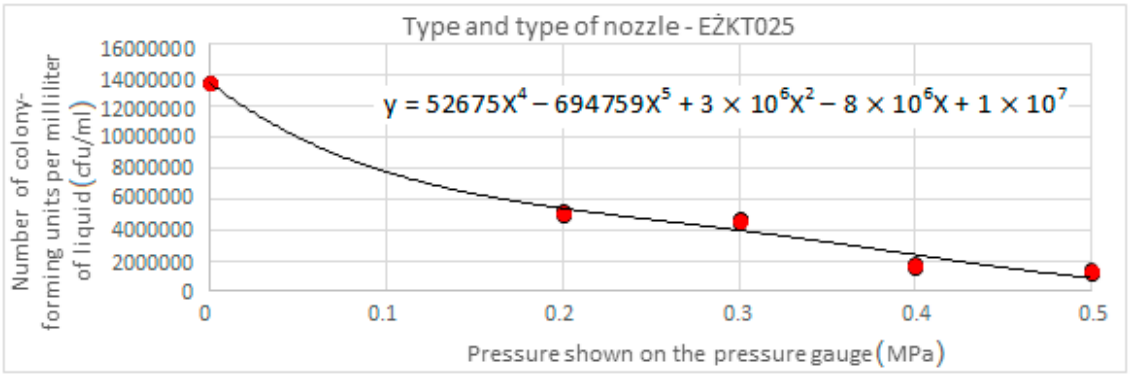

Figure 9. The influence of pressure on survival of cfu/mL of liquid for EŻKT025.

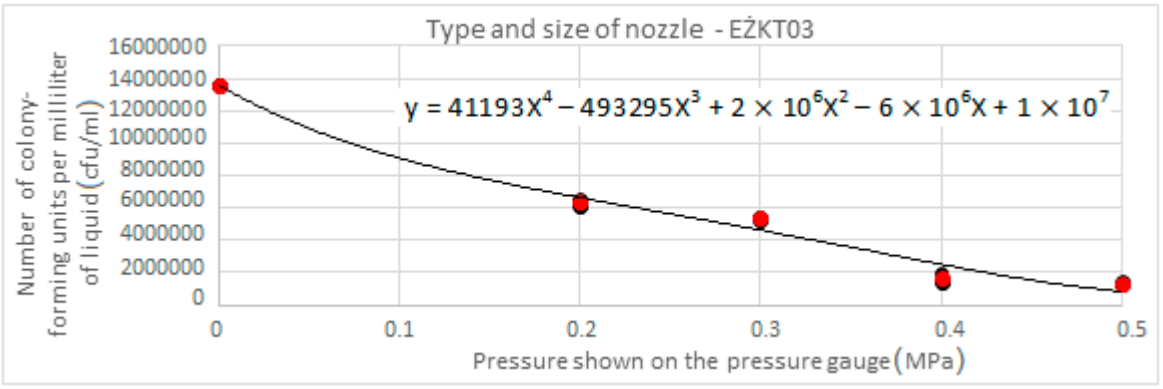

Figure 10. The influence of pressure on survival of cfu/mL of liquid for EŻKT03.

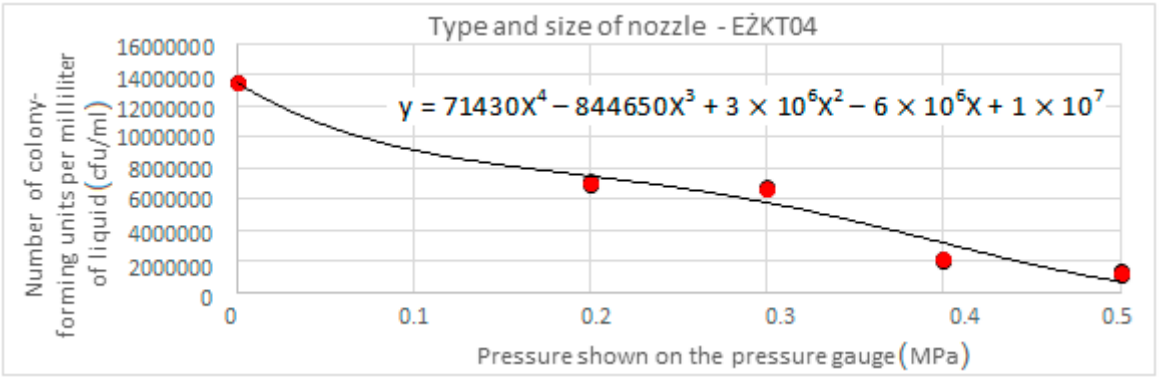

Figure 11. The influence of pressure on survival of cfu/mL of liquid for EŻKT04.

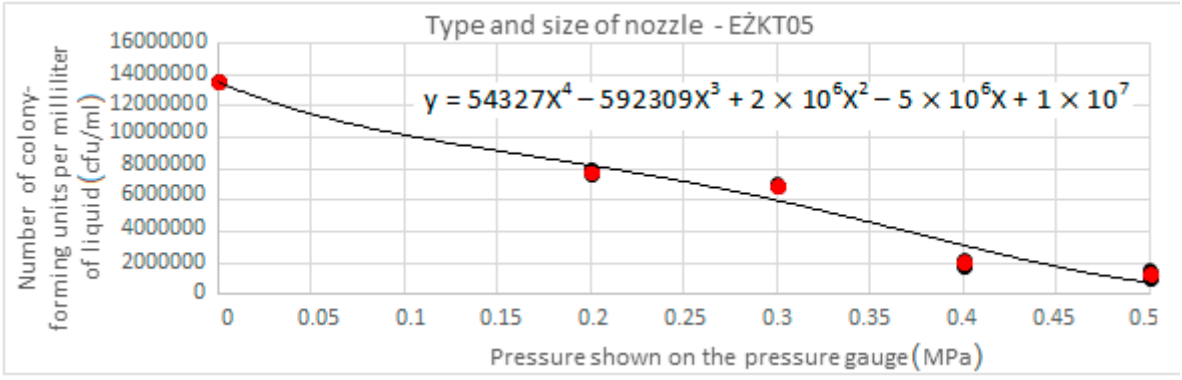

Figure 12. The influence of pressure on survival of cfu/mL of liquid for EŻKT05.

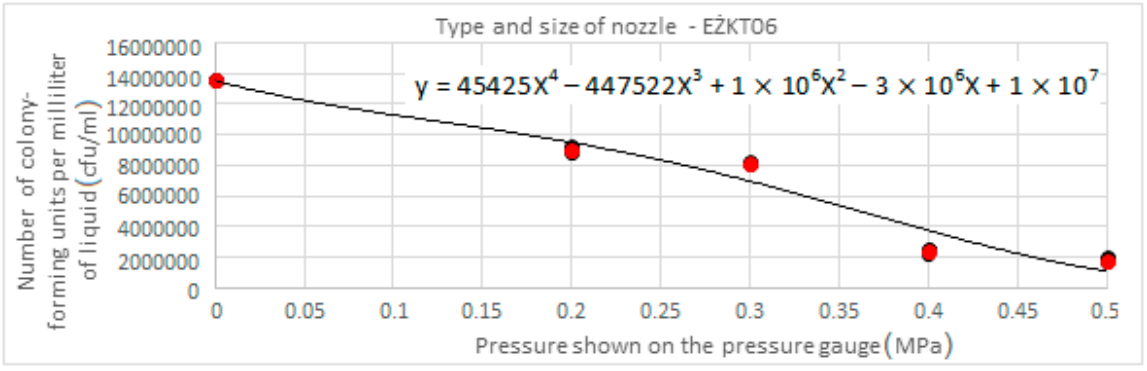

Figure 13. The influence of pressure on survival of cfu/mL of liquid for EŻKT06.

The discussed fertilizing preparations containing beneficial microorganisms are characterized by low efficacy. This can be stated on the basis of the literature review [4,8,13–15,17–25,33,38,39]. It seems that when examining the influence of these substances on the soil, the impact of spraying equipment on microorganisms contained in the substance is overlooked [16].

According to the recommendations of manufacturers of fertilizing preparations with beneficial microorganisms, only the application of preparations with no micronutrients can be efficacious, which is contrary to the results of studies showing that positive effects are obtained only in a combined application of the preparation with composted biomass [20,21].

The conducted research has shown that the highest survival rate is observed in the nozzles with the largest flow rate-06. However, from the farmer's point of view, their size, i.e., the diameter of the outlet orifices, does not allow the user to dose the liquid properly. For example, the EŻKT 06 nozzle at a pressure of 2 bar has a flow rate of 2.23 L/min and at a pressure of 4 bar it amounts to 3.56 L/min. As a result, the flow rates per 1 ha at a speed of 4 kph amount to 739 L and 1168 L respectively, and these values are not satisfactory due to the capacity of the sprayer tank [16].

The following two of the tested field sprayer nozzles that can be really useful for a farmer are characterised by the highest survival of beneficial microorganisms: EŻK04 at the pressure range 0–0.3 MPa with the mean value of 6.9×10^6 cfu/mL and EŻKT04 at the pressure range 0–0.3 MPa with the mean value of 6.6×10^6 cfu/mL. These values represent almost half of the number of surviving microorganisms contained in the tested substance in relation to the control sample. What is more, the flow rate of these nozzles is of 642–709 L per 1 ha, which is still challenging for the user, however, it is more possible to be achieved than the previously discussed components [16].

One of the most important conclusions of this study is the negative impact of increasing the number of outlet orifices. When comparing directly two component with the same flow rates (e.g., EŻK02 to EŻKT02 etc.), it was noticed that at the pressure range of 0–0.3 MPa the difference in survival rate was 7.2% on average, while at the pressure range of 0.3–0.5 MPa it was, on average, 44.3% more living organisms in a single jet nozzle [16].

On the example of the tested nozzles, in selected flow rates it was observed that the bigger the diameter of the outlet orifice, and thus the higher the flow rate of the nozzle, the higher the survival of microorganisms in the preparation used [15,16,33]. Having obtained the above test results, it can be concluded that from the perspective of the end user applying the fertilizer preparation the most effective is the use of a single jet nozzle of a flow rate of 05 at a pressure in the range of 0.2–0.3 MPa, as the survival of microorganisms is in this solution at the highest possible level of 47.7% of initial value.

The obtained values of correlation and determination coefficient for different variants of the tested nozzles are presented in Tables 1 and 2.

Table 1. The values of correlation and determination coefficient for the EŻK nozzle.

Size of the Nozzle	**02**	**025**	**03**	**04**	**05**	**06**
Value of the r^2 coefficient	0.8223	0.8555	0.9000	0.9258	0.9285	0.9332

Table 2. The values of correlation and determination coefficient for the EŻKT nozzle.

Size of the Nozzle	**02**	**025**	**03**	**04**	**05**	**06**
Value of the r^2 coefficient	0.8723	0.8975	0.9310	0.9435	0.9662	0.973

The study on the Pearson correlation coefficient clearly demonstrated that in all the tested variants the relationship is strong and negative. It can be described as strong because the value of r coefficient ranges between −0.99 and −0.9. On the other hand, the negativity means that as the values on the *x*-axis (pressure) increase, the values on the *y*-axis decrease (cfu/mL). A nozzle with highest value of r coefficient (−0.9868) was the EŻK single jet air induction nozzle of the flow rate of 06, which means that with the increase in pressure, the smallest decrease in the colony-forming units was observed

in this variant. A nozzle with the lowest value of r coefficient (−0.9068) was the EŻKT nozzle of the flow rate of 02, where the decrease in the colony-forming units was the smallest compared to all the tested variants.

When analysing values of the determination coefficient r^2, the highest value (0.9739) was observed in the EŻK twin jet air induction nozzle of the flow rate of 06, while the lowest value (0.8223) was observed in the EŻKT twin jet air induction nozzle of the flow rate of 02. All of the primary data is shown in [16].

4. Conclusions

The aim of this study was to determine the relationship between the pressure applied by the hydraulic manifold and the size of the nozzle outlet orifice in the field sprayer on the survival rate of colony-forming units in a fertilizing preparation. Due to the developed research methodology it was possible to confirm the validity of assumptions and, as a consequence, to draw the following conclusions:

1. The increase in pressure applied by the manifold in both sprayer variants caused a decrease in survival. Based on the example of the EŻK component, it was found that at a pressure of 0.2 MPa, the survival decreased to approx. 40.4% of the initial value; similarly, in case of the EŻKT component this value amounted to 33.8% of the initial number of microorganisms.
2. The lowest survival rate was noted for the EŻKT 02 nozzle, which at a pressure of 0.5 MPa caused death of microorganisms at the level of 91.4% of the initial value.
3. For all tested elements, the lowest survival occurs at a pressure of 0.4–0.5 MPa and ranges between 9–17.6%. The maximum suggested pressure for the user applying the preparation is 0.3 MPa.
4. Low effectiveness of preparations containing beneficial organisms may result from their incorrect application caused by the pressure applied in the field sprayer. Consequently, it is necessary to look for other alternative methods of applying the discussed substances. Based on the conclusions drawn, it is advisable to use components with the largest possible size of nozzle outlet orifices, which do not have any obstacles that inhibit the flow of the liquid.

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