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Research paper

Fungicidal and antibacterial activity of the hydrogel compositions with silver

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Abstract

Contamination of the rhizosphere by pathogenic microorganisms is a universal soil-ecological problem leading to heavy losses in tuber and root crop yield of agricultural crops. Suppression of these microorganisms is possible because of special gel compositions with silver ions and nanoparticles as plant-protecting agents. An efficiency test of gel-silver inhibitors was carried out on two common pathogens, the causative agents of late blight (*Oomycete Phytophthora infestans*) and black leg of potato (*Enterobacterium Pectobacterium atrosepticum*). The study revealed an exponential relationship between inhibitor dose and linear (by diameter) and nonlinear (by area) specific growth rates of colonies of microorganisms. According this model the EC_{50} values of the gel compositions varied from 1 to 86 ppm of the active substance (Ag) and were consistent with other research findings. The fungicidal effect was sufficiently (3-30 times) lower for gel compositions based on ionic silver in comparison with colloidal forms; whereas bactericidal properties for both agents were similar.

Keywords: *Plant Pathogens, Hydrogel compositions, Silver nanoparticles and ions, Plant protection agents, Rhizosphere;*

1. Introduction

Most known plant-protecting agents (PPAs) target a complete surface treatment and pest control of vegetative and genital organs located above the soil. The rhizosphere protection against pathogenic flora in the soil and/or on seed grains is important for yield formation and ensures survival of tuber crops, root crops, young trees and shrubs. Annually, about 20-30% of potatoes growing in Russia have been lost from similar pathogenic agents depending on the cultivar, and in some years, the loss exceeded 50%. In a storage period, rot had destroyed 30-40% of tubers [1]. Average worldwide losses reach 8-10%, which is worth of several billion dollars [2]. In some European countries like Poland, losses can reach 20-25% annually [3]. Pathogenic microorganisms can cause extra damage to seed potatoes because a causative agent of disease spreads in different areas of potatoes growing through infected tubers of seed grains. This results in a serious damage to the industry.

Suppression of such microorganisms is possible through a formation of local gel structures with modern PPAs at the rhizosphere, in particular with an addition of silver [4]. Unlike aqueous solutions and suspensions used in the fight against root rot [5], such structures are firmly fixed in the root area, thus preventing vertical migration and removal of water and water-soluble substances stable in their composition [6-9]. For toxic PPAs, this means a decrease of the ecological risk of contamination of the adjacent environments (i.e.; soil and groundwater). Hydrogels act as augmenting agents that improve the water-retention and absorption capacity of soils. Their use in soil engineering allows extending a period of steady water consumption (without reduction of transpiration) of plants in drought conditions for 2-3 weeks or to achieve 1.5-2-fold water savings during crop irrigation [4,8,9]. Unlike the traditionally used solid-phase carriers (e.g. peat, zeolites, perlite, vermiculite), application of gel compositions for fixation of the PPAs and mineral elements in the root area is more effective, according to the authors, because the PPAs locate in all volume of the carrier. Consequently, the protection of roots and tubers with gel compositions should be more advanced because of a tight and all-round (i.e. not local) contact of plant organs with the PPAs.

The development of new PPA technology using gel carriers should begin with an assessment of their efficiency as inhibitors of pathogenic flora. Thus, the research objective focuses on a comparative analysis of the effects of similar PPAs in the form of gel structures with colloidal and ionic silver on the colonial growth of fungal and bacterial flora. Such causative agents as late blight and black leg of potato were used for the laboratory experiments. Unlike previous studies [2, 10-14], this research involves the assessment of non-water solutions such as gel compositions of the PPA with colloidal and ionic form of silver nitrate ($AgNO_3$). These compositions improve the stability of the nanoparticles, and in the practical use guarantee fixation of PPA in the rhizosphere and its protection from leaching. Ionic silver was selected as a PPA considering that adding special stabilizers is not needed, therefore the practical implementation is convenient and cheaper. Two main objectives of the research are as follows: 1) to assess the antifungal and antibacterial effects of gel compositions with silver in order to determine the range of PPA doses for subsequent trials under field conditions; 2) to compare the

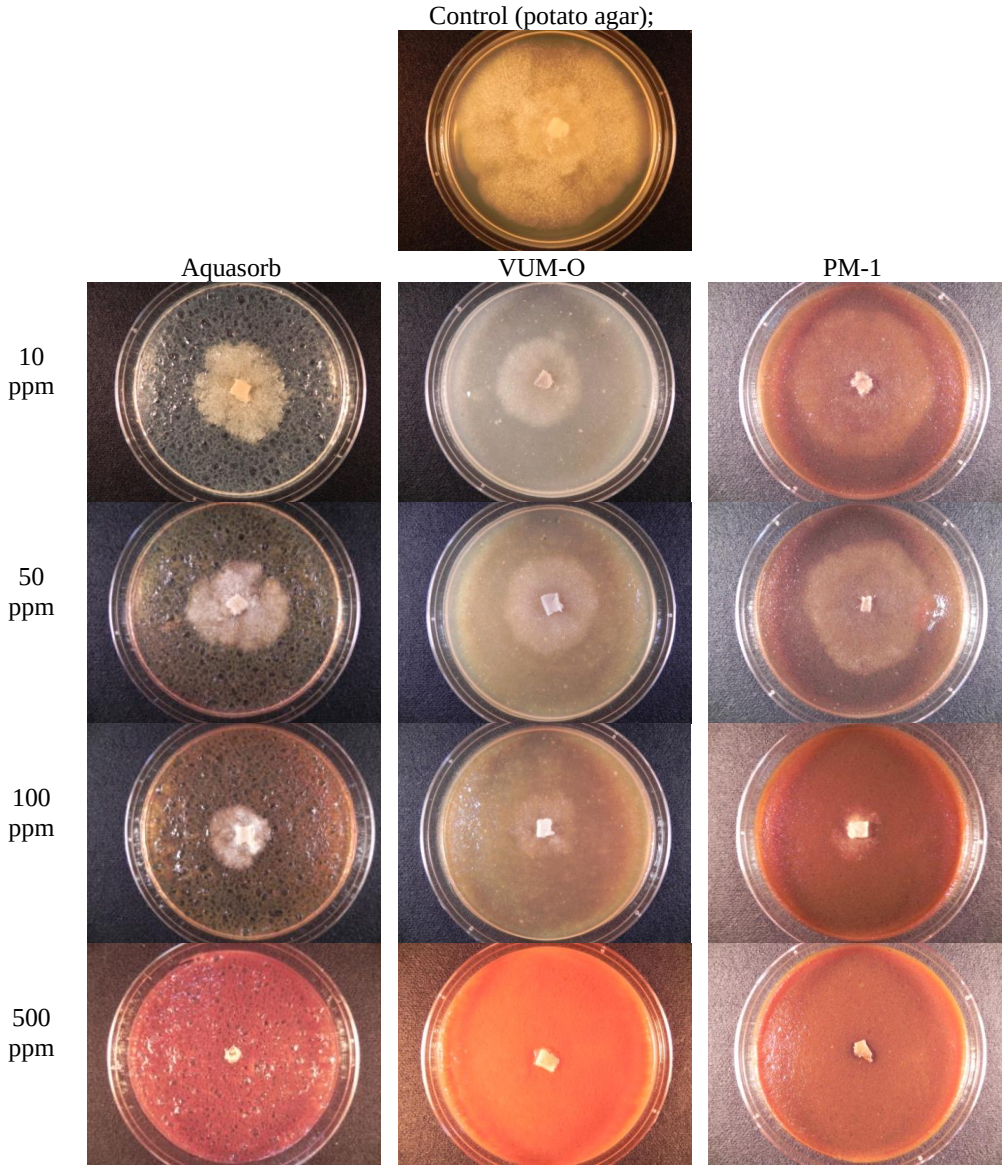

Fig. 1. The growth of colonies of *Phytophthora* with an application of the protective gel composition with silver nanoparticles.

effectiveness of different forms of silver and different types of hydrogels. Based on the previous studies [2, 10-16], the dose of silver should not exceed 100 ppm, which is economically sufficient considering an average price of silver 15-30$ per Trinity oz.. This study compared the efficiency of the two agents, as well as several types of hydrogels: a convenient "Aquasorb" based on polyacrylamide and new hydrogels produced at the Ural Chemicals Factory by our proprietary technology with the filling of the polymer matrix. To compare a new methodology for quantitative evaluation of hydrogel compositions effect on the growth of pathogenic organisms was developed. The methodology was based on the exponential growth model and two quantitative indicators, such as linear (calculated by the diameter of colonies of microorganisms) and nonlinear (by their area).

2. Material and Methods

2.1. Experimental set-up

Pathogenic organisms' strains of late blight (Oomycete Phytophthora infestans (Mont.) de Bary) and black leg of potatoes (Enterobacterium Pectobacterium atrosepticum (van Hall 1902)) used in the microbiological experiments were obtained from the All-Russian Collection of Industrial Microorganisms in State Science Research Institute of Genetics. The assessment of protective properties of the gel compositions was performed on potato agar with addition of silver inhibitors with the concentrations of 10, 50, 100 and 500 ppm (by pure active substance - Ag). Two types of growth inhibitors were used, colloidal silver and ionic silver (chemically pure $AgNO_3$) in the composition with three types of hydrogels (hydrophilic agent Aquasorb (France) based on polyacrylamide, acrylic gel VUM-0 with hydrophilic filler in the form of waste biocatalytic production of polyacrylamide and acrylic gel PM-1 with amphiphilic filler in the form of dispersed peat). The latter is an experimental product of the Ural Chemicals Factory from patent-pending technology [4-17]. The colloidal silver is an experimental product "Zeroxxe" of AgroChimProm GC (http://tdahp.ru/en/,) that contains silver nanoparticles with a size of 10-70 nm superficially modified with environmentally safe biodegradable amphiphilic surfactant (tallow amphopolycarboxyglycinate-stabilizer [18].

To prepare the growth medium for the microorganisms, hydrogels and potato agar were mixed in a 1:1 ratio with a dose of 10 g of each substance per 1 L of medium. The inoculation was carried out in the center of a 100 mm diameter Petri dish, in the form of mycelium excision 5×5 mm (Oomycete). Similar sized areas were created by a microbiological loop (Enterobacterium). The dishes were incubated at room temperature (22-24 ° C) and daylight illumination.

2.2. Microbiological analysis and estimation of antimicrobial effective concentrations

A visual growth estimation of the diameter of the colony (**D**) was carried out in three replications for the control (potato agar) and each experimental variant with different concentrations of silver inhibitors in different gels. A comparative measurement of the diameters was performed when the colonies' sizes reached the diameter of the Petri dish.

Two indicators for the relative growth of the colonies were used. The first indicator is linear showing an increase in diameter (**D/D_0**, where **D_0** – diameter of the colony under control), as used in other studies [13]. The second indicator, proposed here, is based on the radial growth of the colony according to its area. For radial growth of the colony, its biomass and the size of the covered resource is proportional to the volume with constant altitude according to the area of the colony ($\pi D^2/4$). Then the dimensionless indicator **D^2/D_0^2** reflects the relative growth in comparison with the control ($\pi D_0^2/4$). Both indicators, D/D_0 and D^2/D_0^2, are relevant for the calculation of the median or half-maximal (50%) effective concentration (EC_{50}) of the PPAs on the basis of the experimental relationship between these indicators of relative growth and PPA concentration.

Most of laboratory tests of PPA do not consider the growth of pathogens, but rather focus on the efficiency of its suppression (Inhibition Rate, IR) depending on PPA doses [13-19]. For example, in Kim et al. [13] the indicator IR is calculated as:

$$IR = \frac{R_0 - R}{R_0} \quad (1)$$

where **R** is radius of the colony, **R_0** is an analogical value in control (without PPA). It is easy to verify that our rate D/D_0 will be associated with the traditionally used value of IR by the following relationship:

$$IR = \frac{R_0 - R}{R_0} = 1 - \frac{R}{R_0} = 1 - \frac{D}{D_0} \quad (2)$$

It is shown below that for exponential model used in our work, both indicators are identical, and, therefore, the obtained results can be compared with the previous publications using the IR index [13-19].

2.3 Modeling dependence of "the indicator of growth vs. the dose of PPA"

The EC_{50} expresses antimicrobial potency and sensitivity of plant pathogens. To estimate the value EC_{50}, it is necessary to obtain the relationship between concentration (dose) of the PPA and indicator of growth of microbial colony and to approximate this dependence of the most appropriate mathematical model. Substitution in such a model the value of dimensionless indicator of growth D/D_0 or D^2/D_0^2 equal to 0.5 will give the required value EC_{50}. For this purpose, a 4-parameter logistic model is used in toxicology and plant pathology [20]. However, an extensive experimental dataset is needed to fir this model especially in areas close to zero (PPA dose = 0) and maximal inhibition processes [21]. In this study, we discovered that a simpler 2-parameter exponential model could be used to estimate a relationship between the PPA concentrations and both indicators of relative microbial growth. The following relationship is proposed:

$$C = a \cdot \exp(-b \cdot x), \quad (3)$$

where C - the concentration of silver inhibitor, [ppm], x – the dimensionless indicator of colonies growth (D/D_0 or D^2/D_0^2), a, [ppm], b [dimensionless] – the empirical parameters of the model.

In semi-logarithmic coordinates, this model gives a linear trend. This means that from a mathematical point of view, our approach is similar to a linear log method (linear regression of microbial growth inhibition vs. logarithmic concentration) proposed by [20]. Such approach could be based presumably on lognormal data distribution obtained from [22] during a statistical analysis of the fungicide EC_{50} values. As shown in [20] a linear logarithmic method could be implemented instead of more complicated models of the EC_{50} calculation, including a 4-parameter logistic model and standard software (IBM SPSS ®, GraphPad Prism® and DPS®) on its basis for computer processing of toxicological data. The exponential model (3), unlike the 4-parameter logistic, allows calculating both the EC_{50} and total maximal (100%) effective concentration (ECt) of the complete growth inhibition when D = 0 value is substituted in the exponential equation. In this case, the EC_t will be numerically equal to the parameter of model a.

In fact, using either growth indexes or more common in the literature values of growth inhibition IR will lead to the same estimates EC50 or ECt for the exponential model (3). If $x = D/D_0$ in model (3) or it satisfies the relationship between the dose of PPA and dimensionless index of specific growth of pathogens (D/D_0), the latter could be expressed via IR from (2) and substitution in model (3), after simple algebraic transformations. The new model is as follows:

$$C = a \cdot \exp\left(-b \cdot \left(\frac{D}{D_0}\right)\right) = a \cdot \exp\left(-b \cdot (1 - IR)\right) = A \cdot \exp(b \cdot IR) \quad (4)$$

Where $$A = \frac{a}{\exp(b)} = \text{const} \quad (5)$$

The resulting model (4) for the variable IR is also represented by an exponential dependence with the same exponentially index b, only with opposite sign. But in this case, the calculation EC_{50} or EC_t for both models (3) and (4) will give the same results. Indeed, a substitution in (3) of the value of $D/D_0=0.5$ to calculate EC_{50} gives

$$EC_{50} = a \cdot \exp(-0.5b). \quad (6)$$

If $D/D_0=0.5$, the index IR according to (2), is also equal to 0.5. Substituting IR=0.5 in (4), we take into account the ratios (5):

$$EC_{50} = A \cdot \exp(0.5b) = \frac{a \cdot \exp(0.5b)}{\exp(b)} = a \cdot \exp(-0.5b) \quad (7)$$

or the value identical to (6).

Substituting in (3) the value of $D/D_0=0$ to calculate EC_t, required value as mentioned above, will be equal to a (ECt=a). Indicator IR at D/D0=0 will be equal to its maximum value – unity, according to (2). But in this case, model (4) by substituting IR=1 gives the following expression:

$$EC_t = A \cdot \exp(b) = \frac{a \cdot \exp(b)}{\exp(b)} = a \cdot \quad (8)$$

In this case like in model (3) EC_t=a as required.

Thus, regardless of whether a direct measure of the relative growth of D/D_0 or a reverse value IR of growth inhibition is used, an exponential relationship of any of the two parameters with concentration (linear relationship with the logarithm of concentration) will be true for the other indicator.

The same will obviously be true for the proposed new criterion of relative growth by area of colony D^2/D_0^2. The same dimensionless index IRs here can be estimated in a first approximation according to the formula $IRs=1-D^2/D_0^2$ and then all of previously obtained reasoning and ratio (4) – (8) remain in effect.

Statistical and mathematical processing of the results, including the selection of the model (3) parameters were produced in the program Sigma Plot 9 with the built-in algorithms for nonlinear regression "Regression Wizard".

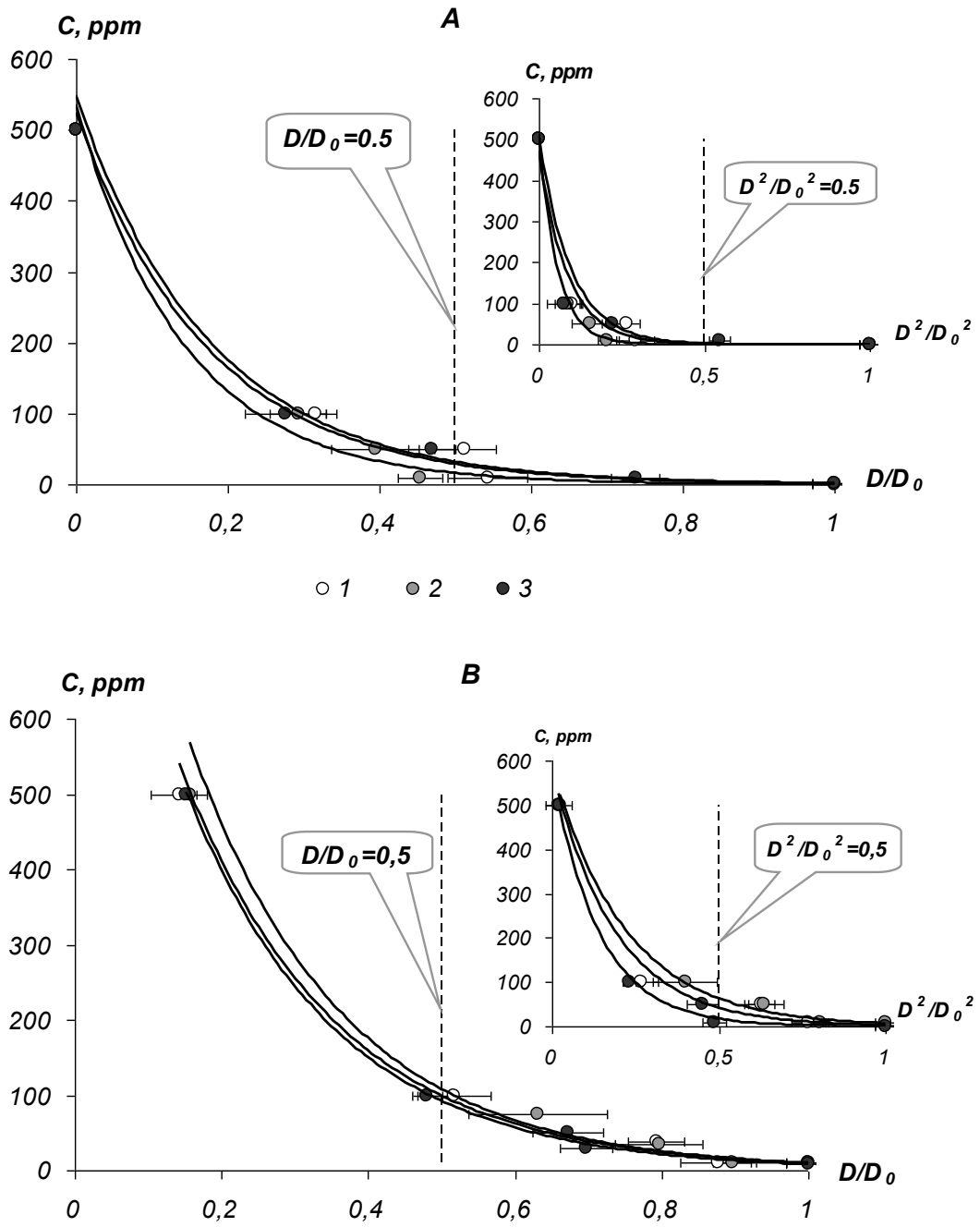

Fig. 2. Exponential model describing the relationships of doses of the inhibitor (***C***) and the specific growth of *Phytophthora* (to the definition of EC_{50}). Hydrogels: *1* – Aquasorb, *2* – VUM-0, *3*– PM-1;

A – colloidal silver, on the main figure, growth indicator is shown in relation to the diameter of colonies (**D/D_0**), equation of the exponential model: *1* – $y = 525.07 \cdot \exp(-5.8009 \cdot x)$ $R^2 = 0.94$; *2* – $y = 534.66 \cdot \exp(-7.014 \cdot x)$, $R^2 = 0.97$; *3* – $y = 547.88 \cdot \exp(-5.6776 \cdot x)$, $R^2 = 0.99$; insert figure shows the growth indicator in relation the area of colonies (**D^2/D_0^2**), equation of the exponential model: *1* – $y = 471.56 \cdot \exp(-11.63 \cdot x)$, $R^2 = 0.99$; *2* – $y = 513.14 \cdot \exp(-17.756 \cdot x)$, $R^2 = 0.99$; – $y = 363.85 \cdot \exp(-8.2973 \cdot x)$, $R^2 = 0.94$).

B – ionic silver; on main figure, an equation of the exponential model is shown: *1* – $y = 1061.1 \cdot \exp(-4.7387 \cdot x)$ $R^2 = 0.96$; *2* – $y = 1215.0 \cdot \exp(-4.8404 \cdot x)$, $R^2 = 0.96$; *3* – $y = 1058.7 \cdot \exp(-4.8801 \cdot x)$, $R^2 = 0.99$; insert figure, an equation of the exponential model is shown: *1* – $y = 583.27 \cdot \exp(-5.258 \cdot x)$, $R^2 = 0.94$; *2* – $y = 583.04 \cdot \exp(-4.4487 \cdot x)$, $R^2 = 0.97$; – $y = 577.86 \cdot \exp(-7.0555 \cdot x)$, $R^2 = 0.96$).

3. Results

3.1 Estimating the hydrogel's anti-fungal and bactericidal effect

The gel-silver specimens evaluated during the experiment demonstrated fungicide and bactericide properties with respect to the pathogenic flora using late blight and black leg of potato. Several photos of the experiment (**Fig. 1**) illustrate the visual anti-fungal effect from the composition with silver nanoparticles, which begins inhibiting growth and developing late blight with doses of the active substance as small as 10 to 50 ppm.

The resulted anti-fungal effect as well as bactericide properties of the hydrogel's composition with silver were characterized quantitatively using two dimensionless indicators for colony growth (D/D_0 and D^2/D_0^2). Regardless of index selected, the data correlat-

ed well with the inhibitor concentration (R^2 = 0.94-0.99) according the exponential model (3) (**Fig. 2**). All parameters of the model were statistically significant at the level of $p < 0.01$. The EC_{50} criteria calculated in accordance with the model characterizing a nonlinear relationship between the dose of PPAs and the pathogenic organism growth. Based on this relationship, small concentrations of the PPAs intensively inhibited the growth of the pathogenic flora reducing it in more than 2-3 times compared with the control (Fig. 2). However with further increases of PPA dose, the efficiency of pathogenic agents' inhibition decreases. Total suppression of pathogenic organisms, according to the obtained dependence occurs when the PPA dose becomes equal to EC_t or parameter *a* in model (3).

3.2 Comparison EC_{50} and EC_t values between different PPAs and pathogens

Calculated values for the EC_{50} and EC_t for the gel compositions with ionic and colloidal silver at two variants of growth estimation, by diameter and by area of the colony, are shown in **Table 1** and **2**. The EC_{50} of the studied PPAs varied within the range of 0.9±0.1 and 118.1±51.5 ppm. The gel compositions based on colloidal silver in the case of late blight had the lower-range values (1-30 ppm). Similar compositions with ionic silver were characterized by higher EC_{50} values of 20 -100 ppm relative to the late blight, i.e. lower fungicide effect. With respect to *Enterobacterium*, the gel compositions based on $AgNO_3$ appeared to be generally more efficient than silver nanoparticles. The EC_{50} concentrations for the hydrophilic gels Aquasorb and VUM-0 varied from 1.1±0.5 ppm to 21.7±4.3 ppm, while the values for the compositions with nanoparticles were higher, ranging from 2.4±0.4 to 35.0±23.4 ppm. In contrast to hydrophilic gels, the bactericide effect of the composition with amphiphilic hydrogel PM-1 and silver nanoparticles was 1.4-2 times higher than the ionic analogue. However, significantly higher EC_{50} values (3-10 times) were observed here in contrast to the hydrophilic compositions based on Aquasorb and VUM-0. Therefore the type of hydrogel (hydrophilic or amphoteric) may affect the bactericidal properties of the composition. Apparently, the amphiphilic peat filler of the hydrogel PM-1 serves as an adsorbed substance for silver ions and reduces their activity in the liquid phase of the gel composition.

The effective concentrations of the EC_{50} calculated based on the linear growth (diameter) and the area of the colony growth were different despite similarities in the model relation between the specific growth indicators and PPAs' dosages. In the case of late blight, the EC_{50} values calculated based on the diameter exceeded the calculation by area by 2-20 times. Similar excess by 3-15 times was observed in the case of the bacterial culture of *Pectobacterium atrosepticum*.

Complete inhibition of pathogenic agent activity was characterized according to the exponential model with the ECt values. Its estimation for all types of the protective compositions and calculated variants ranged between 363.9±14 and 1215±107 ppm in the case of late blight and between 99±8 and 1701±554 ppm for black leg of potato. These values were 10-50 times or higher and exceeded the estimations of the PPA efficiency based on the EC50 alone. Furthermore, the ECt concentrations were different by1.5-3 times between different variants of the gel compositions and based on the calculation approach (by diameter or area). The values were comparable with similar differences for the EC50 indicator reaching 10-20 times or higher. Thus, the ECt value was found to be a more steady efficient criteria of the PPA in comparison with the EC_{50}.

4. Discussion

The estimated by diameter EC_{50} values (16.0±5.9 - 86.4±39.0 ppm) for the compositions with colloidal silver were close to obtained estimations (1-50 up to 100 ppm) for silver nanoparticles in other studies [2, 11-16]. This is indicative of quite high fungicide and bactericide efficiency of similar PPA. This approach also confirms a high antimicrobial effect of the gel-silver compositions indicated by the area of colonies (index D^2/D_0^2) that gives sufficiently lower EC_{50}. While we have no information which approach is a better estimation of the growth of microorganisms, according to a linear increment of the diameter of the colony or by its area. If our approach is correct considering that the index D^2/D_0^2 better describes the radial growth of colonies with the increase of its biomass in proportion to its area, the values EC_{50} will be significantly less. In this case, the double inhibition of the growth of *Pectobacterium atrosepticum* will require no more than 2-5 ppm of silver in any form (nanoparticles or ions) in the hydrophilic compositions (gel Aquasorb or VUM-0). And more cheaper by the inclusion biocatalytically wastes new hydrogel VUM-0 not how much inferior to the well-known brand Aquasorb. The practical application of amphiphilic composition with gel PM-1 to suppress the growth of *Enterobacteria* is obviously not effective as it requires significantly higher values EC_{50} from 20 to 40 ppm. To combat late blight is better to use a gel composition with silver nanoparticles; all types of hydrogels produced here similar results. Assessment of EC_{50} by the growth area of the colony gives a value of 2-6 ppm, similar to those obtained above for *Enterobacteria*.

Such low concentrations (2-6 ppm) are suitable from economic and technological perspective, and they are in full compliance with known information about the antibacterial properties of silver in the aquatic environment. For example, the EC_{50} values of silver ions and nanoparticles for bacterial and fungi microflora in natural water systems and in cultural liquids usually do not exceed 100-200 ppb [10, 23, 24]. Bactericidal concentrations of silver in water of 10-1000 ppb also are far below dangerous thresholds for humans, according to [23] in contrast with the silver PPAs with the concentrations of tens of ppm.

However, we must emphasize that in our calculations small doses (2-6 ppm) are obtained by only using the index D^2/D_0^2 of specific microbial growth. Traditional evaluation by the linear growth of the colony gives value EC_{50} from 16 to 32 ppm for *Oomycete* in the case of using any types of hydrogels with silver nanoparticles and EC_{50} from 14 to 35 ppm for *Enterobacteria* in the case of using any forms of silver (ionic or colloidal) with a hydrophilic type of hydrogels (Aquasorb or VUM-0). The same values were obtained by a similar laboratory microbiological methods (silver ions or nanoparticles in the composition of dense, gel-like agar medium or in the foams as saponins) in the studies [15] – EC_{50} from 30 to 50 ppm; [12] – diapason EC_{50}– EC_t up to 111 ppm; [13] – EC_{50}– EC_t from 50 to 100 ppm; [16] – EC_{50} from 13 to 50 ppm; [14] – EC_{50} from 10 to 15 ppm. Smaller doses were received only for aquaculture, or when using water solutions and dispersions of silver at the surface of agar medium for the growth of microbes as, for example, EC_{50} = 5-50 ppb of silver ions in Slade and Pegg [10], 2-8 ppm of silver nanoparticles in Min et al. [11] or Ali et al. [2]. Perhaps a large difference between the values of the EC_{50} in water and gel systems, including agar mediums for microbial growth, are associated with a decrease in ionic and nanoparticle mobility in gel systems.

The 99% effect according to the 4-parametric model of the growth inhibition of pathogenic microflora, apparently, is achieved at doses of 75-100 ppm when using aqueous solutions or dispersions of silver [2, 25]. However, for the gel media a higher doses EC_t may be required (Table 2). Leaving aside the economic problem of high doses introducing, we touch briefly its environmental aspects. For aquatic plants and aquaculture twofold decrease in growth occurs at 10-20 ppm (up to 150 ppm for green algae) of silver ions or nanoparticles [23, 26]. In the soil, for which protective hydrogel compositions are designed, the value EC_{50} for plants vary in the range of 50-1000 mg/kg of the solid phase [27]. In terms of soil solution it gives at the range from 250 to 5000 ppm (20% water content). Shclich et al. [28] reported a similar order of EC50 values from 200 to 400 ppm for earthworms in the soil at experimental doses of ionic and colloidal silver from 15 to 1000 mg/kg or from 75 to 5000 ppm at 20% soil moisture.

Taking into account the obtained results, we can assume that the most effective dose of silver in protective gel compositions for rhizosphere should be around 100 ppm. Such doses guarantee the suppression of pathogenic microflora and does not violate much the growth and development of plants and soil invertebrates. Are doses of about 100 ppm effective in real field conditions? How to make protective composition: in the form of a mixture with the soil or just in the form of pure hydrogel? What should be the minimal mass of hydrogel introduced into the rhizosphere? Will there be a silver-based inhibitors in protective gel compositions more effective than traditional organic pesticides? All these questions remain open and require further testing in the field. Based on the obtained results (Tables 1 and 2), it will be necessary to use a wide range of silver ions and nanoparticles at least from 10 to 500 ppm.

Table 1. The effective concentration for two-fold suppression of pathogenic microflora growth (EC_{50})

Hydrogels	EC_{50} (estimation by diameter)	EC_{50} (estimation by area)
1. *Phytophthora*		
Ionic silver		
Aquasorb	99.3±20.3	42.1±4.4
VUM-0	108.8±18.8	63.0±9.6
PM-1	92.3±10.8	17.0±5.4
Colloidal silver		
Aquasorb	28.9±7.6	1.4±0.2
VUM-0	16.0±5.9	0.9±0.1
PM-1	32.0±11.4	5.8±2.4
2. *Pectobacterium*		
Ionic silver		
Aquasorb	13.8±3.1	1.1±0.5
VUM-0	21.7±4.3	5.1±1.9
PM-1	118.1±51.5	40.3±14.0
Colloidal silver		
Aquasorb	31.1±19.1	2.7±0.5
VUM-0	35.0±23.4	2.4±0.4
PM-1	86.4±39.0	21.0±9.3

Table 2. The effective concentration for total growth inhibition of pathogenic microflora (EC_t)

Hydrogels	EC_{50} (estimation by diameter)	EC_{50} (estimation by area)
1. *Phytophthora*		
Ionic silver		
Aquasorb	1061±223	583±14
VUM-0	1215±107	583±36
PM-1	1039±26	578±62
Colloidal silver		
Aquasorb	525±20	472±24
VUM-0	535±21	513±16
PM-1	547.8±20	363.9±14
2. *Pectobacterium*		
Ionic silver		
Aquasorb	100±10	99±8
VUM-0	103±13	102±5
PM-1	1701±554	663±81
Colloidal silver		
Aquasorb	483±22	583±14
VUM-0	496±17	583±36
PM-1	1449±704	643±90

5. Conclusion

1. Hydrogel compositions based on colloidal and ionic silver can be an effective means of protecting the rhizosphere of plants against pathogenic microorganisms. Three types of hydrogels were tested using collection strains of pathogenic microflora of potato *Oomycete Phytophthora infestans* and *Enterobacterium Pectobacterium atrosepticum*.
2. For the studied range of concentrations of silver ions and nanoparticles from 10 to 500 ppm an exponential relationship between dose and the specific growth rate of colonies of the pathogens was detected. Along with traditional linear criterion of specific growth of the colony by increase of its diameter (a), a new nonlinear criterion by the increase of the area of microbial colony (b) was proposed.
3. Evaluation of the median effective concentration EC_{50} by criterion (a) according to the obtained exponential model gives the average range from 16 to 109 ppm for *Oomycete* and from 14 to 118 ppm for *Enterobacteria*. Assessment criterion (b) gives the values EC_{50} at 2-20 times less.

4. Silver nanoparticles are more efficient in the growth inhibition of *Phytophthora* than ions. The bactericidal properties of colloidal and ionic silver against black leg of potato were actually the same.
5. New hydrogels with fillers in the form of biocatalytic wastes and of dispersed peat practically did not differ from a known brand "Aquasorb" in fungicidal experiments with *Phytophthora*. In bactericidal compositions, the gel with dispersed peat gave the worst results, presumably due to the adsorption of silver by the surface of the peat.
6. A widely used indicator EC_{50} may underestimate the possibility of growth of the studied pathogenic microorganisms, whose complete suppression requires doses EC_t of 100-500 ppm.

Acknowledgments

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